Towards Wastewater Biorefineries:

Integrated Bioreactor and Process Design for Combined Water Treatment and Resource Productivity

Final Report

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EXECUTIVE SUMMARY

In this project, we have investigated the concept of the wastewater biorefinery (WWBR): the integrated processing of a wastewater stream or streams to generate products of value, including "clean" water, and remediate the effluent simultaneously. In this approach products of variable value are produced concomitantly with production of clean water as a product, typically through multiple unit operations, with a preference to generate products of sufficient value to make the biorefinery economically viable. Our focus has been on both developing and testing this concept as well as evaluating its applicability to and potential in the South African context.

We are at a time in our anthropological history in which both water and wastes are being considered in new ways. A strong focus is in place to consider the re-use of resources through the concepts of industrial metabolism and the circular economy. The early projects re-visioning water and waste together are beginning to emerge in Europe. The overview in this project shows that there are equivalent possibilities in South Africa. However, implementation of these potentials is difficult because maintaining the integrity of the basic water treatment infrastructure and its optimal performance is already a struggle in South Africa, with its burgeoning urban populations and limited financial and skills-based resources. By integrating the goals of water treatment with the goals of the Bioeconomy, there is great potential to transcend these challenges and create a new industry.

In this project we review the relevant research done both internationally and nationally. We present extensive literature reviews with regard to the different aspects of WWBR, addressing both the global and local context. We investigate specific aspects through interviews with industry stakeholders. This is followed by a review on the wastewater streams available in South Africa. We examine some potential products as well as the bioreactors required to produce them. We begin to examine some of our findings on bioreactors and products through laboratory studies. To contextualise the findings in an integrated system, a generic flowsheet and mass balance model is developed. We explore the features of the integrated biorefinery using this model to assess a few conceptual case studies.

The application of wastewater biorefineries does not generally need new technologies, but rather the integration and optimisation of existing technologies for multiple benefits. Throughout this work it is evident that there are both perceptual and practical hurdles around implementation of wastewater biorefineries. These may be due to risk aversion, policy constraints and lagging technological adaptations. This represents a constraint in the development of WWBR and sociological intervention is needed alongside the technological advances.

It is pleasing to see increased cooperation between academia, state entities and industry on specific technologies, to a greater extent internationally, but also increasingly so in South Africa. This is a critical requirement for the implementation of improved resource utilisation and the combined approach to value addition and water treatment and needs to be nurtured. Globally, and in South Africa, there has been a huge amount of focused work done on wastewater treatment and beneficiation using specific, isolated technologies, but very little work has been done on the integration of these into overall solutions.

Producing products more valuable than energy products is required for the WWBR to be economically feasible, with the conventional bioenergy products produced from residual organics. This requires a mindset change about investment, risk and associated returns, as well as an identification of the relevant product range and comparison between conventional processing routes and what is possible from the wastewater. Waste may need to be re-classified to be used as a raw material.

The products listed in this report represent a fairly intensive overview of the products possible from the heterotrophic bacterial bioreactor, but the products possible from the algal, macrophyte and solids bioreactors have not been extensively reviewed. We identify that function-based products specific to

niche industries, particularly those from which the wastewater comes, are of substantive interest owing to their streamlined market uptake. These may be peculiar to those industries. They depend on both the composition of the wastewater in that industry complex and the needs of the market surrounding it. There is a tension between producing “drop in” products with an existing market and novel function-based products with commodity uses in niche industries closely associated with the industry producing the wastewater. Biopolymers are particularly promising. We feel that the spectrum of potential products has not been completely investigated, even globally. A better overview can only be obtained through focused case studies, including integrated industry pilots coupled with market engagement.

Not all products can be produced in the non-sterile dilute environment. Ecological selection through bioreactor design and operation is a suitable way to direct productivity given the large, dilute volumes of substrate. These products may have the stigma of “waste” associated with them that could limit their application in some areas. For example, wastewaters from food-related industries need to be considered for production of products other than food-based products. This still allows reasonable possibilities for application to associated industries.

Wastewaters are characterised in this report according to volume, concentration and complexity. Of these, complexity introduces the most uncertainty, which includes changing unpredictably over time. Appropriate bioreactor design is required to accommodate this uncertainty. Volume and concentration can be used in combination to determine the potential of the source. Super-imposition of regional location onto this data assists with the identification of wastewater resources with potential as raw material feedstock.

This project has highlighted the need for WWBR bioreactors to function in variable, dilute environments, without the need for sterilisation. The requirements for bioreactors for WWBR are defined both from a design and operational perspective. The bioreactor needs to contribute to provision of an environment that favours the product of interest. Decoupling hydraulic and solid residence time is required, as is the need to create an ecological niche for self-selection of the desired microbial community. Enabling effective product recovery should be included as a design feature. Some existing bioreactor designs are suitable and should be studied in an integrated system as well as coupled with product recovery.

Laboratory-scale studies are valuable in providing proof of concept with respect to product generation from specific wastewater streams, with concomitant delivery of compliant water. Some existing wastewater treatment works may easily be retrofitted to contribute to WWBR due to their location, their choice of reactor systems, or their willingness to experiment, and should be investigated on pilot scale. Industry champions need to perform pilot scale experiments using the real situations commonly encountered on a plant. Laboratory scale investigations of critical parameters, like aeration, need to be pursued through scale-down studies in the context of the WWBR.

There are a variety of downstream processing (DSP) options available that are very well developed, but these have been employed in situations different to the WWBR. These processes, including product recovery, need to be investigated further in the context of WWBR. This includes learning from industries that also deal with dilute complex material, like the minerals industry. DSP options that fully exploit the benefits of retained biomass and decoupled production hold particular promise. These need to be explored in conjunction with the bioreactor designs.

The need to integrate multiple unit operations to ensure compliant water as well as produce a bioproduct or bioproducts is key. The four groupings of unit operations considered in this project were chosen because they each contribute to the functioning of the WWBR as a system. The heterotrophic microbial bioreactor, of which the bacterial biocatalyst is used as a representative example, is helpful for removing a high proportion of the organic carbon. A wide range of commodity products with market potential is known to be produced through heterotrophic microbial systems. The photo-mixotrophic bioreactor represented by the algal bioreactor is present to scavenge high proportions of nutrients, particularly nitrogen and phosphorus and these systems are also known to produce commodity products. The macrophyte bioreactor targets the polishing of the exiting stream in terms of nitrogen, phosphorus and particulates to ensure compliant, fit-for-purpose water as a product, with a macrophyte-based byproduct. The solids bioreactor is a new perspective on beneficiation of bio-slurries and the solid phases recovered during WWBR operation, generating products of value including biosolids.

In this project, a model was developed of these unit operations integrated into a single system generating material inventories across the system. This can be used to enable evaluation of possible scenarios in an integrated context through the use of the generic flowsheet and mass balances. We demonstrate that integrative studies of the unit operations are critical. In the same way as we have developed criteria for the bioreactor design, so we need to develop criteria for the functionalities required from the complete set of unit operations included and an understanding of their interactions. This is difficult to do for a general case and rather requires specific case studies.

Consistent data on wastewater is troublingly lacking. This is the clearest need emerging from this study in terms of assessing the national position, both for the development of the WWBR and for conventional environmental management. There is a need for specification of the approach to data collection, including the manner of measurement, frequency of recording and responsibility for reporting and collection. The data required, and its form, must be specified. This includes the development of appropriate instrumentation.

The economic considerations around specific WWBR cases cannot be considered in a generic manner, yielding a universal solution, but is dependent on regional locality, product market needs, logistics and other factors at play. A lack of sufficient data to inform economic decisions is an expected concern at this stage. Detailed techno-economic studies coupled with integrated pilot studies need to be performed on a case study basis to provide additional insight.

To further the progress of integrated systems, we need to harness the expertise of different disciplines, including anthropologists who understand how new paradigms are adopted, policy makers, lawyers and economists, as well as people who can deal with multiple disciplines in an integrative way to build bridges. At UCT, the Future Water Institute has been established to achieve just this.

Over the duration of this project, wastewater biorefineries have developed from a nascent concept to a groundswell exciting many different groups. It has been a privilege to be a part of that journey.

NAVIGATING THIS REPORT

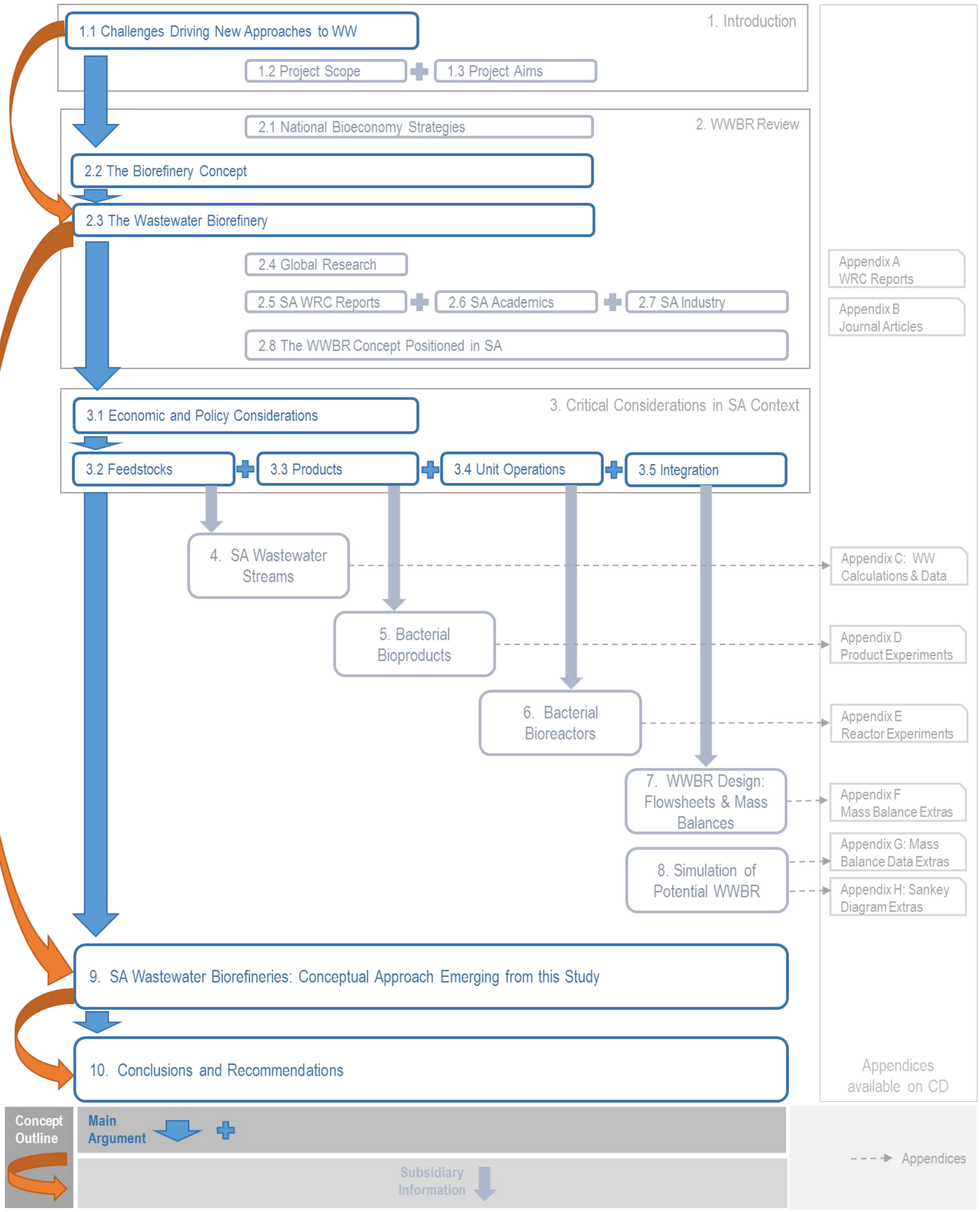

Figure. i Navigating the Report “Towards Wastewater Biorefineries”

This project is a relatively comprehensive review of an emerging concept. As such the report is designed as a document which can be used at multiple levels for different purposes. For a policy-maker coming on the idea fresh from a more traditional space, using the introductory sections preparatory to reading the concluding chapters will provide a conceptual outline (curved orange arrows, extreme left in Figure. i). For a decision-maker wanting to explore this concept with a view to possible application the main argument should be followed more fully, including the critical considerations for the South African Context (thick blue arrows in Figure. i). In the event of the need to examine one or more aspects of the wastewater biorefinery (WWBR) more carefully, the relevant detailed chapter can then be used (medium-width blue-grey arrows in Figure. i). For the researcher wishing to place the study within the flow of the development of the concept the other parts of Chapters 1 (the Introduction) and 2 (the WWBR Review) will be helpful. Finally, anyone wishing to take up the challenge of further research and implementation of the WWBR concept will find helpful supplementary details in the Appendices (thin light grey dashed arrows to right hand block in Figure. i).

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Note: The Appendices listed in this Table of Contents are published in electronic format only and are available on a CD from the WRC.

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ACRONYMS & ABBREVIATIONS

AD	Anaerobic Digestion
AGS	Aerobic Granular Sludge
AOB	Ammonia Oxidizing Bacteria
AOX	Adsorbable Organic Halogen
ARD	Acid Rock Drainage
AS	Activated Sludge
ASSAf	Academy of Science of South Africa
ASU	Arizona State University
AUW	Africa Utility Week
BBR	Billund BioRefinery
BC	British Columbia
BCET	Bio-Chemical Engineering and Technology
BE	Biological Efficiency (yield in mushroom industry)
BIC	Biotechnology Innovation Centre
BNR	Biological Nutrient Removal
BOD	Biological Oxygen Demand
BOE	Barrels of Oil Equivalent
BOT	Build-Operate-Transfer
BTB	Biocatalysis and Technical Biology Research Group
BWWTW	Biological Wastewater Treatment Works
CAD	Computer-Aided Design
CAPEX	Capital Cost
CAWP	Coalition Against Water Privatisation
CBB	Chemical Building Block
CDP	Carbon Disclosure Project
CDW	Cell Dry Weight
CeBER	Centre for Bioprocess Engineering
CFD	Computational Fluid Dynamics
CHP	Combined Heat and Power
CoCT	City of Cape Town
COD	Chemical Oxygen Demand
CPUT	Cape Peninsula University of Technology
CSTR	Continuous Stirred-Tank Reactor
CT	Cape Town
CTBE	Nacional de Ciência e Tecnologia do Bioetanol (Brazilian Bioethanol Science and Technology Laboratory)
cwe	carcass weight equivalent

DAFF	Department of Agriculture, Fisheries and Forestry, South Africa
DO	Dissolved Oxygen
DoHET	Department of Higher Education and Training
DSP	Downstream Processing
DST	Department of Science and Technology, South Africa
DTI	Department of Trade and Industry, South Africa
DUT	Durban University of Technology
DWA	Department of Water Affairs, South Africa (to 2013)
DWAF	Department of Water Affairs and Forestry, South Africa (to 2009)
DWS	Department of Water and Sanitation, South Africa (present)
EBRU	Environmental Biotechnology Rhodes University
EC	Electrical Conductivity
EFC	Eutectic Freeze Crystallization
EGS	Environmental and Geographical Science
EPS	Exopolysaccharide
EU	European Union
FAO	Food and Agriculture Organisation, United Nations
FBBR	Fluidized Bed Biological Reactor
FF	Furfural
FOG	Fat, Oil and Grease
FTN	Flavor Threshold Number
FTW	Floating Treatment Wetlands
GHG	Greenhouse gases
GIZ	Deutsche Gesellschaft für Internationale Zusammenarbeit
GWP	Global warming potential
HPLC	High Pressure Liquid Chromatography
HPS	High Pressure Steam
HRAP	High Rate Algal Ponds
HRT	Hydraulic Retention Time
HVLV	High Value-Low-Volume
IAPS	Integrated Algae Ponding Systems
IEA	International Energy Agency
IfBB	Institute for Bioplastics and Biocomposites (University of Applied Sciences and Arts Hannover, Germany)
INRA	Institute for Agricultural Research (France)
IWA	International Water Association
IWR	Institute for Water Research
IWWT	Institute for Water and Wastewater Technology
Kp	Annual Operating and Maintenance Cost
KT	Total Capital Cost

LCA	Life Cycle Analysis
LCI	Life Cycle Inventory
LLE	Liquid-Liquid Extraction
LVHV	Low-Value-High-Volume
MBBR	Moving Bed Biofilm Reactor
MBR	Membrane Bioreactor
MME	Modified Medium E
MPO	Milk Producers Organisation, South Africa
MSW	Municipal Solid Waste
MXC	Microbial Electrochemical Cells
NatSurv	National Industrial Water and Wastewater Survey
nl	not listed
NOB	Nitrite Oxidizing Bacteria
NREU	Non-Renewable Energy Usage
NTU	Nephelometric Turbidity Units
NWA	National Water Act, South Africa
NWRS	National Water Resource Strategy, South Africa
NWU	North-West University
OHO	Ordinary Heterotrophic Organism
OPEX	Operating and Maintenance Costs
PAO	Phosphate Accumulating Organisms
PBR	Packed Bed Reactor
PBS	Polybutylene succinate
PBT	Polybutyleneterephthalate
PE	Polyethylene
PGA	Polyglutamic Acid
PHA	Polyhydroxyalkonoates
PhaP	Putative HLA-DR-Associated Proteins
PHB	Polyhydroxybutyrate
PLA	Polylactic Acid
POCIS	Polar Organic Chemical Integrative Sampler
PST	Primary Settling Tank
PTT	Polytrimethyleneterephthalate
PUR	Polyurethane
Q	Volumetric Flow Rate
R&D	Research and Development
RBC	Rotating Biological Contactor
RDI	Research, Development, and Innovation
RECORD	Renewable Energy Centre of Research and Development
RMRD	Red Meat Research and Development, South Africa

RO	Reverse Osmosis
RRB	International Conference on Renewable Resources and Biorefineries
SAB	South African Breweries
SABC	South African Broadcasting Corporation
SABIA	South African Biogas Industry Association
SALGA	South African Local Government Association
SANEDI	South African National Energy Development Institute
SAPIA	South African Petroleum Industry Association
SAPREF	South African Petroleum Refineries (Shell and BP)
SASA	South African Sugar Association
SBR	Sequencing Batch Reactor
SC	Solids Content. Mass of solids (dry mass) in sludge / mass of sludge
SCP	Single Cell Protein
SEV	Specific Effluent Volume
SIIT	Sirindhorn International Institute of Technology
SOL	Soluble Organic Loading
SS	Suspended Solids
SSF	(Bio)Solid Substrate Fermentation
SSI	Smallholder System Innovations
SWOT	Strength, Weaknesses, Opportunities and Threats
SWPN	Strategic Water Partners Network
TBR	Trickle Bed Reactor
TC	Total Carbon
TF	Trickling Filter
TKN	Total Kjehldahl Nitrogen
TN	Total Nitrogen
TOC	Total Organic Carbon
TOL	Total Organic Load
TP	Total Phosphorus
TUD	Delft University of Technology
TUT	Tswane University of Technology
UASB	Upflow Anaerobic Sludge Blanket
UCEWQ	Unilever Centre for Environmental Water Quality
UCT	University of Cape Town
UKZN	University of KwaZulu-Natal
UV	Ultraviolet
VFA	Volatile Fatty Acid
VOC	Volatile Organic Compounds
VSS	Volatile Settleable Solids
VTU	Vandsektorens Teknologiudviklingsfond, Denmark

WEF	Water Environment Federation, US
WISA	Water Institute of Southern Africa
WOSA	Wines of South Africa
WRC	Water Research Commission, South Africa
WRCU	the number of non-bovine species equivalent to one bovine cattle unit in terms of water usage during processing
WRN	Water Research Node (WRC)
WSUD	Water Sensitive Urban Design
WWBR	Wastewater Biorefineries
WWT	Wastewater Treatment
WWTW	Wastewater Treatment Works

GLOSSARY OF TERMS

Beneficiation	concentration or enrichment of a valuable product from its raw material
Bio-based chemicals	substitutes for petrochemicals or novel products derived from renewable biomass sources (recently fixed CO_2)
Bio-based economy	an economy that integrates the full range of natural and renewable biological resources and the processing and consumption of these bioresources The bio-based economy encompasses agriculture, forestry, fisheries, food and industrial sectors. It makes more use of biomass to replace fossil based resources using biotechnology for the production of fine chemicals and pharmaceuticals.
Bio-based products	non-food products derived from biomass (plants, algae, crops, trees, marine organisms and biological waste from households, animals and food production) may range from high value added fine chemicals such as pharmaceuticals, cosmetics, food additives etc., to high volume materials such as bio-polymers or chemical feedstocks, including platform chemicals
Bioflocculant	bio-based substance which causes aggregation of fine, dispersed organic particles and even microorganisms
Bioprocess	specific process that uses microorganisms or enzymes to obtain desired products
Biorefinery	integrative, multifunctional over-arching concept that uses biomass as a diverse source of raw materials for the sustainable generation of a spectrum of intermediates and products while ensuring the minimization of waste products (see Section 2.2.1)
Bioremediation	cleaning contaminated soil or water using microorganisms or plants
Biosurfactant	diverse group of surface active molecules and chemical compounds synthesised by microorganisms that reduce the surface tension, stabilise emulsions, promote foaming, are non-toxic and biodegradable
Circular economy	an alternative to a traditional linear economy (make, use, dispose) in which we keep resources in use for as long as possible, extract the maximum value from them whilst in use, then recover and regenerate products and materials at the end of each service life www.wrap.org.uk/about-us/about/wrap-and-circular-economy
Commodity products	also bulk products large-volume, low-price, homogeneous, and standardized chemicals produced in dedicated plants and used for a large variety of applications, petrochemicals, basic chemicals, heavy organic and inorganic chemicals (large-volume) monomers, commodity fibres, and plastics http://www.worldcat.org/wcpa/servlet/DCARead?standardNo=9780470050750&standardNoType=1&excerpt=true
Drop-ins	bio-based products chemically identical to their petrochemical counterparts
Economy of scale	reduction in cost-per-unit-produced resulting directly from increased size of production facility
Feedstock	raw material used as the basis for an industrial process

Fine chemical	complex, single, pure chemical substances produced in limited quantities in multipurpose plants by multistep batch chemical or biotechnological processes, identified according to chemical formula http://www.worldcat.org/wcpa/servlet/DCARead?standardNo=9780470050750&standardNoType=1&excerpt=true
Industrial ecology	systematic study of material and energy flows in products, industrial processes, and economies focussing on the interaction of industrial and the ecological systems of which they are a part
Macrophyte	aquatic plant (growing in or near water) – emergent, submerged or floating
Meta research	research systematically combining and integrating data and analyses from multiple studies in order to develop more powerful conclusions and resolve or highlight conflicting areas includes research studying research practices including methods, reporting, reproducibility, evaluation and incentives
Non-renewable resources	natural resources of economic value that cannot be replaced by natural means on a level equal to consumption
Novel bio-based products	new chemicals and materials from renewable raw materials with unique characteristics that are often impossible or very difficult to produce from petrochemical raw materials
Platform chemical	used as feedstock in subsequent chemical or biochemical industrial processes to manufacture a range of consumer products
Renewable resources	natural resources of economic value that are replaced through cultivation, natural growth or deposition at a rate commensurate with consumption
Resource recovery	process of obtaining matter or energy from waste materials
Sankey diagram	a type of flow diagram in which the width of the arrows is proportional to the flow quantity
Soil conditioner	organic or inorganic materials added to soil to improve its properties (cation exchange capacity, pH, water holding capacity, compaction)
Specialty chemicals	formulations of chemicals containing one or more fine chemicals as active ingredients identified according to performance properties for example: adhesives, agrochemicals, biocides, catalysts, dyestuffs and pigments, enzymes, electronic chemicals, flavours and fragrances, food and feed additives, pharmaceuticals, and specialty polymers http://www.worldcat.org/wcpa/servlet/DCARead?standardNo=9780470050750&standardNoType=1&excerpt=true
Valorisation	process of using chemical or biological methods to increase the value of a material by changing it – in particular here, producing products of value from a feedstock otherwise regarded as waste
Wastewater biorefinery	a biorefinery (see above) operating in the wastewater arena and designed to generate products of value from waste nutrients and simultaneously produce clean or 'fit for purpose' water as the non-negotiable product (see Section 2.3)

1 INTRODUCTION

1.1 Challenges driving new approaches to wastewater

It is well recognized that humankind is using the earth's resources and creating a waste burden at a faster rate than nature can replenish these. The seminal analysis of Wackernagel et al. (2002) demonstrated that the balance of resources used or degraded versus those available per capita passed the balance point around 1980 and, since then, a steady degradation of our natural capital is occurring. Some nations are exploiting this natural capital in excess of 5 fold the rate of its replenishment. For many developed countries, this ratio is around 3 fold. For South Africa, it lies between 1.2 and 1.8 fold, yet many in the nation do not experience an acceptable quality of life.

These findings may be super-imposed on the increasing shift from the closed natural cycles to an open system of consumption and waste generation, demonstrated in Figure 1-1. To address environmental burden on our planet, end-of-pipe treatments and, increasingly, waste minimisation have been considered. However, the ongoing urbanisation and industrialisation drive the geographic separation of resource requirement and waste generation, including wastewaters, requiring a new approach.

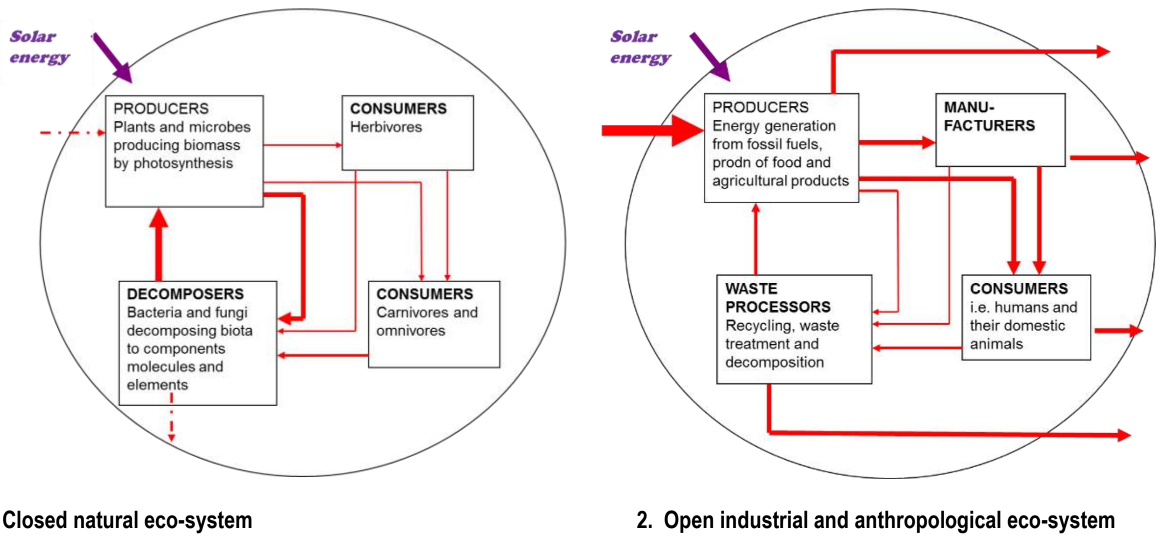


Figure 1-1: Demonstrating the need for closure in our anthropological and industrial eco-systems

With the growing demand for water, driven by the increasing population and its quality of life, water demand is expected to outstrip water supply through conventional approaches. Further, impacts of climate change may aggravate this on a regional basis. Hence, alternative sources of water are sought to address water scarcity. One of these alternative sources is the increasing use of water recycle.

In addition, with ongoing depletion of natural resources, as well as the growing demands of our expanding and developing society, alternative, renewable sources of materials, chemicals, energy and fuels to the traditional fossil-fuel based resources are sought. As a result of this push away from fossil-fuel based materials, there has been a surge of interest in the use of biological systems for the production of biofuels, energy and chemicals. This transition has been nurtured by several countries putting in place 'Bioeconomy Strategy' or "Bio-based Economy Strategy" policies specifically geared towards growing this sector of the economy, giving rise to many widely used bioprocesses. This is premised on a reduced utilisation of fossil resources and increasing dependence on renewable resources.

Traditional examples of bioprocesses geared towards replicating and replacing existing petro-chemical based systems include bioethanol production particularly in Brazil, biodiesel production in the European Union, and poly-lactic acid or starch-based polymers replacing plastics. Typically, these processes use agricultural 'virgin' products or by-products as feedstocks. Examples of the use of fairly pure, and relatively expensive, feedstocks include glucose- or sucrose-syrups for ethanol production, vegetable oils for biodiesel production, or glucose for a variety of bioprocesses producing commodity products. The requirement for these clean feedstocks puts pressure on these processes: economically as the feedstocks often account for a majority of the operating cost of the process (Grotkjær, 2016), environmentally with agricultural feedstocks contributing significantly to the environmental burden (Harding, 2009; Harding, et al., 2007), as well as through socio-economic implications due to the competition between food production and the production of agricultural feedstocks for bio-based chemicals or biofuel.

In contrast to these 'traditional' bioprocesses, biological systems can make use of by-product streams and streams that form the effluents or waste products of other processes. These are, by nature, streams of variable composition, variable flow rates, multiple and changing components. Traditionally, for the most part, bioprocesses for synthesis of products of value and those processing effluents and waste streams have been considered separately, using quite different processing approaches. With the former, the product is all important and the feedstock a cost. With the latter, the quality of the water is all important and the feedstocks present are considered as contaminants to be removed from the liquid phase to gaseous or sludge components that are benign, but without value. The notable exception to this is biogas-producing anaerobic digestion (AD) in which waste organic materials are converted into methane for use as an energy source and, potentially, VFAs as a feedstock for remediation (Harrison, et al., 2014) and commodity processes (Kleerebezem, et al., 2015). Currently the separation between these two types of bioprocesses is significant: on the traditional bioprocessing side, pure feedstocks are used to produce specific products; on the waste-water treatment side, varied streams are processed to produce clean water with little focus on the products of the conversion of the C, N and P resources within the feedstock or recovery of value within the waste stream.

A key need exists to ensure both the maintenance of our water resources (preventing their degradation through pollution) and the maximization of resource productivity i.e. maximizing the use of each resource we exploit while minimising environmental burden. The integration of these two goals with associated improved efficiencies and productivity is the motivator for the development of wastewater biorefineries in which water treatment and optimizing resource productivity are integrated through the sustainable processing of the waste water into a spectrum of marketable products (chemicals and materials), energy and clean water (adapted from IEA Bioenergy Task 42 (n.d.)). The opportunity for this approach is clear when one considers that typical municipal wastewater contains some nine fold the chemical energy required for its treatment (Shizas & Bagley, 2004), yet we commonly input a significant fraction of the municipal energy to treat the water with no combined products. This was confirmed by the analysis of South African wastewaters in 2007 in WRC K5/1732 (Burton, et al., 2009) in which it was seen that energy recovery from waste water could provide a significant contribution to the SA energy provision and that a variety of technologies, including heat recovery, biomass production with subsequent combustion and gasification, biogas production, bioethanol production and microbial fuel cells, could contribute towards energy products.

This project aims to outline and examine a relatively new thinking at the intersection between traditional bioprocessing and wastewater treatment to utilise waste streams as a valuable substrate for commodity bioproducts, rather than a liability simply to be sufficiently cleaned. This concept can be termed the "wastewater biorefinery" where focussed on liquid effluents or more generically the "waste biorefinery". While the concept of the biorefinery within the literature has generally focussed on biorefineries which convert cellulosic biomass as a feedstock, the subject of this study can be defined as distinct by focussing on a substantial and under-utilised resource: wastewaters. The consideration of wastewaters for biorefining has been a recent development in the literature, being tabled for the first time only around

2007/8 (Werker, 2008; Mooibroek, et al., 2007). However, significant research and some preliminary implementation has taken place, for the most part in a European setting.

The implementation of wastewater biorefineries moves industrial production towards closing resource cycles by re-capturing those components of wastewaters which have value and re-inserting them into economic circulation while at the same time remediating wastes and recovering clean water as a product, thus creating a circular economy. This approach is consistent with both the concepts of industrial ecology and cleaner production.

In this document, we focus on the potential of wastewater biorefineries in South Africa and the development of key aspects of these. The potential to view wastewater streams as both a potential water resource and a resource of nutrients to fuel bioprocesses for commodity product formation is considered. Local and global research centred on or applicable to wastewater biorefineries is reviewed. Wastewater streams with potential to be used as feedstock are highlighted along with the technical requirements for such, including which biological systems and reactor configurations may be appropriate. A number of examples of potential products from the wastewater biorefinery are presented. A generic wastewater biorefinery flowsheet and an associated material balance model have been compiled. This is used to explore several hypothetical wastewater biorefinery flowsheets for several South African wastewaters. The compilation of these findings allows the potential value of the wastewater biorefinery in South Africa to be considered.

1.2 Project Scope and Limitations

The provision of water of suitable quality and the treatment of wastewater are a high priority, both globally and specifically in water scarce South Africa where currently 8 of 9 provinces are declared drought disaster zones (Reuters, 2016). In most countries compliance requirements have become more stringent with increasing recognition of the impact of poor water quality on the quality of human life and the environment. There is an impending water scarcity in many parts of the world, including South Africa, with growing water demand and increasing wastewater generation resulting from an increase in human population, standard of living and industrialisation.

The first priority with respect to water use and wastewater generation should always be to minimise both waste production and water use through cleaner production approaches and the integration of closed systems. Where this cannot be achieved or is only partially achieved, the concept of wastewater biorefineries has potential for further minimisation of waste generation and water use, within a larger ‘system boundary’.

Typically, wastewater treatment is considered an expense, with its associated treatment and energy costs. It is focused on the remediation of water to environmental quality rather than to direct application for particular use. Approaching wastewater from a different perspective, we consider wastewater as a potential feedstock for the production of both compliant water or water ‘fit for purpose’ for its next use and for the production of other products using the organic carbon as well as nitrogen and phosphorus nutrient components of the wastewater streams. Such perspectives are aligned with water sensitive urban designs and with the principle of industrial ecology.

The wastewater biorefinery is designed and optimised with the aim of ensuring both the effective treatment of the water to the necessary standard (yielding “clean” and “fit for purpose” water as a product), and the conversion of the components removed from the wastewater to products of economic and/or social value.

Due to the dilute nature of wastewaters, many typical bioprocess configurations and bioreactor designs are not appropriate. Because of the non-sterile nature of the wastewater environment and impracticality of sterilisation, use of mixed microbial consortia is required. Hence it is imperative that the microbial community of interest is selected through the choice of culture conditions and environment providing selective advantage. This means that wastewater biorefineries are not suitable for all bioproducts.

In order to investigate the potential of wastewaters as a resource for bio-based products in South Africa and to identify potential products suitable for production from wastewater with simultaneous upgrading of the water resource, the wastewater inventory needs to be better understood. This requires good quantification of wastewater generation and of the components in the various wastewaters. In a study by Cloete et al. (2010), information on SA wastewaters was obtained from numerous companies in the industrial, food and beverage, mining and electricity generation sectors; however, the majority of the companies contacted did not perform analyses for the full spectrum of substances in the effluent, limiting the completeness of the data gathered. Currently updated information is being collected through the WRC's NatSurv studies; however the outcome of these is not yet available. At time of writing there is still no system in place to regulate exactly what information on effluent production must be gathered by metropolitan councils, especially with regard to chemical composition. As a result, the data obtainable from metropolitan councils is inconsistent and clear comparisons are not possible (Cloete, et al., 2010; WRC SA, October 2015),

To meet the requirements and challenges highlighted for this study, the following components have been considered within the extended scope of the study:

- Review of national and global research relevant to wastewater biorefineries
- Review of potential example feedstocks for wastewater biorefineries with a particular focus on South Africa
- Proposal of some example products of a potential wastewater biorefinery
- Development of criteria for successful biorefineries with particular focus on criteria for appropriate bioreactor systems
- Assessment of bioreactor systems currently used within wastewater treatment plants, or with potential to be used, and their potential for use in the wastewater biorefinery
- Development of a flowsheet framework for wastewater biorefineries to facilitate assessment of their potential
- The building of some illustrative flowsheets using SA feedstocks to provide scenarios for investigation of wastewater biorefineries and their key features
- Description of the WWBR concept with its potential and limitations

1.3 Project Aims

In this study, we have set out to address the following aims:

1. To review current research nationally and globally, focussed on the valorisation of waste through the "waste to resource" concept and biorefinery concept.
2. Building on our earlier studies reported in Verster et al. (2013), to identify a set of appropriate reactor designs for use in the wastewater biorefinery for conversion of dilute organic streams in a non-sterile environment to a product of value, to refine these designs and to specify the factors guiding choice between these reactors.
3. To explore potential of a unit operation for the conversion of soluble organic carbon in a partially treated wastewater stream to a polymer product of value (such as PGA through building on the outcomes reported by Verster et al. (2013)), through appropriate reactor design and microbial ecology – proof of concept at the lab scale.
4. To review the data available on the potential component processes to be included in the wastewater biorefinery to meet its complementary needs of removal of organics, N, P, processing of sludge as well as water polishing, using the open literature and, particularly the WRC database, to inform integration of these operations into the flowsheet and to provide data for material and energy inventories.
5. To identify a potential set of component processes for the wastewater biorefinery, allowing the selection of two to three process flowsheets to be developed.
6. To define the required components incorporated into a wastewater biorefinery conceptual plan relevant to South Africa.

Through discussion with the Reference Group during the course of the project, the experimental reactor studies were de-emphasised. Further, we set about additional research beyond that originally scoped in the project to provide an initial description of examples of important wastewater streams in South

Africa. It must be noted here that the level of available information is currently constrained, limiting the detail of this study. This is expected to be partly remedied through the new NatSurv documents in preparation.

Project deliverables specified at the outset of the project are given in Table 1-1. Any deviations from project scope agreed in discussion with the reference group through the project are given here too.

Table 1-1: Project Deliverables

#	Deliverable	Deliverables specified in proposal	Alterations
1(D)	Overviewing and developing the wastewater biorefinery concept for application in South Africa	Review of global and national R&D trends related to biorefineries and "waste-to-resource" in the wastewater and sanitation space Review of wastewater valorisation research in South Africa, mining R&D reports of WRC in particular Develop examples of the biowaste biorefinery, drawing on both new and existing RSA examples of suitable unit operations with economic evaluations Definition of a wastewater biorefinery conceptual plan(s) relevant to RSA	
	Review of SA wastewaters to provide example feedstocks		Overview of a selection of example wastewaters with respect to composition, abundance was undertaken following discussion with the reference group.
2 (A)	Reactor selection as a key system component for value from waste	The review of reactors with application in wastewater biorefineries will be expanded based on criteria specified Following selection of two reactor designs, design, construction and commissioning will take place at the 5 - 20 litre scale	
3 (B)	Products from dilute organic streams.	Overview of example of polymer products for production from WW Report on performance of polymer process, describing conversion, product quality and recovery, microbial ecology Provide a literature review on microbial polymer production	Focus on the experimental study and proof of concept was de-emphasised to enable the “big picture” study to progress as focal point
4 (C)	Component processes for the integrated biorefinery	Initial biorefineries process flowsheets will be proposed Material and energy inventories on the hypothetical WWBR flowsheet for integration into biorefinery Provide integrated process flowsheet of two to three process configurations of the wastewater biorefinery	

2 WASTEWATER BIOREFINERY REVIEW: GLOBAL AND NATIONAL PERSPECTIVES

The wastewater biorefinery (WWBR) concept is gathering interest and is increasingly recognised for its potential contribution to the bioeconomy or bio-based economy as well as its impact on the move towards cleaner production, an industrial ecology and circular economy. In this section, the Bioeconomy Strategy of certain countries is introduced, the biorefinery concept explained and the WWBR introduced. Thereafter examples of global projects utilising wastewater are summarised. These reviews lead to questions which need to be addressed in order to determine the possible application of WWBRs, their potential and their design.

2.1 National Bioeconomy Strategies

2.1.1 Establishing global bioeconomy strategies

Many countries, including Ireland, Sweden, Norway, the Netherlands, Canada, USA, Germany and the European Union have developed bioeconomy strategies (Bioeconomy in Action, n.d.), anticipating increased focus on biotechnology-based value generation. The first of these was published by the Netherlands in 2004 with most of the remainder published between 2011 and 2014. These strategies, with the focus of each summarised, are enumerated in Table 2-1.

Table 2-1: Other countries that have published Bioeconomy Strategies with the main focus of each (www.bioeconomy.dk)
See also Section 2.1.2 The South African bioeconomy strategy

Country	Bioeconomy strategy	Focus of strategy
Canada [4]	In July 2011, a "Bio-economy Committee" was formed in British Columbia (BC), Canada.	The Bio-economy Committee formulated a set of recommendations for government to hasten productive economic development of BC's bio-economy sector. The main pillars of the recommendations are: • Establish a clear, long-term bio-economy vision. • Improve access to fibre and feedstock. • Establish a technology development strategy. • Develop markets for BC bioproducts and aggressively market BC's advantages. • Integrate the bio-economy's infrastructure needs into provincial initiatives.
Europe [8]	On February 23rd 2012, the European Commission released its strategy "Innovating for Sustainable Growth - A Bioeconomy for Europe".	
Finland [7]	The Finnish Bioeconomy Strategy was published in August 2014.	The strategic goals of the Finnish Bioeconomy Strategy are: • A competitive operating environment for the bioeconomy, • New business from the bioeconomy, • A strong bioeconomy competence base, • Accessibility and sustainability of biomasses.
Germany [6]	The German BioEconomy Council released its first recommendations in 2009 and second recommendations in 2011. It also has published a number of reports on bioeconomy potential and growth.	The research strategy lays out five priority fields of action: • Global food security, • Sustainable agricultural production, • Healthy and safe foods, • The industrial application of renewable resources, • The development of biomass-based energy carriers

Country	Bioeconomy strategy	Focus of strategy
Ireland [1]	Ireland published its Foresight Report in 2008 “Towards 2030 – Teagasc’s Role in Transforming Ireland’s Agri-Food Sector and the Wider Bioeconomy”.	The four pillars are: • Food production and processing • Value-added food processing • Agri-environmental products and services • Energy and bio-processing.
Netherlands [5]	The Netherlands “Bio-based Economy” strategy was launched in 2004, funded through the profits of North Sea oil.	The working paper of The Netherlands focuses on: • The integrated approach • The whole value chain of biomass • Opportunities for agriculture • A level playing field
Norway [3]	Norway has published a preliminary research programme from 2012-2022 on “Sustainable Innovation in Food and Bio-based Industries”, BIONÆR.	The following cross-cutting perspectives will apply to all research activities under the BIONÆR programme: • Sustainable production and consumption, emissions reductions and adaptation to climate change. • Improved resource efficiency in new and existing biomass production and full utilisation of all biological resources in closed-loop systems. Focus on reducing food loss and discard and on using residual raw materials as a resource • Further refinement of existing and development of new types of value-creating cross-utilisation between resource streams. • Further refinement of existing and development of new processes, products and services. • Enhanced value creation and competitiveness in the bio-based industries, with a focus on market orientation and innovation in all segments of the various value chains.
Sweden [2]	The “Swedish Research and Innovation Strategy for a Bio-based Economy” was published in March 2012 by the Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS).	The following research and development needs were defined: • The replacement of fossil-based raw materials with bio-based raw materials • Smarter products and smarter use of raw materials • Change in consumption habits and attitudes • Prioritisation and choice of measures
USA [9]	In the USA in April 2012, the Obama Administration released the bioeconomy strategy "US National Bioeconomy Blueprint".	The USA strategy has many similarities with the European, and has five major objectives: • Support R&D investments that will provide the foundation for the future U.S. bioeconomy. • Facilitate the transition of bioinventions from research lab to market, including an increased focus on translational and regulatory sciences. • Develop and reform regulations to reduce barriers, increase the speed and predictability of regulatory processes, and reduce costs while protecting human and environmental health. • Update training programs and align academic institution incentives with student training for national workforce needs. • Identify and support opportunities for the development of public-private partnerships and precompetitive collaborations—where competitors pool resources, knowledge, and expertise to learn from successes and failures.

[1] http://www.teagasc.ie/Foresight/. Date accessed 15 May 2014.
[2] http://www.formas.se/PageFiles/5074/Strategy_Biobased_Ekonomy_hela.pdf. Date accessed 15 May 2014.
[3] www.http://bioeconomy.dk/Norway_Bionaer_programme.pdf. Date accessed 15 May 2014.
[4] http://bioeconomy.dk/BritishColumbia_Bioeconomy_Report.pdf. Date accessed 15 May 2014.
[5] http://www.bmbf.de/pub/Natinal_Research_Strategy_BioEconomy_2030.pdf. Date accessed 15 May 2014.
[6] http://www.bmbf.de/pub/Natinal_Research_Strategy_BioEconomy_2030.pdf. Date accessed 15 May 2014.
[7] http://biotalous.fi/wp-content/uploads/2014/08/The_Finnish_Bioeconomy_Strategy_110620141.pdf. Date accessed 08 August 2016.
[8] http://ec.europa.eu/research/bioeconomy/pdf/201202_innovating_sustainable_growth_en.pdf. Date accessed 15 May 2014.
[9] http://www.whitehouse.gov/sites/default/files/microsites/ostp/national_bioeconomy_blueprint_april_2012.pdf. Date accessed 15 May 2014.

2.1.2 The South African bioeconomy strategy

South Africa has built on its National Biotechnology Strategy of 2001 (South Africa. Department of Science and Technology, 2001), launching a Bioeconomy Strategy in 2013. While this strategy was drafted by the Department of Science and Technology (DST), it has the buy-in of multiple ministries including Department of Higher Education and Training (DoHET), Department of Water Affairs Fishery and Forestry (DWAFF), Department of Trade and Industry (DTI) and others. In this strategy, cooperation between industry, science councils, government departments and academia is highlighted to ensure that bioprocesses, biotechnology and bioinnovations are market relevant for easy application in South Africa. The three foci of the strategy are Health, Agriculture, and Industrial Bioprocesses and the Environment (DST SA, 2013). All three areas have implications in water use and treatment, as bioprocesses and biotechnology are fundamentally water-based. Water, waste and the environment are specifically addressed in the implementation plan under development for the Industrial Bioprocess and the Environment component of the SA strategy.

2.2 The Biorefinery Concept

2.2.1 Defining the concept

A biorefinery is characterised as an integrative, multifunctional, over-arching concept that uses biomass as a diverse source of raw materials for the sustainable generation of a spectrum of intermediates and products (chemicals, materials, bioenergy and fuels) whilst including the fullest possible use of raw material components (i.e. maximising resource productivity) and ensuring the minimisation of waste products. Co-products can also be food or feed. These objectives necessitate the integration of a range of different methods and technologies. The biorefinery process chain includes the pre-treatment and preparation of biomass, the separation of biomass components (primary refining), subsequent conversion and processing steps (secondary conversion) as well as subsequent separations (De la Fuente, 2014).

Most commonly, biorefineries refer to the use and beneficiation of biomass and consider lignocellulose as a main starting material (Fernando, et al., 2006; Kamm, et al., 2006). Many initiatives for biomass valorisation focus on fermentation of the whole raw material to low-value energy carriers such as biogas or ethanol, also known as Low-Value-High-Volume (LVHV) products. It is, however, potentially more economically sustainable to produce High Value-Low-Volume (HVLV) products from this biomass and its associated side-streams and use residual fractions for conversion to biogas or other energy-carriers (Wolkers, et al., 2011).

2.2.2 Classification of biorefineries

Biorefineries can be classified in a number of ways, based on their system components (IEA Bioenergy, n.d.), viz platforms, products, feedstocks, and conversion processes as explained below:

- **Platforms**: These refer to intermediates which connect different biorefinery systems and their processes. The number of platforms is an indication of the system complexity. There are five platform biorefineries currently recognised (Nieddu & Vivien, 2013):
    - Sugar platform
    - Thermochemical/ Syngas platform (gasification of biomass feedstocks),
    - Biogas platform (produces cooking gas – CO_2 and methane),
    - Carbon-rich chains platform (produces biodiesel)
    - Plant products platform (the plant is operated as a biorefinery)
- **Products**: Two biorefinery product groups are recognised, namely
    - energy products like bioethanol, biodiesel and synthetic biofuels
    - material products like chemicals, materials, food and feed
- **Feedstocks**: These can be grouped as
    - energy crops from agriculture (e.g. starch crops, short rotation forestry)
    - biomass residues from agriculture, forestry, trade and industry (e.g. straw, bark, used cooking oils, waste streams from biomass processing)

   - wastewater, wastewater-associated sludges and solid waste as a feedstock is not presented in any of the work from which this analysis was drawn, but is recognised by the authors as a key opportunity
- **Conversion processes**: Currently there are four major groups of conversion processes involved in biorefinery systems, namely
   - biochemical (e.g. fermentation, enzymatic conversion)
   - thermo-chemical (e.g. gasification, pyrolysis)
   - chemical (e.g. acid hydrolysis, synthesis, esterification)
   - mechanical (e.g. fractionation, pressing, size reduction)

2.2.3 The three biorefinery generations

First generation biorefinery processes were dedicated to the production of biodiesel and ethanol, on the basis of "a single raw material, a single major product". These systems are limited since by-products are produced and therefore the management of these co-products must be considered. For example, biodiesel production generates a major by-product, glycerol, which must be used for the approach to succeed.

Second generation biorefinery processes are also based on the processing of a single raw material, but focus on creating a range a products and using all the biorefinery co-products, thereby extracting a whole range of products for energy, chemicals and other materials. The feedstocks may also be termed first, second or third generation: arable crops, non-food biomass (such as agricultural residue, woody biomass and lignocellulose), and algae or waste materials respectively. The existing second generation biorefineries utilize less than 20% of the biomass feedstock for ethanol production. Major side-streams are produced, such as pentose and lignin waste streams that are used for biogas and energy production. Converting the carbon from these waste streams into added-value products is expected to increase the otherwise low profitability and improve the environmental benefits of the biorefineries (Biorefine2g.eu, 2013).

Third generation biorefineries have been proposed and are in an emerging concept, set to reach maturity as an integrated process around 2020. Sharing the same multi-product approach as the previous generation, the third generation integrated biorefinery can incorporate a combination of any of the five platforms. Third generation biorefineries diverge in two ways from second generation biorefineries. Firstly, third generation biorefineries should be capable of using different types of raw materials and transformation technologies. Secondly, they should be capable, depending on price developments, of modifying the technical itineraries to reverse the hierarchies between key-products and sub-products. This approach of flexibility to select the most profitable combination of raw materials and processes relies on a vision of the ideal production tool that is fully adaptable to market fluctuations.

WWBRs complement conventional biorefineries by providing additional resources to enable this value-addition. The WWBR would, of necessity, be a third generation biorefinery because of the complex and variable nature of the raw material. Following the de la Fuente definition (De la Fuente, 2014), it would make the fullest possible use of all raw material components, producing clean or 'fit for purpose' water as one of the products.

2.3 The Wastewater Biorefinery

A WWBR, as an example of a third generation biorefinery, needs to both generate products of sufficient value to be economically viable, as well as produce products of variable value concomitantly with production of clean water as a product, typically through multiple unit operations. This concept views wastewater treatment as an integrated system rather than a unit process. It potentially provides a link between the users of water and those responsible for its management where resources are recovered in closed loop cycles, thus contributing towards the concept of a circular economy (IEA Bioenergy, n.d.).

In contrast to these 'traditional' bioprocesses, biological systems can also use complex or 'dirty' streams i.e. non-sterile streams of variable composition and flow rate with multiple and changing components.

Conventional biological systems have been developed to remediate wastewater streams with the principal aim of producing sufficiently clean water and with little regard for producing any other products. A notable exception to this is the biogas-producing anaerobic digestion (AD) which converts waste organic materials into methane for use as an energy source while decreasing the organic loading as part of water treatment.

A WWBR operates in the wastewater arena; however, it is designed to generate products of sufficient value from the waste nutrients for economic viability while simultaneously producing clean or 'fit for purpose' water as the non-negotiable product. This concept positions wastewater treatment as part of an integrated system rather than an 'end of process' unit. The WWBR consists a set of processes which can be assimilated into the operations of either the wastewater producers or the wastewater treatment.

The WWBR, or even "global and national R&D trends related to biorefineries and 'waste-to-resource' in the wastewater and sanitation space" (IEA, 2014) exists only as a nascent concept at this stage, put forward by a small number of research groupings. Implementation is yet to be realised. Most approaches recorded towards the WWBR are currently still in the 'first' and 'second' generation biorefinery process stages, where the products from wastewater and sanitation are directed towards a combination of biogas, compost or biofertiliser.

This project seeks to contribute to the definition of and development of the WWBR concept. Figure 2-1 is an illustration of a potential WWBR process flowsheet using municipal wastewater as its raw material. This conceptual development was initiated as part of the study conducted in WRC K5/2000 and reported by Verster et al. (2014). The unit processes typically found in a functional WWTW using biological nutrient removal are adapted to facilitate product recovery. Typically, multiple unit processes are present in the WWTW to enhance overall process performance and resilience. It is proposed that some of these unit processes are adapted for commercial production, depending on the characteristics of the incoming waste stream(s), surrounding market needs and similar factors.

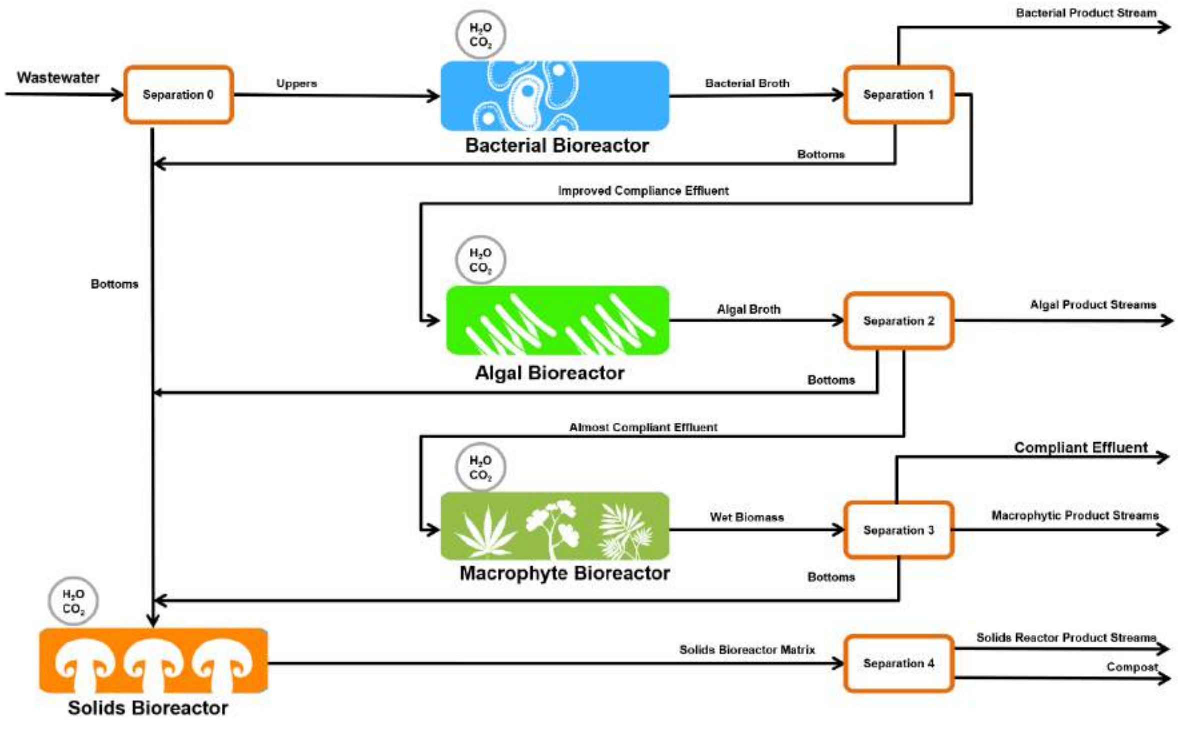


Figure 2-1: A simplified flowsheet of a potential wastewater biorefinery

2.4 Global Research around Wastewater Biorefineries

2.4.1 Conferences and initiatives relevant to WWBRs

International Conference on Renewable Resources and Biorefineries (RRB)

The annual RRB is used here as an indication of the progression of research in this field. This conference series started in September 2005 to allow delegates from university, industry, governmental and non-governmental organisations and venture capital providers to present their views on industrial biotechnology, sustainable (green) chemistry and agricultural policy related to the use of renewable raw materials for non-food applications and energy supply. The conference further aims to provide an overview of the scientific, technical, economic, environmental and social issues around renewable resources and biorefineries in order to give an impetus to the bio-based economy and to present new developments in this area. The conference provides a forum for leading political, corporate, academic and financial people to discuss recent developments and set up collaborations (www.rrbconference.com).

The RRB conference focus has been on:

- starch biorefineries
- the integration between agriculture and chemistry for biorefineries
- and the production of biopolymers as well as cellulosic biorefineries

At the fourth RRB in 2008 the production of products of value from wastewaters was first mentioned:

RRB-4 2008; a single presentation on value from wastewaters:

- Alan Werker from AnoxKaldnes Biopolymer (Sweden) presented “Production of biopolymers as a by-product of industrial wastewater treatment”

RRB-7 2011; two presentations on value from wastewaters:

- Bernelle Verster from the University of Cape Town (South Africa) “Producing poly-glutamic acid from wastewater, using *Bacillus* - considerations when moving from bioprocess to environmental engineering”
- Jean-Philippe Steyer from INRA (France) “Anaerobic digestion for waste/wastewater treatment and bioenergy-production: Shouldn't we get inspired by Mother Nature?”

RRB-8 2012: five presentations relating to the biorefinery concept, but no specific mention of wastewater biorefineries:

- PhD short communication: Sofia Tsakona from the Agricultural University of Athens (Greece) "Production of generic fermentation feedstock from flour based industrial waste streams"
- John A. Posada from Utrecht University (Netherlands) "Integrating the concept of biorefinery on a biodiesel production plant: fuel, chemical and energy production"
- Geneviève Doreau from Maguin (France) "The concept of a biorefinery: Looking to the future"
- Valérie Vandermeulen from Ghent University (Belgium) "Industry’s expectations regarding the transition towards a biobased economy"
- Han Vervaeren from Howest (Belgium) "Wastewater treatment with microalgal bacterial (MaB) flocs: From lab to pilot scale"

RRB-9 2013; five presentations on using wastewater and 3rd generation biorefineries:

- Kees Roest from Watercycle Research Institute (Netherlands) “Water resources”
- Carl Dewaele from NuReSys (Belgium) “P recovery from municipal WWT plants”
- Michel Eppink from the University of Wageningen (Netherlands), “Biorefinery of microalgae: Production of high value products, bulk chemicals and biofuels”
- Erika Cristina Francisco from the University of Campinas (Brazil),”Production of high cell density of *Cyanobacterium phorimidum* sp. using cassava wastewater”

- Antonis Kokossis from the National Technical University of Athens (Greece) “Integrated designs of micro-algae biorefineries using a fixed selection of halophytic algae”

RRB-10 2014, relevant presentations increased to seven:

- Germán Buitrón from the Universidad Nacional Autónoma de México (Mexico), “Biohydrogen production using tequila vinasse wastewater”
- Bernelle Verster from the University of Cape Town (South Africa), “Wastewater biorefineries: Recovering value while producing cleaner water”
- Boudewijn Meesschaert from Leuven University (Belgium), “Calcium phosphate precipitation in anaerobic effluent of potato processing industry is promoted by preceding nitrification”
- Cedric Tarayre from Gembloux University (Belgium), “Biorefine: Recovery of nutrients and metallic trace elements from different wastes by chemical and biochemical processes”
- Wilhelmus J. Mulder from Wageningen University (Netherlands), “Biorefinery and the potential of proteins from side streams”
- Alexandre Besson from LISBP (France), “Microalgae harvesting for biorefineries valorisations — Scale up of an autoflocculation-flotation process”
- Paulien Harmsen from Wageningen University (Netherlands), “Biorefinery of seaweed (or macro algae): Which way to go?”

RRB-11 2015 saw an even greater emphasis on waste biorefineries, with increased focus on the peripheral requirements like integration and downstream processing, with twelve relevant oral presentations:

- Paulo Coutinho, Braskem, Brazil, “Brazilian Biorefinery: An evolving model” presented in the opening plenary session
- Paulo Coutinho, Braskem, Brazil, and Stefanie Kluge, AVT-RWTH Aachen University, Germany, “Enzymatic production of acetylated cello-oligomers from water-soluble cellulose acetate”
- Oscar Avello, Centro de Estudios en Alimentos Procesados, Chile, “Microalgae cultivation using liquid waste streams from fruit and vegetable processing plants as growth medium for biomass and biofuel production”
- Vania Zuin, Federal University of Sao Carlos, Brazil , “Downstream processing and biorefinery separation challenges: new perspectives on chromatographic methods”
- Anthony Lloyd, Novasep Process, UK, “Separation and purification, the missing link between biomass deconstruction and commercial product”
- Diogo Queiros, University of Aveiro, Portugal, “Short-chain fatty acids production through acidogenic fermentation of hardwood sulphite spent liquor”
- Davide Mainero, ACEA, Italy, “The ACEA Pinerolese experience in the management of municipal biowastes and research on their valorization as source of biofuel and added value products”
- Rommie van der Weide, WageningenUR, Netherlands, “Recycling nutrients and valorise side streams in local biorefineries”
- Mette Lubeck, Aalborg University Copenhagen, Germany, “Mixed cultures of fungi for conversion of lignocellulose into bioproducts”
- Md Ariful Haque, City University of Hong Kong, “Valorization of food waste for bio-colorant (Monascus dye) production”
- Federica Zaccheria, ISTM CNR, Italy, “Beyond dedicated crops: The waste biorefinery”
- Merten Moralsed, ETH Zurich, Switzerland, “Integrated biorefinery concepts for polypropylene production from palm oil and wood residues”

RRB 2015 also featured 11 relevant posters:

- Y. Hu, et al. (Hong Kong & China), “Conversion of food waste into polylactic acid fibre”
- Alexandra Lanot, J. Sloan, Y. Li, S. McQueen-Mason (UK), “MultiHemp: A knowledge-driven effort to develop a hemp-based biorefinery”
- Margarita María Andrade-Mahecha et al. (Columbia), “Use of sugar cane bagasse for the improvement of the mechanical properties of paper”

- Gianluca Ottolina, S. Gandolfi, L. Pistone, P. Xu, S. Riva (Italy & China), "Hemp hurds biorefinery - Production of L-(+)-lactic acid"
- Xu Zhang, T. Tan (China), "From waste bioresources to bioenergy and chemicals"
- Sofia Raikova, C. Chuck, V. Ting (UK), "Valorisation of microalgae used in remediation of mine waste"
- S. Grivot, K. Tomono, Thierry Talou (France & UK), "VFWV wheel: An interactive model illustrating EUB is network valorisations of Vegetables & Fruits Wastes"
- Sophie Roelants, et al. (Belgium) "Microbial biosurfactants: Closing the gap in the innovation chain"
- Ferrer, (Spain), "Bioenergy and bioproducts from microalgae grown in wastewater"
- V. Liakou, (Greece), "2,3-butanediol production from fruit and vegetable waste streams"
- Nefeli-Maria Georgaka, M. (Greece), "Development of an advanced biorefinery concept based on valorization of winery wastes"

Reneseng

This is a Renewable Systems Engineering initiative launched towards the end of 2013 with funding from a European Consortium to the value of €4.2 million. The aim of the project is to contribute to research and training of engineers with project experience in biorefineries and emphasis on advanced process design, synthesis, model-based screening and analysis and process integration. To this end, two workshops have been held, in April 2014 and March 2015. The first workshop was an introduction to biorenewables and biorefineries, with attention given to the logistics of the project itself. The focus of the second was the use of Process Systems Engineering tools in the biorefinery space. A course entitled "Renewable Systems Engineering" was held at TU Delft in November 2014. The Reneseng Project has eleven full partners, seven academic and four private. The project is scheduled to conclude after 4 years, towards end of 2017. (Reneseng, n.d.)

International Water Association (IWA) Resource Recovery Conference (IWARR)

In late 2015, the first International Water Association (IWA) Resource Recovery Conference (IWARR2015) was held in Ghent, Belgium (IWARR2015, 2015). This promising development was the first conference dedicated to the interface between water treatment and resource recovery. Two presentations focused on data gathering, which remains one of the main challenges when moving towards resource recovery:

- M Papa from the University of Trento (Italy) "How far are we from closing the loop of resource recovery? A real picture of municipal wastewater treatment plants"
- JP Van der Hoek from Waternet/TU Delft (Netherlands) "Wastewater as a resource: strategies to recover resources from Amsterdam's wastewater"

The main resource recovery routes remain focused on a limited number of products, namely struvite, biogas and PHA, illustrated in Mark van Loosdrecht's (TU Delft, Netherlands) presentation "Wastewater: What are the potentials for resource recovery". Other potential product streams include cellulose and alginate. Veolia is an industry leader with regards to PHA production, and was strongly represented:

- Alan Werker from Veolia (France) "Bridging Upstream and Downstream Stakeholder Needs for Regional Biopolymer Value Chains Built on Residuals Management Services"
- M Hjort from Veolia Water Technoligies - AnoxKaldnes (Sweden) "PHA as municipal wastewater treatment by-products: a polymer production demonstration project - PHARIO"

While the conference aimed to contribute to the circular economy concept, the field still struggles to define what this means. Kees Biesheuvel from SmartDeltaCluster (Netherlands) illustrated a successful case study "Industrial symbiosis, a human challenge".

A compendium report on resource recovery from water was released at the same time, by the IWA Resource Recovery Cluster (http://www.iwa-network.org/cluster/resource-recovery-from-water-cluster [accessed 10 February 2016]). This document gives an overview of the state of the industry and aimed at "creating awareness of the issues involved, and are particularly meant to activate readers from different backgrounds towards the conceptual but also pragmatic approaches of “cleantech” in the water business” (Holmgren, et al., 2015, p. 2). The report incorporates data gathered from a survey targeting water professionals in academia and industry. Unfortunately participation from stakeholders in Africa was low (Holmgren, et al., 2015).

2.4.2 European facilities creating value from wastewater

In European countries, especially Netherlands and Denmark, several pilot or industrial scale facilities have been developed in recent years and are operating and creating value from wastewater. These examples of global progress are reviewed in Table 2-2. Researchers in the Netherlands and Denmark are international leaders in the field.

Table 2-2: Companies in Europe producing value from wastewater

Country	Company	Industries	Wastewater	Product	Scale (demo, pilot, industrial,)	Volume if applicable
Netherlands [1]	Plant built by Paques BV		Chocolate wastewater from Mars factory	Bioplastic poly-hyroxyalkanoates (PHAs)	Pilot plant (Nov 2012 - end 2013)	-
Denmark [2]	Kalundborg (example of integrated biorefinery)	Symbiosis between 5 companies: the Asnæs power station, plasterboard makers Gyproc, pharmaceutical and biotechnology firms Novozymes & Novo Nordisk, soil cleansing company Soilrem and the Statoil refinery	-Wastewater from Novo Nordisk and Novoenzymes -Sludge from municipality's water treatment plant	-Biofuel (see next entry) -Fertilisers distributed to local farmers -Final product used a an additional soil nutrient	Industrial	150 000 tonnes of fertilisers were produced in 2010
Denmark [3]	Novozymes and Novo Nordisk	Novozyme & Novo Nordisk in Kalundborg facility	Wastewater from the factories in Kalundborg	Biofuel (Biogas)	Industrial	Biogas reactor produces up to 47 000 MWh of electricity p.a
Denmark [4]	Krüger A/S, Billund Vand A/S, Billund Municipality, Danish Ministry of Environment and VTU Fonden (Water Sector Technology Development Foundation)	Billund BioRefinery (BBR)	Domestic, industrial and agricultural wastewater	Biogas, organic fertiliser, bioplastic	Demo Plant	
Netherlands [5]	Nijhuis Water Technology	Outsourcing wastewater technology available to companies	Wastewater from industries	Biogas, fertiliser	Industrial	

[1] DELTA, 2013. *Living on water from Mars* [Online], Available at: http://delta.tudelft.nl/artikel/living-on-water-from-mars/26740, [Accessed 8 October 2014].
[2] Global Lamp Index, 2011. *The Kalundborg Symbiosis A model of progressive resource exchanges* [Online], Available at: http://www.lampindex.com/2011/10/the-kalundborg-symbiosis/, [Accessed 8 October 2014].
[3] Novozymes, 2013. *Novozymes utilizes wastewater to produce biogas* [Online], Available at: http://novozymes.com/en/news/news-archive/Pages/novozymes-utilizes-wastewater-to-produce-biogas.aspx, [Accessed 8 October 2014].
[4] Billund BioRefinery, 2014. *Billund BioRefinery.* [Online], Available at: http://www.billundbiorefinery.dk/en/, [Accessed 2014 October 2014].
[5] Nijhuis Water Technology, 2014. *Nijhuis Water Technology.* [Online], Available at: http://www.nijhuis-water.com/default.aspx?taal=true, [Accessed 8 October 2014].

2.4.3 European researchers in WWBRs

European researchers are international leaders in wastewater biorefineries. Some of their progress is summarised below.

Marc van Loosdrecht's group at TU Delft works on biofilms and granular sludge systems, microbial storage polymers, nutrient removal processes and the microbial ecology of engineered systems. The development of the Aerobic Granular Sludge system, commercialised as the Nereda, is instrumental in widening possibilities for wastewater biorefineries. One publicised example of this is the pilot plant operating in the Netherlands, producing polyhydroxyalkanoates (PHA) from chocolate wastewater (http://delta.tudelft.nl/artikel/living-on-water-from-mars/26740).

Jean Phillipe Steyer of the National Institute for Agricultural Research (INRA), France, works on anaerobic digestion of algae (http://www.inra.fr/en/Partners-and-Agribusiness/Results-Innovations-Transfer/All-the-news/The-Algotron).

Frank Rogella, Aqualia's Innovation and Technology director in Madrid, works on high rate algal treatment systems with biofuels as co-products. He leads a consortium, All-Gas, which was awarded one of the large projects of the European Union (EU) to demonstrate ´Algae to Biofuel´ implementation on a scale of 10 ha. The consortium maintains that the costs of its plant are well below those for a conventional system. (http://www.reuters.com/article/2013/06/26/us-spain-bioenergy-idUSBRE95P0JG20130626; http://www.futureenergyevents.com/algae/2011/04/19/speaker-profile-frank-rogalla-director-of-innovation-and-technology-aqualia-gestion-integral-del-agua-s-a/)

Jose Porro is a Senior Researcher at Universitat de Girona, in Madrid, Spain and serves as a consultant for Arcadis in New York City. He is interested in sustainable and integrated urban water design, and currently doing his PhD at Ghent, where he is developing qualitative models for risk assessment of biological operational problems in urban water systems (http://www.sanitas-itn.eu/project/fellows/#ER1; http://modeleau.fsg.ulaval.ca/no_cache/en/people/phd_students/phd_students_details/professeur/140/2166/; http://www.novedar.com/ecoSTP/programme-plenaries.asp).

In the field of wetlands, Polprasert, Kadlec and Rittman are global leaders. *Dr. Bruce Rittmann* is Director of the Swette Center for Environmental Biotechnology at Arizona State University (ASU). He approaches environmental biotechnology from the perspective of "managing microbial communities that provide services to society." "The concept of wastes or waste products is obsolete - the focus and the future are used resource recovery." This is achieved through cross-disciplinary and team-based research in the areas of engineering, science, sustainability, and biological design. Research topics include fundamental studies and practical applications such as microbial electrochemical cells (MXCs), microbial photobioenergy, and bioremediation. In order to make "research meet practice," Dr. Rittmann's research teams integrate microbial ecology, chemistry and process kinetics through mathematical modelling, and they regularly partner with practitioners (http://rittmann.environmentalbiotechnology.org/).

Chongrak Polprasert is based at the School of Bio-Chemical Engineering and Technology (BCET), Sirindhorn International Institute of Technology (SIIT), Thailand. His research foci include water pollution control, waste recycling and recovery, and hazardous wastes engineering and management. He authored "Organic waste recycling: technology and management" (Polprasert, 2007). Amongst others, he co-authored two articles that could contribute to wastewater biorefineries: "Phosphorus Recovery from Human Urine and Anaerobically Treated Wastewater Through pH Adjustment and Chemical Precipitation" (Kemacheevakul, et al., 2011), and "Treating Swine Wastewater by Integrating Earthworms into Constructed Wetlands" (Nuengjamnong, et al., 2011) (http://www.siitthailand.com/web/professor_en.php?id=32).

2.5 The South African Water Research Commission and WWBRs

The Water Research Commission (WRC) of South African is custodian of a large body of research into water and wastewater. This was analysed in order to position the potential for the valorisation of wastewater through the use of the WWBR concept. Reports commissioned by the WRC were reviewed. In the following two sections, research from academic institutions (Section 2.6) and trade literature (Section 2.7) is similarly reviewed, bringing together already existing knowledge and skills in South Africa while identifying knowledge and skills gaps.

A list of all reports from 1984 to 2015 was obtained from the WRC, totalling 2274 documents. Of these, 252 reports were deemed relevant to WWBR and grouped into 6 main categories (Table 2-3; Figure

2-2). These were further divided into more specific subcategories where appropriate. These are discussed below and the most promising reports highlighted in each category.

The reports are listed in Appendix A sorted into the six categories and further by year of publication.

Table 2-3: Number of WRC reports relevant to WWBR in each of six categories

Category	Number of reports in category	Percent
A. Wastewater management	112	43.1%
B. Wastewater treatment technology	89	34.2%
C. Cleaner Production	14	5.4%
D. Products from wastewater	18	6.9%
E. Products to be used in wastewater	16	6.2%
F. Wastewater Biorefineries	11	4.2%
Total	**260**	

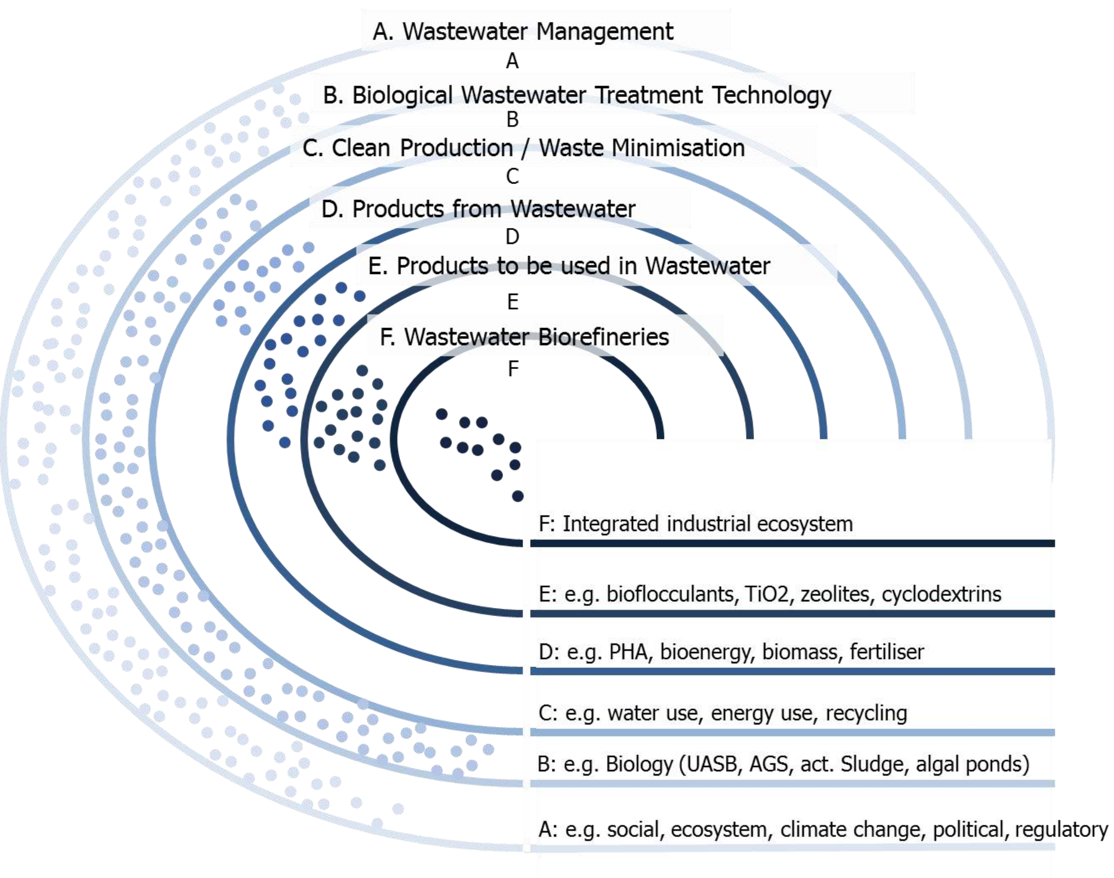


Figure 2-2: Graphical illustration of the context of wastewater biorefineries related research listed in Appendix A

2.5.1 Category A: wastewater management

Almost half of the relevant reports dealt with the management of wastewater. This reflects thinking from about 2005 onwards and suggests that this is may be the most challenging part of WWBR. The demand-side of water resource management focuses attention on how to manage water demand and use. This shift is influenced to an extent by various social advocacy movements, but is also influenced by increasing recognition of resource scarcity, heightened interest in sustainable development considerations, post-modern philosophies and increased prominence of environmental justice, equity and democratisation of resources (Siebrits, et al., 2014).

Table 2-4: Number of WRC reports relevant to WWBR with regards to wastewater management (Category A), in each of six sub-categories

Category A: Wastewater Management	Number of reports in sub-category	Percent of Category A
1. General	40	35.7%
2. Analysis and Characterisation	12	10.7%
3. Health	9	8.0%
4. Meta research	22	19.7%
5. Economics	18	16.1%
6. Solids: Landfill and Rural	11	9.8%
Total	**112**	

The classification of the reports on wastewater management is given in Table 2-4. Some highlights of the general reports in Category A are listed in Table 2-5 to illustrate the type of projects undertaken.

Table 2-5: Most relevant WRC reports from Category A: wastewater management

Title	Year of pub	Value of research	Gaps / Further research required for application to WWBR
2085/1/14: Mitchell SA ,de Wit MP, Blignaut JN, Crookes D, Waste water treatment plants: the financing mechanisms associated with achieving green drop rating	2014	Financing mechanisms of wastewater treatment plants	Improve the performance of WWTWs through providing an incentive to the works in the form of a scoring system. Limited applicability to WWBR, except as an operational incentive mechanism.
TT 588/13: Armitage N, Fisher-Jeffes L, Carden K, Winter K, Naidoo V, Spiegel A, Mauck B, Coulson D, Water Sensitive Urban Design (WSUD) for South Africa: Framework and guidelines	2014	Biological and chemical treatment of associated contaminants, drainage and the management of industrial effluents. Water-Energy-Food Nexus. Wastewater re-use and minimisation.	Big picture of WWBR and beyond.
TT 564/13: Wall K, Ive O, Social Franchising Partnerships for Operation and Maintenance of Water Services: Lessons and Experiences from an Eastern Cape Pilot	2013	An investigation of the business model that could occur in the sanitation sector	The project is aimed at a more social responsiveness and community level. It would be interesting to see if this can be extended to a bioproduction facility context.
TT 518/12: Schulze RE, A 2011 perspective on climate change and the South African Water Sector	2011	The effect of climate change on hydrological responses. Predictive scenarios, indicating risk levels, for the biophysical changes associated with projected climatic change for climatically divergent catchments in South Africa were then developed.	Could be placed into context of WWBR and how WWBR contributes to mitigating climate change and builds resilience.

Analysis and characterisation

Reports dealing with analysis and characterisation of wastewaters are important for this project, but most of these were published more than five years ago. Of particular interest are techniques that can be used to analyse the more complex requirements with regards to composition of a WWBR stream. The most notable of the existing reports are listed in Table 2-6.

Table 2-6: Most relevant WRC reports from Category A.2: analysis techniques that may be of use in WWBR

Title	Year of pub	Value of research	Gaps / Further research required for application to WWBR
KV 249/10: Garcin CJ, Nicolls F, Randall B, Fraser M, Griffiths M, Harrison STL, Development of LED-photodiode-flow cell for online measurement of dissolved substances in liquids	2010		WWBR process control and analysis
TT 405/09: Leopold P, Freese SD, A Simple guide to the chemistry, selection and use of chemicals for water and wastewater treatment.	2009		
1286/1/07: Pillay B, Dechlan, Development and application of prokaryotic biosensor systems for the evaluation of toxicity of environmental water samples.	2007		Potential way to evaluate incoming wastewater to prevent system failure

Meta-research

Meta-research reports are of primary importance in this study because of the need to position this research in the current South Africa wastewater management setting. Further, meta-research is a key resource at the start of any focused project for accessing introductory information quickly, and for locating the initial material for more specific investigation. Some of the more helpful overview reports are listed in Table 2-7.

Table 2-7: Most relevant WRC reports from Category A.4: meta-research reports

Title	Year of pub	Value of research	Gaps / Further research required for application to WWBR
2199/1/12: Pouris, Anastassios, A Pulse Study on the State of Water Research and Development in South Africa	2012	A quantitative account of key R&D trends in the water sector. The analysis identifies that the field is performing above expectation in comparison with the country's research size.	Overview of the research landscape in South Africa with regards water. However, little attention has been paid to the increasingly important water reuse/recovery/beneficiation concepts.
TT 514/11: Claassen M, Funke N, Nienaber S, The Water Sector Institutional Landscape by 2025	2011	Project to build knowledge about key drivers and uncertainties related to SA water sector institutions, with a focus on water resource management.	WWBRs can feed into water resource management as a water source, thus this report outlines some key stake holders in the space.
1547/1/10: Cloete TE; Gerber A, Maritz L, Inventory of water use and waste production by industry, mining and electricity generation	2010	The overall objective of this project was to compile a first order inventory of the amount of water used and effluent produced by the South African industrial, mining and power generation sectors, and to assess the impact these might have on water quality, but existing data sets were of limited value and outdated.	Much of the data is out of date, and some data is inconsistent. It is of great concern that many of the surveyed industries do not conduct any chemical analyses on the effluents that they produce and that where chemical analyses are done. They very seldom go beyond a few basic parameters like COD, phosphate and nitrate.

Solid waste management

Reports dealing with solids were of particular interest, as the first objective of product recovery is decoupling solid and liquid residence time. As this report is concerned with the more technical aspects of WWBR, the distinction of solids were used rather than rural (vs urban), or sewered vs non-sewered. The most relevant reports are given in Table 2-8.

Table 2-8: Most relevant WRC reports from Category A.6: solid waste management as it relates to wastewater

Title	Year of pub	Value of research	Gaps / Further research required for application to WWBR
1240/1/04: Marx CJ, Alexander WV, Johannes WG, Steinbach-Kane S, A technical and financial review of sludge treatment technologies	2004	The aim was to give a clear indication to metropolitan councils, municipalities and other sludge producers of the technologies available and applicable under local conditions, as well as an indication of the cost and economy of scale applicable to each process. The study includes an overview of current sludge management practices in South Africa, as well as an estimate of sludge quantities and qualities and a brief description of commonly used sludge treatment and disposal methods.	Applicable to sludges used / produced in WWBR, including the legal framework, using as a basis the Sludge Utilisation or Disposal Decision Flow Diagram (SUDDFD), as presented in the Addendum No 1 to the Permissible Utilisation and Disposal of Sewage Sludge (Edition 1), (Department of Agriculture et al 1997).
TT 107/99: Ceronio AD, Van Vuuren LRJ, Warner APC: Guidelines for the design and operation of sewage sludge drying beds	1999	Sludge drying / 'preprocessing'	Needs further work to consider drying beds as solid substrate bioreactors

2.5.2 Category B: wastewater treatment technology

The 81 WRC reports relevant to WWBR with regards to wastewater treatment technology, Category B, were further grouped into nine sub-categories taking account of specific unit technologies (Table 2-9).

Table 2-9: Number of WRC reports relevant to WWBR with regards to wastewater treatment technology (Category B), in each of nine sub-categories

Category B: Wastewater treatment technology	Number of reports in sub-category	Percent of Category B
1. General	42	47.2%
2. Anaerobic digestion (AD)	7	7.9%
3. Biological Nutrient Removal (BNR)	5	5.6%
4. Algae	1	1.1%
5. Wetland	4	4.5%
6. Membrane	18	20.2%
7. Solid Substrate Fermentation (SSF)	7	7.9%
8. Nanotechnology	3	3.4%
9. Upflow Anaerobic Sludge Blanket (UASB)	2	2.2%
Total	**89**	

In this category, reports detailing reactor technology that can deal with solid substrate were of particular interest, and are listed in Table 2-10. It is pertinent to develop WWBR technologies and feasible business models that focus on solid wastes because of the large number of non-sewered disposal routes, particularly in developing countries. Further, the first step in a WWBR is to decouple the solid and liquid residence times, which necessitates specific consideration of the solids component.

Table 2-10: Most relevant WRC reports from Category B.7: solid waste reactor technology as it relates to wastewater

Title	Year of pub	Value of research	Gaps / Further research required for application to WWBR
766/1/05: De Jesus AE, Heinze PH, Muller JR, Nortje GL, Utilisation of earthworms and associated systems for the treatment of effluent from red meat abattoirs.	2005	Utilisation of earthworms and associated systems for the treatment of effluent from red meat abattoirs.	Use of vermicompost or vermiculture for pre-treatment of biosolids before the Biosolids reactor
1129/1/04: Burton SG, Ryan DR, van Wyk L, Bioreactor systems using the white rot fungus Trametes for bioremediation of industrial wastewater	2004	Immobilised biofilm reactors in the form of a Transverse Flow Membrane Bioreactor and a Trickle Bed Reactor were identified as suitable for growth, enzyme production and phenolic removal by T. versicolor.	Investigate the value of this work in context of WWBR - does it optimise well as a unit process in the treatment train? Take the system to larger scale and demonstrate its effectiveness at pilot scale.
333/1/97: Whyte DC, Swartz CD, The removal of suspended solids from pulp and paper effluents by employing the combined sedimentation flotation process	1997	The most significant conclusions of this study are that high percentages of removal for suspended solids can be obtained with the combined SEDIDAF process; the settling stage of the process contributes most to the overall removal of solids from the effluent.	Investigate the application potential for WWBR. Has this been applied to industry since publication of this report?

2.5.3 Category C: cleaner production

Cleaner production is the precursor to wastewater biorefineries and, as such, is important to consider as a category. The key reports in this area are listed in Table 2-11. Important work has been done in this field, notably by Prof Chris Buckley at the Pollution Research Group at UKZN.

Table 2-11: Most relevant WRC reports in Category C: cleaner production

Title	Year of pub	Value of research	Gaps / Further research required for application to WWBR
TT 546/12: Mvuma GG, Hooijman F, Brent AC, Oelofse SHH, Rogers DEC, Volume III: Development and assessment of technological interventions for cleaner production at the scale of the complex	2012	Key factors that influence the environmental sustainability of a large inland industrial complex: The Secunda Industrial Complex.	Assessment of cleaner production options and environmental assessment by LCA
1625/1/08: Majozi T, Gouws JF, Development of a complete process integration framework for wastewater minimisation in multipurpose batch plants.	2008		Important thinking for WWBR
TT 283 & 4/07: Barclay S, Buckley C, Waste minimisation clubs in South Africa (Facilitation and Training Manual)	2007	Cleaner production initiative	

2.5.4 Category D: products from wastewater

Products that can be produced from wastewater depend on the ecological advantage that the product gives to the organisms producing them. It is also crucial to consider the market needs, ensuring that the market can absorb products from the wastewater. In order to maintain economic feasibility, the productivity needs to be high enough to cover operational costs. Category D contains WRC reports that address these aspects, and some highlighted reports are listed in Table 2-12.

Table 2-12: Most relevant WRC reports in Category D: products from wastewater

Title	Year of pub	Value of research	Gaps / Further research required for application to WWBR
1724/1/12: Tesfamariam EH, Annandale JG, de Jager PC, Mbakwe I, van der Merwe P, Nobela L, van der Laan M, Sustainable Agricultural Use of Municipal Wastewater Sludge, 1724/2/12: The potential of sludge amended combustion coal ash residues	2012	An investigation of use of sludge (both municipal waste derived, and petro-chemical waste derived) for agriculture.	Recovery and re-use of N and P from sludge for agriculture. More work needed on the requirements of processing that would allow the products to be considered, or acceptable for use as originating from sludge (more than just soil additive).
1937/1/11: Burton SG, Mupure CH, Horne KA, Jones S and Welz PJ, Beneficiation of Agri-Industry Effluents	2011	Downstream processing of agri-wastes, for recovery of valuable products (phenols, antioxidants and sugars).	A closer evaluation of the (economic) feasibility and market potential of concepts highlighted in this study.
1367/1/05: Christopher L, Bio-remediation and Bio-utilization of pulping and bleaching waste waters.	2005	This technical paper demonstrates the reduction of toxic chemical use when using alternative bleaches, such as enzymatic approaches. Furthermore, valuable products (such as the above mentioned enzymes) can be produced from the pulp wastewaters.	The application of the wastewater technology (cleaning pulp wastewater to produce enzymes) is applicable to WWBRs, more than the first part of the report.

2.5.5 Category E: products to be used in wastewater treatment

Generating products that can be used in the treatment processes used to produce them are promising avenues to illustrate the wastewater biorefinery concept, without needing to address broader logistical issues right away. This approach secures a market for the products.

The WRC reports in this category mostly evaluated specific products. It would be useful to research whether those of the effective products can be produced from the wastewater they are used to treat. Some highlights are listed in Table 2-13.

Table 2-13: Most relevant WRC reports in Category E: products to be used in wastewater treatment

Title	Year of pub	Value of research	Gaps / Further research required for application to WWBR
KV 248/10: Lutchamma-Dudoo C, Biologically Enhanced Primary Settlement:	2010	Investigation into using biological agents as settling agents to replace the more commonly used ferric chloride, to allow rural communities to become more self-reliant with regards waste water treatment.	The technology could be applied in WWBRs, although its capabilities and limitations need to be further explored.
1363/1/08: Binda M, Gounder P, Buckley CA, Promotion of biodegradable chemicals in the textile industry	2008	Development of a score system for textile industry effluent. A pilot study of implementing the score system at volunteer factories.	This methodology could be applied to WWBRs usefully, for influent and effluent analysis. However, more work is required to apply the methodology to other industrial effluents.
1377/1/05: Taljaard L, Venter A, Gorton D, An evaluation of different commercial microbial or microbially-derived products for the treatment of organic waste in pit latrines.	2005	Investigated the claims of 16 microbially-derived products for the treatment of organic waste in pit latrines to speed up the process, control odour and reduce the bulk of the organic material.	Results did not seem particularly promising. Designing pit latrines to promote their own healthy ecosystem may be better (ecological engineering).

2.5.6 Category F: reports most closely associated with the WWBR concept

It was encouraging to see some work towards integrating waste streams toward product recovery, paving the way to WWBR. These span a wide spectrum of thinking, from single unit product recovery to incorporating the societal impacts into the proposed beneficiation process. Highlights of the relevant reports are listed in Table 2-14.

Table 2-14: WRC reports most closely associated with WWBR

Title	Year of pub	Value of research	Gaps / Further research required for application to WWBR
1803/1/13: Blignaut J, de Wit M, Milton S, Esler K, le Maitre D, Mitchell S, Crookes D, A Market for Ecosystem Goods and Services Following the Restoration of Natural Capital: Volume 1: Main Report (and 1803/2/13)	2013	Integrated system dynamics model on the likely impact of restoration on the ecology, hydrology and economy of restoration sites	Ecosystem economics possibly applicable to WWBR, although products are not the focus of the model.
TT 399/09: Burton SG, Cohen B, Harrison S, Pather-Elias S, Stafford W, van Hille R, von Blottnitz H, Energy From Wastewater – A Feasibility Study (Essence Report)	2009	An overview of the chemical potential of wastewater, making wastewater biorefineries possible in principle	Only considers energy products. Similar work is required for commodity chemicals, and nitrogen and phosphate containing products
TT 235/04: Rouhani QA, Britz PJ, Contribution of aquaculture to rural livelihoods in South Africa: A baseline	2004	Contribution of aquaculture to rural livelihoods in South Africa: A baseline study	Should aquaculture be considered in WWBR?
1081/1/04: Klusener CW, The development of a protein recovery technology at Sezela for the treatment of furfural plant azeotrope effluent with the simultaneous production of mycoprotein.	2004	Production of bioproduct furfural	Treating industrial effluent and generating income from it. Good example of WWBR
1082/1/03: Christof LP, Further development of a biotechnological approach to the management of waste waters from the pulp and paper industry.	2003	Managing wastewater from pulp and paper industry for subsequent production of bioproducts	Developing more environmental friendly processes to treat wastewater and the use of microorganisms to produce valuable products such as single cell protein and high value fatty acids
939/1/03: Burton SG, Boshoff A, Foster I, Koteshwar K, Luke A, Mhlanga C, Nganwa P, Notshe T, Ryan D, Bioreactor systems for the conversion of organic compounds in industrial effluents to useful products.	2003	Value recovery from industrial effluent	A techno-economic analysis needs to be done
TT 187/02: Rose PD, Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa (Report 1) (and TT 188/02, TT 190/02, TT 191/02, TT 192/02, TT 196/02, TT 409/09)	2002	The BioSure process considers the treatment of acid mine drainage using sewage sludge as electron donor. Potential exist to recover a sulphur product (and metals)	A short explicit value offering would be a better 'marketing tool'
1054/1/01: Abbott G, Cultivation of high-value aquatic plants in restored urban wetlands for income generation in local communities ("new green" database)	2001	Considering wetland plants as value-add products.	More thinking about wetlands as macrophyte bioreactors, and looking at the system as a whole.

2.6 South African Academic Institutions and WWBRs

2.6.1 South African academic research groups with WWBR themes

A search was conducted to identify the academic groups in South Africa studying the recovery of value from wastewaters. The annual reports of the various universities, “The State of Waste to Energy Research in South Africa: A Review” (SANEDI, 2014), “The State of Energy Research in South Africa” (ASSAf, 2014), and internet based searches were conducted to explore the various role players in the water and wastewater research space (Table 2-15). This listing of research groups is not intended to be a comprehensive list, but the start of exploring the work currently being done in the area. While every care has been taken to include relevant research, there may groups that have been missed. A necessary outcome may be the creation of a database that allows users to update their own information.

Table 2-15: South African academic researchers in the water and wastewater sphere

Institute	Name	Directors/ Lead investigators	Projects/ Themes relevant to Wastewater biorefineries (WWBR)	Reference
University of Cape Town	Centre for Bioprocess Engineering (CeBER), Dept Chemical Engineering	Prof Sue Harrison; Dr Madelyn Johnstone Robertson; Dr Rob Huddy	CeBER strives to address environmental issues primarily related to water, with consideration for the potential for value addition. Current projects consider acid rock drainage (ARD) prevention through enhanced management and use of waste materials and remediation, using biological sulphate reduction and sulphide oxidation technologies for ARD treatment, biological cyanide degradation, anaerobic digestion, algal processes, metal removal and the remediation of olive processing wastewaters. Across these projects there is a focus on integrated systems, microbial ecology and the potential for value recovery. At the macro-scale, the Centre has expertise in sustainability and life cycle analyses and emerging technologies for renewable energy generation and greenhouse gas emission reductions.	http://www.ceber.uct.ac.za/index.php?option=com_k2&view=item&id=10:green-biotechnology&Itemid=29
University of Cape Town	Water Research Group, Dept of Civil Engineering	Prof George Ekama; Dr David Ikumi	Research focusses mainly on environmental systems engineering, which seeks to develop an understanding of the fundamental chemical, physical and biological processes operating in various water-related systems, such as water storage (impoundments), transport (rivers, pipes, sewers) and treatment plants (potable and wastewater).	http://www.civil.uct.ac.za/water-research-group
University of Cape Town	Urban Water Management Research Unit	Prof Neil Armitage; Dr Kirsty Carden	• Urban water services • Water-sensitive urban design • Sanitation in informal settlements	http://www.civil.uct.ac.za/associate-professor-neil-armitage#sthash.khZoSSFN.dpu http://www.civil.uct.ac.za/associate-professor-neil-armitage
University of Cape Town	Environmental and Geographical Science (EGS)	Dr Kevin Winter	• Water quality monitoring • Public / government partnerships • Informal settlement upgrading •	**Error! Hyperlink reference not valid.**http://www.ddrn.dk/filer/forum/File/Overview_Report_UEM_Southern_%20Africa_February_2008.pdf http://www.egs.uct.ac.za/staff_files/kevin.html
Rhodes University	Institute for water research (IWR) incorporating the Unilever Centre for Environmental Water Quality (UCEWQ)	Prof Dennis Hughes (IWR) and Prof Carolyn Palmer (UCEWQ)	• Hydrology project • Environmental water quality projects • Water Resource Management projects	http://www.ru.ac.za/static/institutes/iwr/ http://www.ru.ac.za/static/institutes/iwr/publications/IWRAnnualReport2011.pdf

Institute	**Name**	**Directors/ Lead investigators**	**Projects/ Themes relevant to Wastewater biorefineries (WWBR)**	**Reference**
Rhodes University	Biotechnology Innovation Centre (BIC)	Prof Janice Limson	Remediation of wastewater coupled to power generation in microbial fuels	http://www.ru.ac.za/biotech/people/staff/profjanicelimson/ SANEDI (2014) report
Rhodes University		Prof Brett Pletschke	Immobilisation of enzymes on to nanofibers for subsequent application in water/wastewater research.	http://www.ru.ac.za/bm/people/academicstaff/pletschke/research/#d.en.35053
Rhodes University	Institute for Environmental Biotechnology (EBRU)	Prof Keith Cowan	The research focus of EBRU has targeted the advancement of sustainability through remediation and the beneficiation of saline, domestic and industrial wastewater for high value products and bio-fuels, and the exploitation of solid waste for use in agriculture and industry. Projects investigating value-addition are actively pursued and included in this portfolio: • Integrated Algae Ponding Systems (IAPS) for treating organic effluents and generating a treated water that is safe for discharge into the environment; • Recovering commercially valuable metabolites e.g. β-carotene, glycerol and fertilizer from micro-algae; • Exploring the potential of micro-algae biomass as a feedstock for renewable energy production; • Treating mine drainage wastewaters and using this in agro-industrial development as a basis for social, economic and environmental sustainability, esp. applicable following mine closure; • Removing heavy metals from the environment using biological systems; • Using South African hardwood fungi to bioremediate coal and hydrocarbon wastes.	http://www.ru.ac.za/ebru/aboutebru/
University of KwaZulu-Natal	Pollution Research Group	Prof Chris Buckley	The group's main focus is conducting innovative research projects on water resources, waste water reclamation, the impact of effluents on local environments, sanitation systems, and other water related environmental issues. The group has completed many projects commissioned by the WRC relating to wastewater topics potentially relevant to WWBR. Current projects include (among others): • Co-digestion of sewage sludge and industrial concentrates (WRC – K5/2001) • Integration of aquatic chemistry with bio-process models (WRC -K5/2125) • Integrating agriculture in designing low cost sanitation technologies (WRC -K5/2220) • Micro-nutrient requirements for anaerobic digestion of concentrated industrial effluents (WRC - K5/2228) • Water and waste water management in the soft drink industry (WRC - K5/2286) • Development of an aerobic membrane bioreactor for treating Illovo wastewater (funded by Illovo)	http://prg.ukzn.ac.za/ http://prg.ukzn.ac.za/home http://prg.ukzn.ac.za/projects
University of KwaZulu-Natal	Centre for Research in Environmental, Coastal and Hydrological Engineering	Prof Christina Trois	• Wastewater management • Wastewater treatment • Renewable energy from waste • Greenhouse gas control from zero waste	ASSAF (2014) report http://civeng.ukzn.ac.za/Research.aspx

Institute	Name	Directors/ Lead investigators	Projects/ Themes relevant to Wastewater biorefineries (WWBR)	Reference
University of KwaZulu-Natal	Water, Environment and Biodiversity	Various researchers from UKZN	• Hydrology • Waste, Water and Sanitation Management • Micrometeorology and Agrometeorology • Hydrological Engineering • Limnology • The Smallholder System Innovations (SSI) Research Project	http://research.ukzn.ac.za/ResearchFocusAreas/WaterEnvironmentandBiodiversity.aspx
University of Stellenbosch	Water Institute (involving several departments)	Prof. Gideon Wolfaardt (Director) Prof Eugene Cloete (Chairperson)	• Effluent management • Nanotechnology and filtration • Sustainable water management • Water & agriculture • Water & food • Water & health • Water & society	http://water.sun.ac.za/
North West University (NWU)	School of Chemical and Minerals Engineering, Potchefstroom Campus	Prof Sanette Marx	Membrane technology	http://www.nwu.ac.za/sites/www.nwu.ac.za/files/files/pfe/documents/cv/Prof.%20S.%20Marx%20-%20Associate%20Professor.pdf
University of Pretoria (UP)	Chemical Engineering Biochemical Engineering Water Utilisation	Dr Willie Nicol Prof Evans Chirwa	Bioreactors (biofilm and membrane-recycle bioreactor)	http://www.up.ac.za/chemical-engineering/article/1913314/bioreaction-engineering http://www.up.ac.za/chemical-engineering/article/1913286/biochemical-engineering http://www.up.ac.za/chemical-engineering/article/1913292/water-utilisation
Durban University of Technology	Institute for Water and Wastewater Technology (IWWT)	Prof Faizal Bux Dr Sheana Kumari Dr N Ramdhani	The focus is largely based on developing and optimising technology for the treatment of water and wastewater and to satisfy the needs of industry and the community. Projects are mainly aimed at helping industries to maintain acceptable levels of effluent discharges, thus reducing negative environmental impact and commercialisation of products generated from waste streams.	http://www.dut.ac.za/iwwt SANEDIi (2014) report ASSAF (2014) report
Vaal University of Technology	Centre for Renewable Energy and Water, Chem Eng Dept	Prof. Ochieng Aoyi	• Application of adsorption technique in point-of-use potable water purification and in wastewater remediation • Storm water management and hydrology • Biological wastewater treatment and environmental pollution control • Application of computational fluid dynamics technique in reactor optimization • Application of nano-materials in pollution management	SANEDI (2014) report http://www.vut-research.ac.za/index.php/higher-degrees/higher-degrees-studies/payment-options/44-vut/research/179-water-and-bioenergy-centre

Institute	Name	Directors/ Lead investigators	Projects/ Themes relevant to Wastewater biorefineries (WWBR)	Reference
Tswane University of Technology (TUT)	Department of Environmental, Water & Earth Sciences	Prof Maggie Momba	Various aspects of water with emphasis on: water and wastewater management, health, related water microbiology, biotechnology and molecular biology.	http://www.tut.ac.za/Students/facultiesdepartments/science/departments/environscience/Documents/Maggie%20Momba%20Simple%20removed%20photos.pdf
Cape Peninsula University of Technology	Biocatalysis and Technical Biology research group (BTB)	Dr Marilize le Roes-Hill	The main focus of the research group centres on the discovery and use of robust industrial biocatalysts in applications that range from bioremediation of industrial wastewater to antioxidant synthesis. The research areas range over enzyme discovery, enzyme mutation studies and actinobacteria biology to bioreactor design for wastewater treatment	http://www.cput.ac.za/files/images_folder/researchdictorate/Research%20Report%202011%20smaller.pdf
Cape Peninsula University of Technology	Biotechnology and Water treatment	Prof Marshal Sheldon	• Colour removal from textile wastewater using a pilot-scale dual-stage MBR and subsequent RO system • Water re-use using a dual-stage membrane bioreactor for industrial effluent treatment. • Membrane bioreactor application within the treatment of high-strength textile effluent. • Treatment of paper mill effluent using an anaerobic / aerobic hybrid side-stream membrane bioreactor.	http://www.cput.ac.za/files/images_folder/researchdictorate/Research%20Report%202011%20smaller.pdf

2.6.2 Journal articles with WWBR themes published by South African researchers

The Elsevier abstract and citation data-base of peer-reviewed literature, Scopus (Elsevier, n.d.), was used to discover journal articles in the international literature published by South African scientists and engineers. The keywords “wastewater treatment” with “SA” as affiliate delivered 924 articles, which is a very broad base of research. However, “value from wastewater” with “SA” as affiliate, yielded a much narrower 165 entries. The final selection for further scrutiny was made using the keywords “water, wastewater, effluent, industrial” and South Africa as affiliate. This search produced 124 publications spanning 1978 to 2015. These publications were further analysed and reduced to the 48 most relevant (see B). Using the same approach as in Section 2.5 these references were then classified into categories A – F, represented graphically in Figure 2-3. The majority of research focussed on categories B at 47.9%, with D and E 14.6% each, C 10.4%, and F and A 6.25% each.

Table 2-16: Number of journal articles with WWBR themes by South African researchers in each of six categories

Category	Number of reports in category	Percent
A. Wastewater management	3	6.25 %
B. Wastewater treatment technology	23	47.9 %
C. Cleaner Production	5	10.4 %
D. Products from wastewater	7	14.6 %
E. Products to be used in wastewater	7	14.6 %
F. Wastewater Biorefineries	3	6.25 %
Total	48	

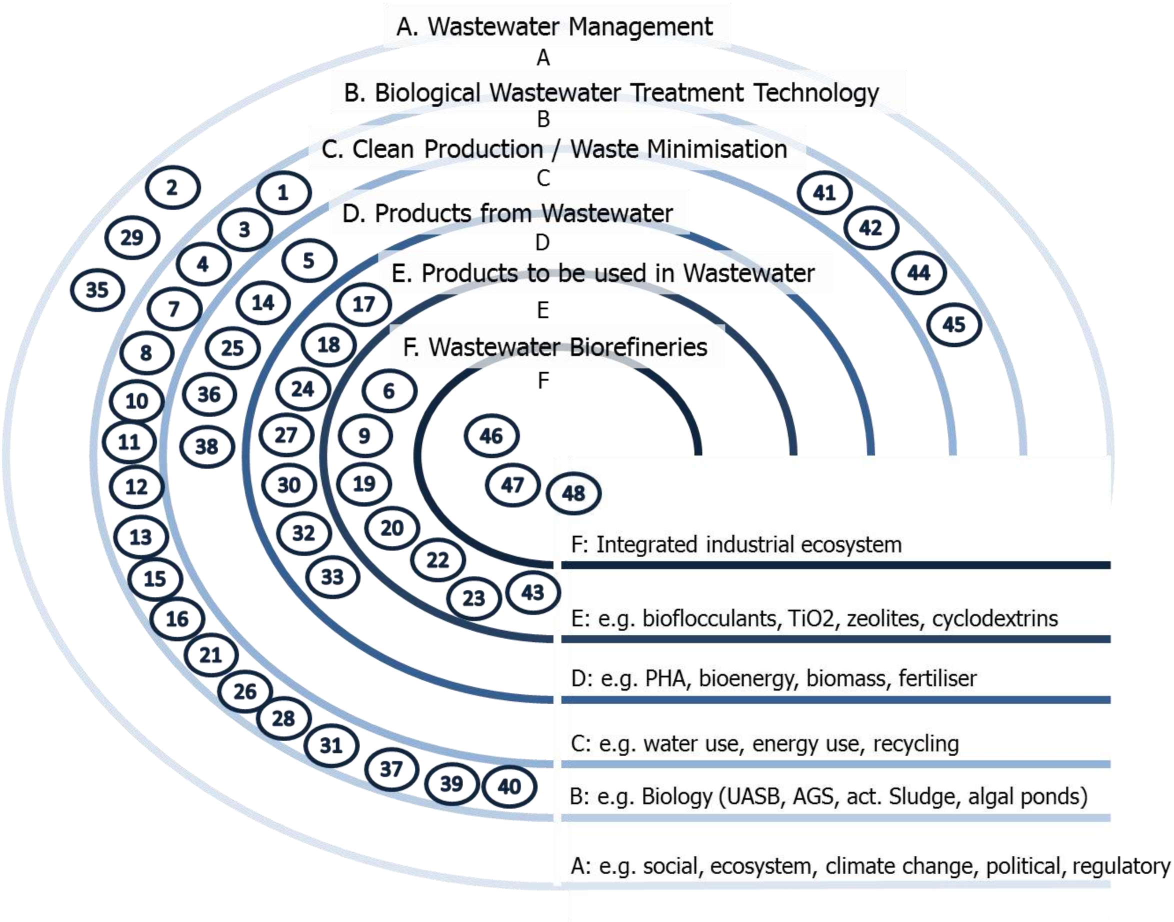


Figure 2-3: Graphical illustration of the context of wastewater biorefineries related to South African research published in journals listed in Appendix B

Pitman & Boyd (number 29 in B (1999)) mentioned the need to remove nutrients from wastewater by biological means and to dispose of the sludge by-products in an efficient manner. This prompted the Greater Johannesburg Metropolitan Council to adopt a new approach to the management of industrial discharges. This is encouraging as it indicates that academic research can and does enable changes in the wastewater industry. The role of the Rand Water research chairs in facilitating this interaction between new knowledge and implementation should be explored.

The Scopus search was refined, using “wastewater biorefinery” and “SA” as affiliate as the keywords; this gave three entries, all from the group of Prof Faizal Bux (IWWT) at DUT. These three entries are listed in Table 2-17 and as references 46 (Singh) (Singh, et al., 2015), 47 (Rawat, et al., 2013), and 48 (Rawat, et al., 2011) in Appendix B. These articles explore the biorefinery approach through the use of wastewater as an algal production medium for CO_2 capture from flue gas to grow algae for the production of biodiesel, other biofuels and value-added products as well as offering environmental protection.

Table 2-17: South African research published in peer-reviewed journals
Sourced through Scopus using the keywords wastewater biorefinery and South Africa (extract from table in Appendix B)

	Authors	Affiliation	Title of journal paper	Year of pub	Journal	Value of research in context of wastewater biorefineries
46	Singh, B., Guldh, A., Singh, P., Rawat, I., Bux, F., Singh, A.	Centre for Environmental Sciences, Central University of Jharkhand, Ranchi, India and Institute for Water and Wastewater Technology, Durban University of Technology	Sustainable production of biofuels from microalgae using a biorefinery approach	2015	Applied Environmental Biotechnology: Present Scenario and Future Trends, Springer, New Delhi, 115-128	The value added product derived from biorefinery basket includes pigments, nutraceuticals, and bioactive compounds. The use of industrial refusals for biomass production includes wastewater as nutrient medium and utilization of flue gases (CO_2) as the carbon source for culture of microalgae. These processes have the potential to reduce fresh water footprint and carbon footprint.
47	Rawat, I., Bhola, V., Kumar, R.R., Bux, F	Institute for Water and Wastewater Technology, Durban University of Technology	Improving the feasibility of producing biofuels from microalgae using wastewater	2013	Environmental Technology, 34 (13-14), pp. 1765-1775.	The use of a biorefinery approach sees the production costs reduced greatly due to utilization of waste streams for cultivation and the generation of several potential energy sources and value-added products while offering environmental protection. The use of wastewater as a production medium, coupled with CO_2 capture from flue gas greatly reduces the microalgal cultivation costs. Conversion of residual biomass and by-products, such as glycerol, for fuel production using an integrated approach potentially holds the key to near future commercial implementation of biofuels production.
48	Rawat, I., Ranjith Kumar, R., Mutanda, T., Bux, F.	Institute for Water and Wastewater Technology, Durban University of Technology	Dual role of microalgae: Phycoremediation of domestic wastewater and biomass production for sustainable biofuels production	2011	Applied Energy, 88 (10), pp. 3411-3424	This paper discusses current knowledge regarding wastewater treatment using HRAPs and microalgal biomass production techniques using wastewater streams. The paper discusses biodiesel production via transesterification of the lipids and other biofuels such as biomethane and bioethanol which are described using the biorefinery approach.

2.7 South African Industry-Based Initiatives in the WWBR Arena

An attempt was made to determine the current state of industry-based initiatives that relate to WWBR in South Africa. However, determining the state of wastewater resource recovery in South African industry proved challenging.

The search specifically excluded wastewater treatment without value-generating products, and only considered treatment that uses bio-based technology. The following steps were taken in exploring the status of industry-based initiatives:

- Web-based searches were conducted using a variety of keywords and following anecdotal leads.
- Industry professionals in South Africa were contacted, drawn from the networks of the research team (see Acknowledgements)

- The WRC project reference steering committee were contacted (see Acknowledgements).

In spite of considerable effort, a rigorous review of the value-from-wastewater space in South Africa was found to be out of reach. Much of the information related to projects which are client confidential, so that contacts were unable to divulge full details. The information that was available was largely derived from quasi-technical news-related articles promoting the environmental awareness of the entity in question.

There are a number of organisations reporting in this arena, and the reports and groupings which were found to be helpful are enumerated. Information relating to industrial wastewater, municipal wastewater, technology development and service providers is given, focusing on a single project in each case. Finally some brief conclusions are drawn.

2.7.1 Overview reports and organisations

There are a number of reports germane to the current levels of industrial implementation of WWBR-relevant technology.

At a global level these give helpful insights in terms of placing South Africa within the global framework of concern about water security.

- CDP (Carbon Disclosure Project) Global Water Report 2015 (CDP, 2015)
- IWA (International Water Association) Resource Recovery from Water, 2015 (IWA Resource Recovery Cluster, 2015)

The Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) is involved in a number of high level reports with other (South African) organisations such as the South African National Energy institute (SANEDI), Renewable Energy Centre of Research and Development (RECORD) and the South African Local Government Association (SALGA).

- “The State of Waste to Energy Research in South Africa”, August 2014 (GIZ, RECORD & SANEDI, 2014)
- “Biogas potential in selected waste water treatment plants”, March 2015 (Ferry & Giljova, 2015)

The Strategic Water Partners Network (SWPN, 2013; 2015) has more than twenty partners, but their publications are mostly rhetoric at this stage.

The South African Biogas Industry Association (SABIA) is a relatively new grouping relevant to biogas within the WWBR. Since biogas is the dominant product currently produced from wastewater in South Africa, this information is helpful.

GreenCape (GreenCape) was established in 2010 as a regional resource centre in the Western Cape. They provide useful regional insight with respect to aspects of the “green economy”. There may be similar organisations in other regions.

2.7.2 Industrial wastewater

A selection of examples of industrial wastewater approaches are given in this section.

SABMiller, with seven breweries in South Africa, has committed itself to “working towards zero waste operations” (SAB, 2013). This places it firmly in the area of application for opportunities presented by the WWBR concept (see Sections 2.2.1 and 2.3). In 2009, four breweries (SAB; Burton, et al., 2009) had anaerobic digestion wastewater facilities producing biogas which is used as an energy source in the brewing process. Up until ten years ago the biogas from these units was flared at all units (Burton, et al., 2009), so the value-recovery aspect is fairly recent. The Newlands brewery, Western Cape, has sponsored at least two postgraduate research projects at UCT (Nkadimeng, 2015; Cohen, 2006) assessing opportunities in biogas production and use. Their biogas is used to power their steam boiler, allowing substantial electricity savings to be accrued. "Currently, the steam generated … accounts for around 10% to 12% of the total steam required in the brewing process with the balance generated from electricity" (Nkadimeng, 2015).

The iBhayi brewery, Eastern Cape, have partnered with Rhodes University and two organisations based in India to expand this approach to wastewater. High Rate Algal Ponding (HRAP) and Constructed Wetland technology are used in addition to AD. The system produces hydroponic lettuce and fish (SAB, 2014; Crous & Britz, 2010; SAB). The wetland uses considerable land which means that the technology is not transferable to all sites (Seggie, 2011). The treated water remains saline and can only be released into naturally saline waterbodies such as estuaries (Seggie, 2011). The use of multi-stage processing together with the production of several products (biogas, fish and lettuce) places this system close to the WWBR concept.

Since the brewing process is already a biological process, it is appropriate that the brewing industry be an early adopter of biological WWT and therefore has good potential as a frontrunner for the WWBR. In essence this move would make the brewery into a biorefinery *per se*, where the initial feedstock is not a wastewater stream but the subsequent (bio)processes produce value-added products downstream of the brewing.

There are several other industries with customised wastewater processes in South Africa. The pulp and paper industry is water-intensive and invests considerable resources in wastewater treatment (Ndaba, 2011; SAPPI, 2014). Currently, it seems that the only value-recovery across most of the industry is in re-using treated water to reduce the overall water footprint (Mac Donald, 2004). At least three companies in the petrochemical industry are known to have invested heavily in wastewater treatment. Chevron refinery, Western Cape, uses a moving bed bioreactor followed by clarifying steps before re-use (Chevron; Petroleum Africa, 2008; Veolia). The SAPREF refinery in Durban, Kwa Zulu-Natal, uses recycled water from the Durban Water Recycling Plant, which also undergoes additional clarifying for certain uses (Veolia; eThekwini City; Ndaba, 2011). Neither of these are involved in value-recovery, however SASOL currently has an innovative project for biogas and associated electricity production, described in Section 2.7.4. Until recently the focus in the mining industry has been entirely on mitigation of environmentally damaging factors in the wastewater; however, there is an increasing body of research into the possibility of value-added products (Harrison, et al., 2014). To date there are no known examples of implementation.

Anecdotally there is an awareness that in the agriculture and food processing industries there is use of anaerobic digestion units with biogas collection. At the South African International Renewable Energy Conference, held in October 2015, Tiepelt (2015), speaking of biogas from waste, claimed that there are approximately 350 small scale units, 280 units at WWTW, and 70 units in commercial operation, although he did not specify the size cut-off of "small" units. Notes of information given by speakers at African Utility Week 2016, 17-19 May Cape Town (Global Utility Week Series, 2016), indicate that this is accurate at order of magnitude level.

It seems that at this point there is no commercial production of value-added products from wastewater in South Africa, other than biogas and water itself. Biogas production remains limited, with even less of the second step of biogas-to-electricity.

2.7.3 Municipal wastewater

The most often cited example of resource recovery is Johannesburg Water's Northern Treatment Works near Diepsloot where a unit was installed in 2012 that generates electricity from biogas produced in the WWTW. The project involved refurbishing and upgrading existing anaerobic digesters, implementing high performance mesophilic AD with pre-thickening and cell lysis (Naidoo, 2013). The energy installations are combined heat and power (CPH) with the heat used for heating the AD units and the electricity used in other WWTW operations such as aeration. The improved AD process increases biogas production and quality, achieving the quality required by the power units. The added benefits are reduction in corrosion of equipment together with production of a sludge that meets the standards for organic compost (Franks, et al., n.d.; City of Johannesburg, n.d.).

A number of significant challenges have to be overcome with this installation, as noted in a GIZ-SALGA report (Franks, et al., n.d.). The major challenge was performance under-capacity, with sludge production running at about half the expected volume and an average methane content of 62% of that expected. As a result the CHP units are running well below capacity, with an electricity production of 1,600 MWh/year instead of a hoped for 5,000 MWh/year. This report intimated that the four high performance AD units would be supplemented with a further two in an attempt to rectify this.

The original reports provide information about roll-out in the other City of Johannesburg WWTWs; however, no reports could be found of implementation in other locations except for one listing (Muldersdrift WWTW) in the South African Biogas Industry Association (SABIA) project database dated June 2014 (SABIA, 2014).

The SABIA project database for biogas lists numerous ‘planned’ projects and a number of existing landfill gas projects. Other than the two major Johannesburg projects, the only existing projects in the wastewater space are six listed for the Western Cape (Table 2-18) which all combine solid waste and sewage. It is likely that this reflects the fact that there was contact with a locally based organisation, GreenCape (see Section 2.7.1), which had collated local information. It is possible that a similar number of projects exist in other provinces, but with no local organisation to broker information, these are not recorded in the open sources.

Table 2-18: Existing wastewater biogas-generating projects listed for the Western Cape (SABIA, 2014).

Province	Local municipality	Technology	Feedstock	Capacity
Western Cape	City of Cape town-Phillipi	AD	MSW organics and sewage	15 t
Western Cape	Cape Winelands-Stellenbosch	AD	MSW organics and sewage	94 t
Western Cape	City of Cape Town-Noordehoek	AD	Volatile animal waste and sewage	60 t
Western Cape	Overberg-Stanford	AD	MSW organics and sewage	117 t
Western Cape	West coast-Riebeck Valley	AD	MSW organics and sewage	164 t
Western Cape	West coast-Riebeck Valley	AD	MSW organics and sewage	88 t

In 2015, results from scoping studies for biogas potential in nine South African municipalities were reported (Ferry & Giljova, 2015). The summary notes that the potential can be limited by low inflows as well as by the wastewater treatment process used. Potential can be increased by proximity to another industry suitable for biogas production.

2.7.4 Technology development

Most of the service providers mentioned are offering designs involving technologies developed elsewhere, in particular in Europe. However, there is one major development project in South Africa relevant to WWBRs.

Toward the end of 2013 Sasol launched a pilot plant on its research and development campus in Sasolburg, Free State. The plant uses an anaerobic membrane bioreactor to produce biogas using the effluent from the gas-to-liquids petrochemical plant, with subsequent conversion of the biogas to electricity. This technology was developed through a collaborative effort, including a sponsored UCT Civil Engineering PhD (Van Zyl, 2008), input from the technology of US-based General Electric and the Sasol research team. The pilot plant has a feed rate of 350 – 1,000 ℓ/h (Tshwarisano, 2016; Industry SA, 2014).

The petrochemicals group announced in April 2015 that the conceptual design for the full-scale commercial process would be available for roll out by the end of that year. The design is for a 60 Mℓ reactor expected to generate up to 40 MW of electricity. Unfortunately the first installation is likely to be at Sasol's proposed gas-to-liquid plant in Louisiana, US (Tshwarisano, 2016; Oliveira, 2015), rather than in South Africa.

2.7.5 Service providers

One of the larger companies offering design, equipment, construction and operation within the South African WWT arena is Veolia. The information they supply mentions decontamination of wastewater, recycling of water, reuse of sludge and recovery of commodities from wastewater (Veolia). They are one of the few service providers who specifically mention by-products (fertiliser and biogas). They also state: "Veolia is also developing the conversion of wastewater treatment process plants into biorefineries capable of producing energy as well as valuable by-products such as biopolymers" (Veolia). This was the only reference to biopolymers or biorefineries found in the service-provider literature.

There are 37 case studies and a number of newer press releases on their website; however, only the latest one specifically mentions biogas recovery. In 2014 Veolia was awarded the contract to design, construct and operate a wastewater treatment facility for Distell (Veolia, n.d.; Bizcommunity, 2015; Western Cape Business News, 2015), a spirits, wine and cider producer situated in Stellenbosch, Western Cape. The Biothane Biobulk® CSTR anaerobic digester will use industrial effluent from the three Distell sites in Stellenbosch as feedstock. The recovered biogas is transferred straight to the boiler producing steam for the distilling process. This plant will be able to treat 1 000 m^3/day of effluent with 8.6 tonnes/day COD and is scheduled to come online in March 2016.

Talbot and Talbot have installed a number of AD plants in South Africa, ranging from feedstocks of 1 to 25 t-COD/day. Applications include those at SAB and Coca Cola. The biogas may be used to raise steam or in CHP operations.

There are, of course, other service providers in the wastewater arena; however, few mention relevant projects on their websites and little technical information provided. For example, Project Assignments Consulting Engineers, who install Paques AD systems, mention installation of an AD producing biogas for CHP at a "large poultry abattoir" (Project Assignments). A much smaller company, iBert supply biogas-electricity installations and mention on their LinkedIn page an abattoir, a cheese farm and a piggery (iBert, 2015). No other service providers were found with biogas-electrical projects featured on their websites.

In addition, it is apparent from personal communications that there are a small number of wastewater beneficiation studies and installations which have been concluded on behalf of unnamed entities. However, client confidentiality means that the details are unavailable. The capital outlay was identified as a hurdle to project implementation by several of the respondents.

There are multiple companies supplying off-the-shelf AD units of various sizes designed for biogas collection.

2.7.6 Conclusions with respect to industrial initiatives

This investigation was challenging as much of the information related to proposed projects and many of the scoping studies and installation projects are client confidential. From the information gathered, it appears that opportunities for valorisation of wastewater are still largely unrecognised in South African industry. A number of front-runners have installed biogas facilities; however, these are not yet a standard feature. Furthermore, the recovery of energy still requires optimisation in several of the installations. This status suggests immense opportunity for value recovery from South African wastewaters.

2.8 The Wastewater Biorefinery Concept Positioned in South Africa

The concept of the wastewater biorefinery is new on the global stage and integral to the shift towards a circular economy. Currently, South Africa has not yet embraced the implementation of the "value from waste" basis of the wastewater biorefinery to any significant extent, despite the presence of a significant body of relevant quality research and discrete examples of implementation. This is perceived to result partly from the lack of information on South Africa's wastewater streams and partly from lack of awareness of the potential for simultaneous value recovery, water treatment and water recovery.

CDP (The Carbon Disclosure Project) was set up in 2000 by a consortium of corporations, to encourage self-reporting and enabling reduction of carbon footprint globally. The CDP 2015 Report states:

> *"There have been some encouraging improvements in the quality of disclosure. Nonetheless, the South African response rate to CDP's water program continues to be low, with just over half the companies responding. This does not reflect the significance of water-related risks in the country, and might suggest that companies are overlooking the severity of these risks.*
>
> *There has been an increase in the number of respondents identifying water-related opportunities, including a particular increase in the number of companies identifying opportunities for enhancing brand value." (CDP, 2015)*

Industry has shown some interest in generating energy from their waste, in the form of biogas. This is an established, and therefore lower risk, technology globally. The increasing electricity prices and energy insecurity makes it an increasingly attractive investment.

The lack of available information on wastewater streams and their handling from industry may indicate a fear of litigation for non-compliance of their wastewater for discharge, but it may also indicate lack of a clearly articulated need for beneficiation of the wastes, with a focus on removal of the waste problem only, rather than realisation of value. For example, in food wastes, the waste is often relocated to animal feed. While this presents a low-value market, it fits into the core business and established supply chain and ecosystem of the producers of the waste. These industries seem reluctant to try new technologies that upset established partnerships. This contrasts to complex wastewaters that do not have an existing outlet, for example municipal wastewater and abattoir wastewaters.

The financial implications for industry to commit to WWBR is very important. Key questions to consider include:

- Is a new plant required or can an existing plant or part thereof be retro-fitted?
- Is technology being bought in or can internal technology be used?
- Is it cheaper or less risky to pay penalties for not complying with effluent standards than to build a WWBR? What is the integrated financial upside?

Despite increasing awareness of the potential savings that can be achieved by more efficient water use and recycling, the level to which opportunities have been implemented varies widely between organisations (Cohen, et al., 2014). Capital cost of implementation and financial return are cited as the primary reasons for not implementing recycle and recovery systems. All investments are justified on the basis of financial return, often regardless of co-benefits for the environment. Water management systems seldom achieve returns comparable to other investment opportunities.

For WWBR to be accepted in the industry context, the value-add has to be significant to offset the greater perceived risk. To use the metaphor of the crude oil refinery, relatively low-value products from wastewater like biogas, fertiliser and animal feed should be considered the equivalent of "heavy vac gas oil" or "asphalt" of biorefineries – the leftovers after the higher value products are refined out of the crude stream. Currently they are considered the only valuable products, which limits the perceived potential of WWBR.

3 CRITICAL CONSIDERATIONS FOR WASTEWATER BIOREFINERIES IN THE SOUTH AFRICAN CONTEXT

In order to assess the suitability of WWBR in South Africa, several critical criteria need to be addressed. This chapter provides an overview of these considerations, with direction for the detail of subsequent chapters in this report. The effects of external factors are discussed in Section 3.1, looking at general economics and government policies. This is followed by three sections surveying the issues which must be accounted for during evaluation: potential wastewater feedstocks (Section 3.2), potential biorefinery products (Section 3.3), and elements of the WWBR process (Section 3.4). Finally (Section 3.5) the dynamics influencing integration of all these aspects into the WWBR are reviewed.

3.1 Economic and Policy Considerations

3.1.1 The effect of economics on the WWBR

The economics of wastewater treatment in South Africa

Van der Berg (2009) studied the South African wastewater market in terms of business opportunities and export promotion for Dutch companies. Promising market segments and a listing of opportunities in South Africa are provided in Table 3-1. Van der Berg (2009) stated that a major factor within the South African economy obstructing development in wastewater was the lack of investment in infrastructure, particularly power supply and water and wastewater infrastructure, with a resulting decline in water quantity and quality. The main elements of the declining water quality are (raw) sewage effluents, eutrophication and acid mine drainage. The most frequently mentioned causes related to the issue of water quality are the lack of enforcement of laws and regulations, non-allocation of funds and the shortage of skills. The non-compliance of wastewater treatment plants presents the most severe problem, having a number of causes and major effects. (Chernick, 2016; Schneider, 2016)

Table 3-1: Technological opportunities in South African wastewater segments (Van den Berg, 2009)

Segment	Opportunities
Collection and sanitation	Upgrading of wastewater pipeline infrastructure and new sanitation concepts
Industrial	Innovative technologies for rehabilitation of industrial wastewater
Domestic wastewater	Wastewater treatment equipment and treatment plants, private sector involvement and upgrade of existing WWTW
Re-use	Membrane technology, domestic water re-use and industrial process water recycling

In terms of water infrastructure, currently South Africa is facing its worst drought in 23 years. This trend is expected to continue due to climate change (Bellprat, et al., 2015). Further, approximately 25% of municipal water is lost through leaks and 55% of municipalities could not provide accurate water statistics (DWS SA, 2015). In this report, it is proposed that the gap between water supply and demand in South Africa must be closed by innovative ways to ensure that more wastewater is treated. Further, water should be conserved through better maintenance of the existing infrastructure. Public awareness of the fragility of water security in South Africa should add impetus both to the ability of government to enforce regulations and to the recognition of responsibility in the private sector.

In the intervening years since Van der Berg's (2009) study, power supply in South Africa has remained unstable, with a combination of intentional rolling blackouts (so-called "load shedding") and major increases in the cost of electricity. In June 2015 academics calculated that the electricity price had doubled in real terms since 2009 (Parsons, et al., 2015). Although Eskom is investing in new power plants to increase the supply of electricity, project delivery is problematic (SABC, 2016) and energy availability is still compromised. The International Monetary Fund, also in June 2015, "singled out delays in easing electricity shortages, and to (sic) policy and regulatory uncertainties, as chief constraints to economic growth" in South Africa (News 24, 2015). While this is essentially an inhibitory factor with regard to investment, it could also make the production of bioenergy more attractive.

Van der Berg's (2009) analysis of the market potential resulted in a list of market drivers and restraints, as well as concrete business opportunities and an overview of competition in the market. One of the most important market drivers is the increased enforcement by the government, which is likely to stimulate spending in this sector in the coming years. Other drivers for this sector are increased feasibility of investments due to increased cost of water and energy, technological developments and the need for improved treatment as a result of increased complexity of wastewaters. In conclusion, Van der Berg (2009) considered the wastewater treatment market in South Africa to be a competitive one, with well-established international competition and many international companies already active in all investigated segments (Section 2.7).

The economics of the WWBR

The WWBR is ultimately a production process and, as such, must be driven by economic considerations. Profitability is the key to the long term viability of the WWBR and depends on the three strands: capital expenditure, operating costs and product value (Bozkurt, et al., 2016). This section presents a qualitative economic analysis of WWBRs with the aim of highlighting critical factors influencing profitability.

Capital expenditure is the up-front financial outlay related to the cost of the design process, processing equipment and ancillaries as well as construction of the biorefinery. These, in turn, are determined by a number of factors, including the process design, the size and the location of the system. In a presentation on biorefinery economics dealing with a biomass refinery, Bohlmann (2006) listed the main capital intensive processes as pretreatment and product recovery. The author recommends the production of coproducts, or a diversified product offering to provide economic synergies, and lists wastewater treatment as a remaining technical challenge. The pretreatment in a WWBR will be less intensive than in a ligno-cellulosic biorefinery, and the wastewater treatment is by definition resolved. The complex, variable and intermittent nature of wastewater streams may impact on the capital cost of producing particular products due to the potential need for specialised systems to handle this variability and deliver consistent product quality.

Operating costs usually include the cost of raw materials and energy as the major costs. The WWBR concept offers the opportunity to utilise cheap or free raw materials as the bulk raw materials; as opposed to a traditional lignocellulosic biorefinery where the cost is composed of: feedstock cost 25 - 40%, reagents 10 - 35%, and transport and logistics 5 - 10% (EuropaBio, 2011). Since most of the microbiological processes considered for the WWBR system use robust naturally-occurring mixed microbial cultures, energy requirements for heating are negligible although cooling may be required. The combination of high volumes and a significant solids component for many wastewaters can result in high costs for pumping. A key factor in operating costs is the cost of downstream processing (DSP). In the large volume wastewater system, this requires careful attention at the design stage. It is recognised that the low (no) cost of bulk raw materials may be offset by the volume-associated pumping, aeration and DSP costs, as well as potential constraints to productivity (Kong, et al., 2010; Theobald, 2015). These require careful scrutiny.

Apart from the capital cost and the ongoing cost of production, profitability is influenced by the selling price of the product. Bioproducts are often classified with respect to their price into high value niche products, intermediate value products and bulk commodity products. High value products are often produced in small quantities (Grotkjær, 2016). Further these products usually demand high purity hence downstream processing costs are significantly reduced by use of simple and consistent feedstocks. Typically, the raw materials form a minor part of the production costs of high value products. These factors suggest that, typically, high value products are less suitable for production in the WWBR, or alternatively form co-products. Bulk commodity products are usually demanded in large quantities. Such commodity products for which purity requirements are also typically less stringent are well suited to the WWBR. In a WWBR, there is the potential to produce lower quantities of intermediate value products upstream of production units for lower value bulk commodity products. A possible negative factor in terms of impact on product economics includes the dilute nature of the raw material, requiring processing of very large volumes of feed streams in order to achieve the bulk needed for the commodity products. It is also important to consider the downstream operations required for product recovery and the positioning of the WWBR relative to the product market. The particular products for the WWBR must be chosen with these multiple factors in mind, however the benefit of the WWBR approach is the potential to offset waste treatment costs against value derived from products.

In Figure 3-1, the impact of the various factors on the profitability of the WWBR, directly or by influencing other factors, is considered. Each interaction is labelled and enlarged on in the list below the diagram.

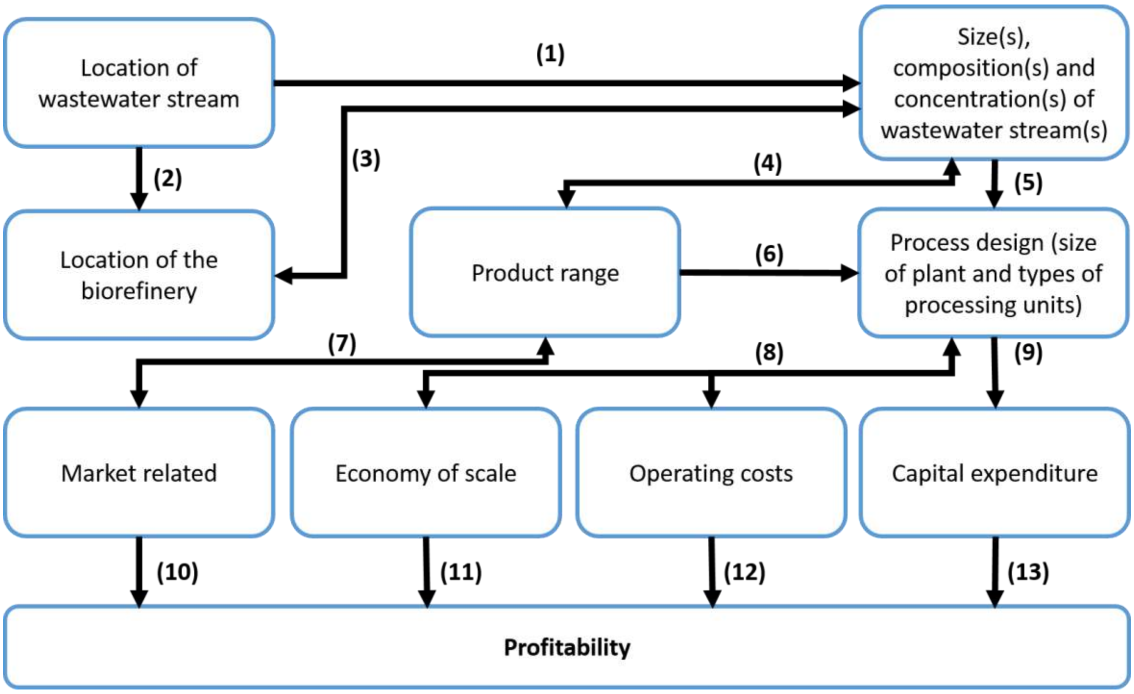


Figure 3-1: Factors affecting the profitability of the wastewater biorefinery

(1) The geographical location of the wastewater stream will determine which other wastewater streams can be used, based on proximity. This determines the size and properties of the inlet stream to the biorefinery.
(2) The location of the wastewater stream influences the location of the biorefinery to avoid pumping or transportation costs.
(3) The sizes and properties of the wastewater streams available in a certain geographical location influence the location of the biorefinery. The location of the biorefinery impacts access to other wastewater streams.
(4) The sizes and properties of available wastewater streams influence the type of products that can be produced.
(5) The sizes and properties of available wastewater streams, and (6) the product range influence the process design.
(7) The product range influences the markets available for infiltration. The size of the market influences the types of products to prioritise i.e. some products are more profitable than others.
(8) The size of the operation influences the economy of scale and the operating costs. The types of processing units and control systems also affect the operating expenditure of the process.
(9) The sizes and types of unit operations as well as the complexity of the process influence the capital requirement.
(10) Market size and product acceptance affect the profitability of the biorefinery directly.
(11) Economy of scale influences profitability directly.
(12) Operating costs influence profitability directly.
(13) Capital expenditure influences profitability directly.

The economics of a WWBR may be influenced by the value of the clean water. This is not accounted for above; however, value can arise from the value of reuse (which leads to lower water consumption) or from mitigation of standards' transgression (Winpenny, et al., 2010). Economic studies may assume offset of wastewater treatment (Fernández-Dacosta, et al., 2015), but in the context of South Africa, or any country with poor enforcement of environmental laws, the scenario against which profitability is measured is one of no treatment rather than one of conventional treatment. The value of the clean water is therefore, at least in part, predicated on governmental policies and regulations with respect to effluent discharge standards as mentioned in Section 3.1.2 as well as on the geographic location determining the value of re-use.

One of the difficulties in positioning expenditure on a WWBR is the emotive issue of spending money on what is still perceived as waste. This is compounded by the unfortunately still-common perception that the cost of waste treatment is an avoidable expense.

3.1.2 The effect of wastewater policy on the WWBR

Standards for treated effluent

In order to establish a good understanding of water effluent criteria, the wastewater treatment standards of South Africa must be considered. The standards listed in Table 3-2 were introduced by the Green Drop Certification in 2008 and are updated annually as part of the incentive-based regulatory model. The Green Drop certification measures the performance of wastewater treatment works and sets a target of 80% compliance with wastewater effluent standards. The 2013 Green Drop Report indicated that 41% of the 914 water supply systems assessed require attention. Similarly, 55% (or 821) of wastewater treatment works require serious, critical and urgent refurbishment (Water and Sanitation, 2015). The model includes strengthening the regulatory approach while re-focusing the Local Government Support Model to improve the problem-solving capacity and move towards preventative maintenance instead of crisis-management (WISA, 2009).

Table 3-2: General Authorisation Standards for treated effluent (DWA SA, 2013)

Substance/Parameter	General Limit	Special Limit
Chemical Oxygen Demand (COD) (mg/ℓ)	75*	30*
Electrical Conductivity (mS/m)	Intake +70; Max 150	Receiving +50; Max 100
Faecal Coliforms (per 100mℓ)	1000	0
pH	5.5 - 9.5	5.5 - 7.5
Ammonia (ionised and un-ionised) as Nitrogen (mg/ℓ)	6	2
Chlorine as Free Chlorine (mg/ℓ)	0.25	0
Fluoride (mg/ℓ)	1	1
Nitrate/Nitrite as Nitrogen (mg/ℓ)	15	1.5
Orthophosphate as phosphorus (mg/ℓ)	10	1 (median); 1.5 (max)
Soap, oil or grease (mg/ℓ)	2.5	0
Suspended Solids (mg/ℓ)	25	10
Dissolved Arsenic (mg/ℓ)	0.02	0.01
Dissolved Cadmium (mg/ℓ)	0.005	0.001
Dissolved Chromium (VI) (mg/ℓ)	0.05	0.02
Dissolved Copper (mg/ℓ)	0.01	0.002
Dissolved Cyanide (mg/ℓ)	0.02	0.01
Dissolved Iron (mg/ℓ)	0.3	0.3
Dissolved Lead (mg/ℓ)	0.01	0.006
Dissolved Manganese (mg/ℓ)	0.1	0.1
Mercury and its compounds (mg/ℓ)	0.005	0.001
Dissolved Selenium (mg/ℓ)	0.02	0.02
Dissolved Zinc (mg/ℓ)	0.1	0.04
Boron (mg/ℓ)	0.1	0.5

The Green Drop Report also highlights that optimising wastewater treatment facilities, for example through energy recovery or energy efficient design (Ferry & Giljova, 2015), has the potential to reduce operational costs or even make the treatment facility financially self-sustainable. This possibility could serve as an incentive for municipalities to consider upgrading their plants while including new technologies for cost recovery (WISA, 2009). One risk of generating economic value from wastewater is that a trade-off may exist between meeting the requisite water quality and maximising economic return. Through this, the compliance of the effluent can become a secondary concern after profit. Verster, et al. (2013) recommended that the production of value should be housed within a separate unit operation to the polishing of final water quality to prevent unnecessary compromise of water quality standards. After the extraction of products, the cleaned water must still adhere to the legislated standards. The WWBR can be incorporated into existing WWTW or operated on the premises of the generator of an industrial wastewater. Some of the challenges are mitigated through the contracting out of plants to private companies, through a variety of Public-Private-Partnership (PPP) or Build-Operate-Transfer (BOT) models (see Section 3.1.3); however, clear cooperation with regulatory requirements is requisite.

Broader policy considerations

In 2015 the WRC published “South Africa’s Water Research, Development, and Innovation (RDI) Roadmap: 2015 – 2025” in collaboration with the Department of Science and Technology and the Department of Water and Sanitation. The RDI Roadmap (WRC SA, 2015) provides a structured framework for focus of the contributions of RDI activity in the implementation of national policy, strategy and planning in water resource management in South Africa. There are four key objectives:

- increase the availability of water
- improve the governance, planning and management of supply and delivery
- enable water and sanitation services to operate as a sustainable "business"
- increase the efficiency and productivity of water use

The water community as a whole was divided into four sectors, namely Agriculture, Industry, Public Sector and Environmental Protection; interventions in each sector were identified in order to provide lists of recommended actions that would satisfy each need. The needs and interventions were categorised into seven clusters, around each of which a ten year programme of action and investment was created. The seven clusters are:

Water supply

1.1 Increase ability to make use of more sources of water, including alternatives
1.2 Improve governance, planning and management of supply and delivery
1.3 Improve adequacy and performance of supply infrastructure
1.4 Run water as a financially sustainable "business" by improving operational performance

Water demand

1.5 Improve governance, planning, and management of demand and use
1.6 Reduce losses and increase efficiency of productive use
1.7 Improve performance of pricing, monitoring, billing, metering and collection

In the report it was highlighted that there exists a need for an increased use of treated effluent, increased use of wastewater, optimisation of the ability to manage water resources from source to source in an integrated way, improved financial sustainability of the water system, improved operational efficiencies, improved cooperative governance with respect to planning and management, optimisation of conjunctive use of water, reduction in volume of water use, improvement in efficiency of water use, increase in levels of water reuse, minimization of output to unrecoverable sources, reduction in volume and toxicity of pollution and minimisation of discharge of poor quality water. The interventions needed in the Agriculture, Industry, Public and Environmental Protection sectors are shown in Table 3-3.

Table 3-3: Clustered needs identified by the four sectors, and their summarised interventions (relevant excerpts from Table 19 from WRC Water RDI Roadmap (2015))

	Agriculture	**Industry**	**Public**	**Environment**
increase use of treated effluent	• implement efficient treatment management system • address public perception issue • catalyse linkages between those that discharge, producers and users – e.g. mines and farms	• improve regulatory frameworks • improve the quality of decision-making information	• improve regulatory frameworks • improve the quality of decision-making information • implement efficient treatment management system • address public perception issue	• investigate treated effluent to artificial recharge of ground water as potential conjunctive source • increase ability to optimise mix for context
increase use of wastewater	• fitness for use	• integrate better with agriculture and energy production	• improve regulatory frameworks to improve the quality of decision-making information • implement efficient treatment management system • address public perception issues	• improve performance and cost of purification •

	Agriculture	Industry	Public	Environment
optimise ability to manage water resources from source to source in an integrated way	• optimise ability to manage water resources from source to source in an integrated way			• refine accountability along the value chain • implement current legislation — WRN • NWA NWRS
improve financial sustainability of the water system			• ring fence — "run water as a business in municipality"	
improve operational efficiencies			• ring fence — "run water as a business in municipality"	
improve cooperative governance with respect to planning and management cross-sectoral	• enable water ordering • improve management of distribution	• systematically increase water independence • map footprint • develop reduction strategy	• provide alignment with NWRS2 in terms of policy instruments and regulations governing licence applications granted or denied	
optimise conjunctive use of water	• balance use of all sources in an integrated manner	• balance use of all sources in an integrated manner • minimise demand on supplier (eg municipality)	• increase the degree of alignment of the quality of water with use	
reduce volume of water use	• use water-saving crops and varieties	• minimise water use, application and losses in primary processes • avoid use of water (e.g. optimised or new no water processes) • recover and recycle condensate • reduce steam leakage • manage water pressure	• stimulate growth more economically (use of water) • highlight the importance of water and its scarcity to encourage consumers to reduce demand • improve dry solution systems and encourage acceptance	
improve efficiency of water use	• encourage uptake of land and water use practices • introduce irrigation systems and improve performance • optimise fertiliser use • increase effectiveness of knowledge transfer • increase levels of rehabilitation	• reduce water in ancillary processes • reduce demand for domestic water		
increase levels of water reuse	• reduce volume of wastewater, recover and recycle	• reduce volume of waste water • increase levels of recovery and recycling		

	Agriculture	Industry	Public	Environment
minimise output to unrecoverable sources	• reduce wastewater released to sewers	• reduce volume of wastewater released to sewers • recycle water streams for water and wastewater treatment		
reduce volume and toxicity of pollution	• reduce rainwater runoff	• minimise production of waste (e.g. cleaner production methods)	• increase number of WWTW with Green Drop certification to >95%	• Maximise natural water resource function (aquatic response)
minimise discharge of poor quality water		• minimise production of effluent (e.g. cleaner production methods)	• increase number of WWTW with Green Drop certification to >95%	•

Many of the interventions enumerated in Table 3-3 can be addressed by applying the WWBR concept to the sector in question. The philosophy of the WWBR includes:

- producing “zero waste” by valorising all elements of a “waste”-water stream
- maximising re-use and recycling of water through adequate extraction of contaminants
- production of energy from residual organic elements in the “waste”-water stream
- economically advantageous treatment of “waste”-water through valorisation
- integration of neighbouring industries in terms of “waste”-water valorisation and re-use
- 100% compliance in water released to environment

In fact, integration of the WWBR is probably the only way to achieve the overall technological remediation envisaged in the report.

3.1.3 The effect of innovative partnership models on the WWBR

The South African water infrastructure is subject to ageing effects associated with internal and external stresses, while inadequate maintenance and lack of capital renewal have resulted in further deterioration (Zhuwakinyu, 2012). The Department of Water and Sanitation (DWS) is struggling with serious capacity and funding problems; it is estimated that an investment of R 1.4-billion is required each year merely to maintain the current infrastructure. The DWS is also faced with a shortage of skilled personnel to implement and supervise maintenance. The problems are further compounded by fading institutional memory, as individuals retire, are retrenched or join the private sector (Water, 2012).

In 2014 the Minister of Water Affairs said the DWA needed an estimated R670-billion capital investment and infrastructure. Since only 45% percent of this was funded by the government budget, controversial options like public-private partnerships (PPP) are under consideration (Kings, 2014). Senior department personnel have frequently mentioned that public-private partnerships are the only way to bridge the funding gap. This is opposed by groups such as the Coalition Against Water Privatisation (CAWP, 2003) who maintain that privatisation policies in the 1990s led to a “dramatic increase in the price of water for the poor across South Africa”. However, many municipalities have faced the collapse of their water and sewerage works because of a lack of funding. Impact of inadequate water treatment on the health of communities has been suggested.

There is evidence that at least some of the PPP result in improved coverage and improved consistency in effluent control (DWS SA, 2015; Donnelly, 2015). An example of this model related to WWBRs is the Johannesburg Northern Works bioenergy project (Section 2.7.3) owned by Johannesburg Water. This was built by WEC Projects who still operate and maintain the energy plant (Franks, et al., n.d.). A similar arrangement is becoming increasingly common in industry. Here a specialist company is awarded the contract to design a water treatment facility, build it and then own-and-operate it for an agreed period. This model addresses the fact that the commissioning entity does not have to envisage expanding into an unfamiliar field or "non-core" business. Further advantages include guaranteed price and availability for any products which are used in house and a set fee for water treatment. An example of build-operate-transfer (BOT) is the agreement between Distell and Veolia for a plant producing biogas and reusable water. Situated in Stellenbosch, Western Cape, the facility is due to be commissioned in March 2016 and will be operated by Veolia for ten years (Bizcommunity, 2015; Western Cape Business News, 2015).

3.2 Evaluating Wastewater Feedstocks

In South Africa, based on a clear need for intensified water re-use, increasing need for alternative sources to supplement electricity supply and a shortfall of funding in the wastewater treatment arena, a major incentive exists for a new approach to wastewater. The approach outlined here sets out to realise the opportunity which wastewater presents as a feedstock for bioproducts and energy, generated through robust bioprocesses. This potential is holistically encompassed by the WWBR concept. To assess its potential, the wastewater streams available as feedstock are evaluated.

3.2.1 Detailing wastewater streams in South Africa

Stafford et al. (2013) and Burton et al. (2009) report on a study exploring technologies for recovering of energy from wastewaters in South Africa. Energy generation through the production of biomass, combustion and gasification, generation of biogas, production of bioethanol, heat recovery and use of microbial fuel cells was considered. A first order desktop analysis of South African wastewaters was used. It was found using data collected in 2007 that there was potential for recovery of 3,200 to 9,000 MWh of energy. This amounts to approximately 7% of South Africa's current electrical power supply. Formal and informal animal husbandry, fruit and beverage industries and domestic blackwater were identified as wastewaters with the greatest potential for energy recovery. Of the technologies reviewed, anaerobic digestion showed applicability to the widest range of feedstocks. Net energy generated, reduction in pollution and water reclamation were identified as the main benefits, with emission reduction, fertiliser production and secondary products as additional benefits.

Cloete, et al. (2010) surveyed the water use and effluent production of South African industrial, mining and electricity generation sector. The report stressed the incomplete data on effluent production which highlights a problem that we face in South Africa in terms of understanding the exact load of waste that is associated with industry. This is a great concern when it comes to managing the impact of effluent production on the environment.

The WRC has commissioned a series of reports attempting to detail the state of water and wastewater management in various industries. Known as the National Surveys or "NatSurv" documents, there are 15 reports in the series (WRC SA, 2015b); these reports are currently being updated, with some of the new reports due to be published in 2016.

The WWBR emphasises recovery and re-use of all elements of the wastewater, especially the carbon, nitrogen and phosphorus nutrients, with energy forming a secondary product. For WWBR purposes, the complete composition of the waste stream is desirable, including variability and complexity. This is more than typically reported. Logistical information is also important, including the volumes available, the distribution and the localities.

3.2.2 Categorising wastewater streams for WWBRs

Wastewaters need to be well-categorized to design the appropriate facilities. The approach taken here is to categorise wastewaters according to three factors; namely, volume, concentration, and complexity. Many of these wastewaters, particularly municipal wastewater, have huge flows, in the order of 50 mega litres every day (CoCT, 2010). These can be quite dilute, with the most common components in the order of milligrams per litre. In addition, wastewaters often exhibit a high level of complexity in terms of the number components, as well as the variability of components and concentrations. The different groupings of wastewaters each have their specific challenges and opportunities, which this project seeks to define and explore.

Volume

The volume classification must be considered from both an individual plant perspective and in terms of national production. Many wastewater sources, like abattoirs (Section 4.3.3) or municipal wastewater (Section 4.2), have relatively few large industrialised plants with large wastewater flows, with many small plants whose wastewater may be poorly managed, or not treated at all. While smaller plants have greater WWBR potential, at least while the concept is still in infancy, because of greater flexibility of operation and smaller volumes, which may translate to lower overall risk, smaller plants often are not regulated effectively. Further, the operations producing the wastewater may not have the funds necessary to invest in adequate waste treatment. Smaller plants may also require cooperation to create the necessary logistics to overcome the limitation of their small size and often scattered or inaccessible locations.

The wastewater treatment plants typically found can be classified as follows according to capacity (DWA SA, 2009; Van den Berg, 2009):

Type of plant	*Capacity*
Micro	<0.5 Mℓ/day
Small	0.5 - 2 Mℓ/day
Medium	2 -10 Mℓ/day
Large	10 -25 Mℓ/day
Macro	>25 Mℓ/day

Figure 3-3 considers the national potential for using wastewater as raw material, hence is focused on an indication of the total volume of wastewater produced per industry. The size and state of the wastewater treatment plants, or volumes of wastewater generated per site is relevant for considerations of economies of scale. This distribution is considered in Section 4.2.

Concentration

The concentration of dissolved solutes in the wastewater influences their beneficiation potential for products other than clean water. For the purpose of this report, high concentrations are above 10 g/ℓ-COD i.e. microbial bioconversions (including growth) can be supported without retained biomass (Nicolella, et al., 2000). Municipal wastewater, for the most part, uses water to transport waste. This necessarily dilutes the components, with a typical value of less than 1 g/ℓ-COD (Henze, et al., 2008), recognised as low concentration. Medium concentration lies between these two values. All wastewaters are likely to have varying concentration over time.

Figure 3-3 considers the potential for using different wastewaters as feedstock. COD values are the most commonly available, hence this metric has been used to compare concentrations. It is noted that this is a limitation for COD-poor, nutrient rich waters. In as far as possible values for all nutrients are reported in Section 4.3 and other relevant components are noted.

Complexity

Potentially, the most problematic characteristic of wastewater is the level of complexity. Some waters, like municipal wastewater, tend to be highly variable changing concentration and, in some instances, composition continuously. These waters are considered by municipal managers as 'receptacles', meaning that the compounds that make their way into the water are not controlled or predictable (Coetzee, 2012).

The complexity can be considered according to the predicted difficulty of treating the wastewater. This relates primarily to the number of different components present, but also to the presence of components that may require more treatment steps, or may interact with each other to prevent treatment, be it through chemical interaction, or through physical interaction. Physical interactions may range from the micro level, like foaming, in the case of fats and oils, to the macro level like the clogging potential of non-dissolved components like feathers or earbuds that may complicate treatment or increase maintenance costs.

The complexity of wastewater is classified according to the authors as:

Low	Composition does not change much, < 5 main components
Medium	Composition changes in predictable manner, 5 – 15 main components
High	Composition changes often and unpredictably, > 15 main components

Figure 3-3 rates the different categories of wastewater using this categorisation to indicate the anticipated difficulty of designing a WWBR which is able to deal with the components present.

3.2.3 A matrix representing wastewaters as feedstock

Figure 3-2 introduces a matrix for qualitative representation of feedstock qualities according to the variables suggested for categorisation in Section 3.2.2: volume, concentration and complexity.

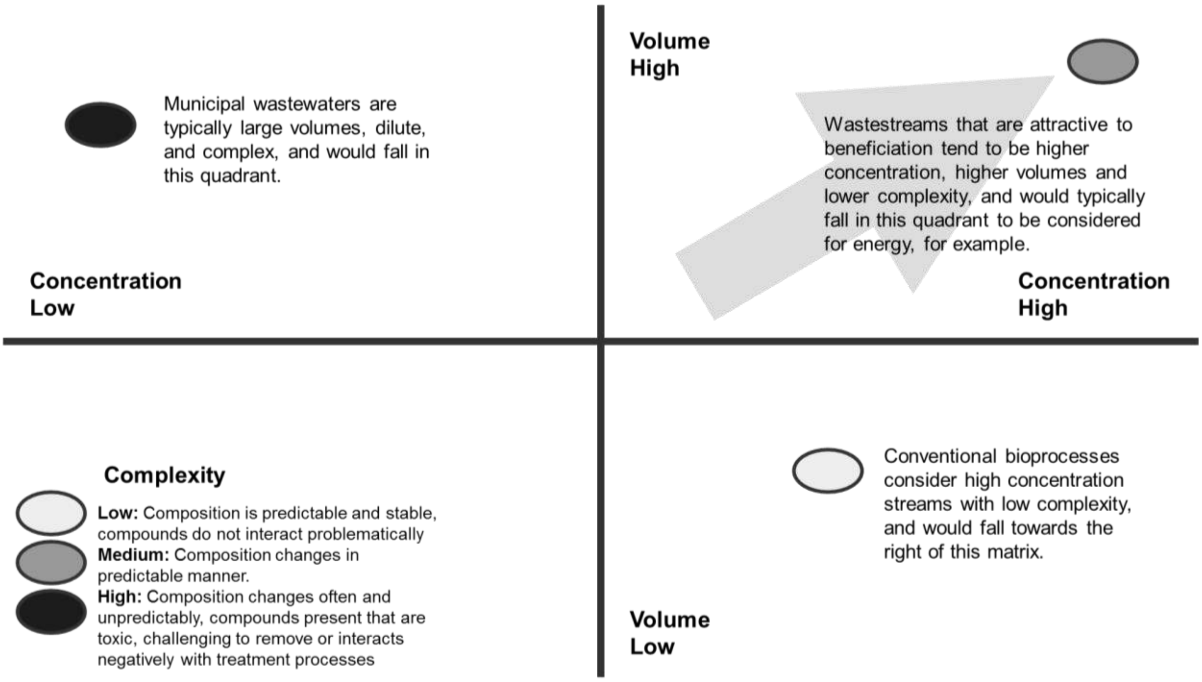


Figure 3-2: Matrix for qualitative representation of feedstock qualities of volume, concentration and complexity

In Figure 3-3 this matrix is used for an initial, subjective comparative categorisation of a broad spectrum of wastewaters in South Africa. For example:

Brewery wastewater is an example of low complexity. The wastewater is well characterised because the preceding process is well understood and controlled from a biological perspective. The components do not interact negatively with each other, and can be treated by few unit processes.

The textile industry is an example of medium complexity. The dye processes change between batches, and the presence of high salt and often of heavy metals complicates treatment. Both physico-chemical and biological treatments are required. The wastewaters are generally produced in a predictable manner, hence an established treatment chain can be applied to different sites with similar results.

While abattoirs have high concentration wastewaters, they contain complex biological molecules like blood and fats, while also having physical components like feathers and skin. While the wastewater produced by large, well-managed abattoirs may be more predictable, smaller plants may combine several waste streams, or use wastes for secondary products, which introduces additional complexity.

Municipal wastewaters are for the most part dilute. They contain a large variety of components, some of which may fall below detection limits. Backyard activities and industrial discharge changes the character of the wastewater across sites and associated treatment required and product potential. Further intermittent disposal aggravates variability.

Chapter 4 contains a quantitative presentation of data collected on different wastewater streams from various industries in South Africa. The later sections in Chapter 4 attempt a more in-depth analysis of the wastewaters in terms of the potential value and possible complications involved in using the wastewater from each industry as feedstock for WWBRs.

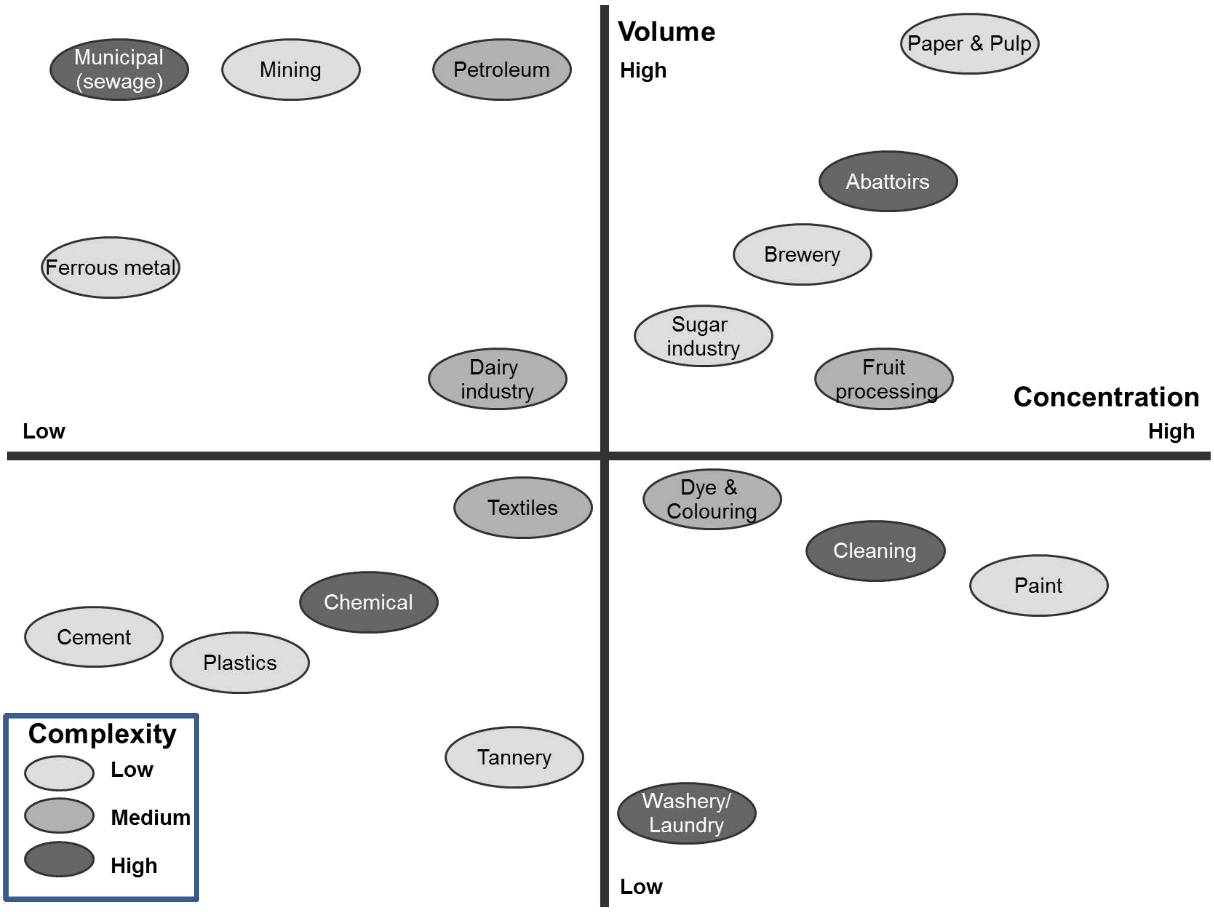


Figure 3-3: Matrix illustrating grouping of wastewater in terms of volume, concentration and complexity

3.3 Evaluating Biorefinery Products

In the evaluation of products for the WWBR, not only technical considerations are important. Aspects of the economics (Section 3.1.1) of the biorefinery are an integral part of decision making. In addition to the typical economic factors of operating cost vs potential income, additional considerations include potential for niche products which require careful market research. Further diversification of products can be economically stressful for the entity producing the wastewater. Policy plays a vital role here too. Firstly, there is the possible necessity for unprofitable products retained for the sake of producing compliant final effluent. Secondly there are limitations with respect to standards required for some products, especially anything associated with human consumption.

3.3.1 Categorising potential products

A wide range of possible products can be formed across the various units of the WWBR. For the purposes of this project the range products are categorised as follows:

- First level products: bioproducts derived from microbial bioreactors
- Second level products: biofuels and bioenergy
- Third level products: processed biomass (fertiliser, animal feed, fibre, compost)
- Fourth level products: Acceptable quality water: fit-for-use, or compliant for discharge

First level products: bioproducts

Bioproducts can further be classed into two categories. The first is those produced by breaking down complex molecules into basic building blocks that can then be used for chemical synthesis. Potential bioproducts in this first category include organic acids, industrial enzymes, volatile fatty acids (VFAs), pigments and alginate (Pandey, et al., 2010). The practical approach for the production of metabolites and enzymes can be related to different areas (paper deinking, paper recycling, agricultural residue utilisation, pesticide biodegradation, fodders, olive and seed oil residues, pruning, fuels, paper pulp production, etc.) and each of them require a different set of biotechnological conditions (Pandey, et al., 2010).

The second category includes function-based products that use complex macromolecules with minimal modification and purification. Examples of these are bioflocculants, biosurfactants or soil conditioners.

Thus the influent wastewater can be classified in terms of potential products:

- very complex, diffuse wastewater from which niche products can be produced, not related to the producers of the waste (e.g. domestic municipal wastewater)
- defined wastewater, but most feasible products fall outside of the market focus of the industry player producing the water (e.g. brewery waste)
- defined wastewater, with potential for conversion into a product used within the process or market focus of the industrial player

Second level products: bioenergy and biofuels

Since considerable amounts of energy are needed in a Wastewater Treatment Works (WWTW) to aerate the aerobic processes and to pump or transport the large volumes of water and biomass from one unit to the next, the greenhouse gas contribution to supply clean water is becoming environmentally unsustainable (Sheik, et al., 2014). Hence energy is a key factor in the WWBR and these “second level” products are important, as they are from almost any biologically based process, particularly one based on waste materials and typically form a key unit operation of the WWBR.

Potential bioenergy products include biogas, algal lipids for biodiesel and biomass for combustion, gasification or pyrolysis. Liquid alcoholic biofuels are only of interest for concentrated product streams. However, since bioenergy production is relatively common as a wastewater treatment strategy and thus well characterised (Bharathiraja, et al., 2014), this project does not investigate the conversion processes for this category of product in detail. These products are, however, considered in the process flowsheet analysis.

Third level products: processed biomass

In order to fulfil the “zero waste” and “zero harm” potential of the WWBR (Sections 2.2.1 and 2.3), the process needs to go beyond these two levels especially in the arena of the macrophyte and fungal processes. These two processes typically produce products such as fibre, hyphae, compost, and agricultural products, as well possible biomass-for-energy and bioproducts. Sludges for fertiliser and associated operations may also be handled in this category. The third level products are largely low-value and non-specialised bulk products, but nevertheless specific to the particulars of the process concerned. More research is needed in this area, since these products have not been addressed in traditional industrial bioprocesses whereas the WWBR concept necessitates them. This project does not explore the production of this level of product in detail, but does consider it in the process flowsheet analysis.

Fourth level product: Water as a product

Water is a key product of the wastewater biorefinery with its final use defining its required properties. This could be "fit for purpose" for recycle back to the industry forming it, "fit for purpose" for an alternative use geographically aligned e.g. irrigation water or cooling water, as potable water or for release into the environment.

3.3.2 Constraints of the WWBR on potential products

The WWBR is established to maximize productivity by ensuring that, not only is the wastewater treated to the necessary standard (yielding the outgoing water product), but that components removed from this wastewater are converted to the selected products which are of value economically, socially or environmentally.

Because of the particular challenges of using wastewater as feed, WWBRs are not suitable for all bioproducts. Due to the (generally) dilute nature of the wastewaters, highly energy intensive production processes are not appropriate. It is also beneficial to select culture conditions and products to contribute a selective advantage to the microbial community of interest (Mooij, et al., 2015; Winpenny, et al., 2010). WWBRs, therefore, are most suitable for products that fulfil a defined role in the microbial ecology allowing natural selection for the microorganism of choice (Verster, et al., 2013). Further, the desired product needs to be easily recoverable from the stream – either produced in a different phase, or be recoverable through a cost efficient process (Verster, et al., 2013).

Products may be favoured that play a role in the functioning of the treatment works or in the industry producing the wastewater. The production of materials required for plant operation from its own waste resources secures a stable market or use for the product and provides additional motivation for introduction to the concept of the WWBR. Moreover, this mitigates the need to expand the core business of the entity in question (Desrochers, 2001).

The regulations and the required level of purity depend on the product. Further, this is impacted by whether the product is for final use or is an intermediate feedstock to a subsequent process (Chen & Zhang, 2015; Ghatak, 2011). Generally speaking, the higher the required purity of a product, the higher the cost of DSP. The required DSP has a major influence on the appropriateness of product selection, as discussed in Section 3.4.5. In addition, the wastewater environment forms a health barrier, actual or imagined, to the direct use of products for human consumption or applications (Dolnicar, et al., 2011; Asano & Cotruvo, 2011).

These considerations will inform the selection of products to impact the economic feasibility of WWBR in South Africa.

3.3.3 Range of potential products for the WWBR

A wide range of potential products and product functionalities can be considered for the wastewater biorefinery. Their market potential is influenced by the source of the feedstock, with particular potential to supply the upstream process with necessary reagents or to supply products into regionally aligned industries. Routes for product recovery and purity requirements are central to the product selection.

Bioproducts

A selection of the wide range of high-level bioproducts that could potentially be produced from dilute wastewater streams, along with appropriate wastewater resources, is listed in Table 3-4, based on the review of Fava et al. (2012). It is noted that, in addition to these “Level 1” bioproducts, bio-energy products, fibre products, fertiliser and soil enhancer products as well as water as a product is expected to arise from each feedstock.

Table 3-4: Overview of types of waste streams, their properties and potential “level 1” bioproducts (adapted from (Fava, 2012))

Type of waste streams	Properties	Potential products
Vegetable and fruit processing by-products, waste and effluents Hydrolysate obtained from by-products/waste pre-treatment of vegetable/fruit waste	High in proteins, sugars and lipids along with particular aliphatic and aromatic compounds -	Fine chemicals: -Natural antioxidants -Antimicrobial agents -Vitamins -Bacterial exopolysaccharide (EPS) (e.g. xanthan gum) Macromolecules: -Cellulose -Starch -Lipids -Protein -Fibres -Plant enzymes -Pigments -Pharmaceuticals -Flavours -Vitamins -Organic acids -Biopolymers -Lubricants -Microbial enzymes
-Sugarcane and beet molasses -Dairy industry (cheese whey effluents) -Vegetable and fruit waste -Effluents of palm oil mill, olive oil mill, paper mill, pull mill -Hydrolysates of starch (corn, tapioca etc.) -Lignocellulosic waste (cellulose and hemicellulose)	High organic content	Microbial polymers (PHA)
Starch processing wastewater	High concentrations of readily biodegradable non-toxic organic compounds with relevant amounts of nitrogen and phosphorus	-Valorised through the recovery of starch and oligosaccharides -Biotechnological production of bio-pesticides, surfactants and amylases -Single cell protein (SCP) production from amylolytic microorganisms and non-amylolytic yeasts
Wastewater with surface contamination problems	High BOD concentrations	-Organic acids (lactic acid and butyric acid) -Alcohols (ethanol and butanol)
Woody (lignocellulosic) waste		-Furfural (from agricultural residues of sugarcane, corn and wheat) -Lignin (from paper pulp production)
Municipal solid waste		-Intermediary chemicals -Proteins -Enzymes

Products such as PHA are produced from monocultures such as *Cupriavidus necator* (previously known as *Ralstonia eutrophia* and *Alcaligenes eutrophus*) (Lopar, et al., 2014). Single cell protein (SCP) bacteria include *Cellulomonas*, *Alcaligenes*, and cyanobacteria such as *Spiruli.* Algae such as *Chlorella* and *Scenedesmus* can produce lipids that can be used for biofuels as well as a range of other products. Molds (*Trichoderma*, *Fusarium*, *Rhizopus*,) and yeast (*Candida* and *Saccharomyces*) may find application in production of organic acids, solvents and furfural (Nasseri, et al., 2011). Biosurfactants are produced from a range of microorganisms such as *Bacillus* sp., *Pseudomonas* sp., *Candida* sp. (Youssef, et al., 2004); the latter two require careful assessment as some members of these genera are pathogenic. Potential bioproducts from fungal action on biosolids include industrial enzymes and organic acids (Pandey, et al., 2010; Chen, 2013).

Biofuels and Bioenergy

The production of methane from organic material by anaerobic digestion is very well characterised (see Sections 2.7.2 and 2.7.3. for examples in South Africa). The technology is widespread and well utilised (Mata-Alvarez, et al., 2000). In this process, an organic carbon source is converted under anaerobic conditions by a mixed consortium of microorganisms via volatile fatty acids and hydrogen, into methane and carbon dioxide. This gas product can be used as an energy source. This technology has been applied to a wide range of organic carbon sources, usually waste streams, such as vinasse (Moraes, et al., 2015) sewage (Seghezzo, et al., 1998), slaughterhouse effluent (Salminen & Rintala, 2002), manure (Nasir, et al., 2012), food waste, beverage wastewaters, waste from the petrochemical industry and a host of other sources.

In addition to methane production from wastes, another possible energy product is hydrogen, produced either through dark fermentation similar to methane-producing anaerobic digestion, or through photofermentation (Hallenbeck & Benemann, 2004). Production of biohydrogen is a much less mature technology than production of biomethane, and has not been as widely applied. However, it does have potential to be an important source of hydrogen for hydrogen-based technologies such as fuel cells (Levin, et al., 2004).

A currently well-utilised biologically based energy carrier is bioethanol. This can be produced via the fermentation of organic compounds (mainly sugars) to ethanol, using yeasts (Gray, et al., 2006). While this technology is utilised on a large scale throughout the world, it is generally not used in wastewater treatment, but rather is based on more conventionally derived organics, such as sugars from sugarcane or maize, or concentrated water streams from agricultural industries, such as molasses. While potential exists for carbohydrate-rich wastewaters to be used in bioethanol production (Hamelinck, et al., 2005), this is not well suited to dilute waste streams owing to the energy requirements and costs of ethanol recovery.

The use of biodiesel is widespread, and often legislated for, particularly in Europe. The majority of biodiesel is produced from oil-bearing plant crops (Ma & Hanna, 1999), or waste oil. In the wastewater biorefinery, an oil producing microorganism could be used to convert waste materials into lipids for conversion into biodiesel. Examples include microalgae (Schenk, et al., 2008) and bacteria (Li, et al., 2008).

Moving into the wastewater space

Many of the potential products have not been demonstrated in the wastewater space. With the WWBR concept still in its infancy, specific research is needed for most of these, particularly studies well-integrated with the proposed feedstock. This is necessary for “Level 1” products, for “Level 2” energy products linked to wastewater feedstocks, but most of all for “Level 3” products associated with the solids and macrophyte bioreactors.

Considering the wide range of products at all levels possible even within the WWBR constraints, selection of products becomes a function of the particular feedstock stream and potential market. This project provides an example of the selection process in the case of first level bioproducts for the bacterial reactor in Chapter 5. Furthermore it considers the integration of multiple products through the flowsheets presented in Chapter 7.

3.4 Unit Operations and Biological Systems for Bioconversion Needs

In this section, the bacterial, algal, macrophyte and fungal reactors as suggested in Figure 2-1 are discussed. Consideration is given to separation units needed for DSP in a WWBR. In observations regarding design of units, the unique challenges of the WWBR must be kept in focus, including economic matters (Section 3.1.1) and policy considerations (Section 3.1.2). The general groundwork for technology selection must be laid, taking account of the opportunities and challenges presented by available feedstocks (Section 3.2) together with a realistic assessment of product options (Section 3.3).

3.4.1 Bacterial bioreactor

In traditional WWTW, a bioreactor cultivating bacteria, yeast or submerged culture fungi is mainly used when there are complex streams with high COD entering the process, or with limited land availability. Since bioreactors for bacteria and unicellular yeast have the most compact footprint and can be operated in the most effective configuration, they are attractive. For simplicity these will be referred to as “bacterial bioreactors” in this report. Typically, a single product bacterial bioreactor also requires skilled operators and may therefore not be suitable for low-maintenance sites.

The critical factors for bacterial bioreactors processing dilute feed streams in the WWTW are biomass retention or the recycle of biomass to achieve higher effective biomass concentration. Recycle of biomass after product recovery may not be feasible depending on the product produced. Further, recycle of biomass demands its flocculation and ready settling as high energy separators are not practical.

Numerous well characterised reactor conformations function with bacterial catalysts, with “off-the-shelf” systems available. For the particular needs of the WWBR, these reactors must be assessed and the most suitable chosen for further evaluation. This process is captured in Chapter 6 and the start of the necessary follow-up experimental evaluation is presented in Appendix E. There may be key modifications needed in order to tailor the design to microbial selection and concomitant product production.

The bacterial bioreactor can produce a high-level value-added product. The bacterial reactor optimised for productivity does not result in depletion of all nutrients. This reactor may provide high quality carbon substrate in the form of a pre-digested feed rich in volatile fatty acids (VFAs) as well as residual combined nitrogen and phosphates for use in an algal reactor. Alternatively, the VFA component may be depleted with concomitant energy production in, for example, an anaerobic digester with the C-depleted, N- and P-containing stream proceeding to an autotrophic algal reactor.

3.4.2 Algal bioreactor

While all algae can grow photoautotrophically, a number of species are mixotrophic, being able to grow on organic carbon or CO_2. These algal cultures may grow more rapidly under heterotrophic or mixotrophic conditions than under autotrophic conditions by a factor 3 to 4 (Kim, et al., 2013), but the potential for contamination also increases under richer nutrient conditions. These mixotrophic algal systems may be useful to scavenge residual organic carbon while simultaneously carrying out nitrogen and phosphorous removal. Algal growth rate and rate of N and P depletion influences the operational costs in the context of wastewater treatment (Kim, et al., 2013). If the algae need to be selected for a dominant (group of) species, factors like the nitrogen and/or phosphate content need to be controlled. To reduce bacterial contamination, the carbon content of the feed stream to the algal bioreactor can be limited through optimisation of the bacterial reactor. Alternatively, if there is a high carbonaceous COD, pre-digestion to produce biogas and thereby remove COD should be considered before entering the algal bioreactor. CO_2 addition has been shown to enhance algal productivity as well as reducing the loss of nitrogen through ammonia volatilisation (Park, et al., 2011). At a WWBR facility the CO_2 produced in the bacterial reactors or anaerobic digester could be re-used at the algal reactor to enhance productivity with a low increase in operating cost.

Literature on the use of algal reactors in wastewater treatment has focused on high rate algal ponds (HRAPs) or adaptations of these. HRAPs are raceway ponds with depth of 0.2-1 m, mixed through by a paddlewheel. HRAPs may be part of an Advanced Pond System including primary bacterial treatment through anaerobic digestion, hence precedent for the application of HRAPs in the wastewater biorefinery context is available (Park, et al., 2011; Rose, et al., 2007). Total COD removal in the order of 31 – 53% in HRAPs combined with Advanced Settling Ponds (ASP) has been reported (Rose, et al., 2007).

Alternatively, wastewater effluents high in N and P are increasingly being sought as nutrient sources for algal production systems for biodiesel, carbon capture, feed supplements and fertilisers (Louw, et al., 2016). The algal bioreactor or ponding systems is mainly used for low COD, high N, P waste streams. In algal biofuel production, N and P nutrient recycling through for example recycling the algal residue after oil recovery or the anaerobic digestate after biogas production, back into the system is desirable to maximise bioenergy production. In a WWBR, it is necessary to have a secondary algal product, such as a fertiliser or soil conditioner, to remove N and P from the system as this is defined as one of the roles of the algal reactor.

Algal product markets include use for bioenergy either on-site or externally, for animal and aquaculture feed additives, algal dyes and soil conditioners and fertilisers (Griffiths, et al., 2016). Nutraceuticals and food products can only be produced when the waste stream is a suitable precursor for food-based products (e.g. waste stream from a food producing facility). An algal ponding system is not suitable when there are space constraints; HRAPs require 50 times greater land area than activated sludge systems (Peccia, et al., 2013). IBhayi Brewery (SA Breweries, Port Elizabeth) experimented with the interfacing of the anaerobic digester and algal and hydroponic ponding systems, demonstrating constraints for urban breweries (Section 2.7.2). Potential exists to expand algal systems to higher intensity closed photobioreactor systems with higher value products for smaller volume wastes.

3.4.3 Macrophyte bioreactor

The macrophyte reactor is positioned as a polishing step in the WWBR, not as the main focus. It is basically a constructed wetland, which means it is characterised by a large land requirement. However, the macrophyte reactor does not equate to a treatment wetland, where a definition is "wastewater treatment technologies that feature passive biological treatment mechanisms with minimum mechanical energy inputs" (WEF FD-16, 2010). The macrophyte bioreactor is designed and constructed with focus for effective product removal (Fosso-Kankeu & Mulaba-Bafubiandi, 2014) as well as compliant exiting water, which is also seen as a product of value. This requires higher maintenance and greater mechanical input to ensure higher productivity. It may approach an agricultural production system.

There are different types of macrophyte bioreactors. The classification is based on hydrology and type of macrophyte growth. There are three possible types of hydrology: open water-surface, horizontal subsurface flow and vertical subsurface flow. Macrophyte growth is usually classified as emergent, submerged, free-floating or floating-leaved. These two parameters are used in various combinations to achieve different results (Vymazal, 2014).

The treatment efficiencies and bioproduction potential of the different macrophyte bioreactor types all lie in the same order of magnitude. The greatest challenge with the macrophyte bioreactor is efficient harvesting and maintenance. To this end, the floating wetland system shows the greatest potential. The matrix that supports the root growth also serves as baffles and as attachment sites for bacterial growth, increasing the effective surface area and active biomass for increased treatment efficiency (WEF FD-16, 2010). The advantage of the floating matrix, provided the holding tank or pond does not dry out and allow the roots to embed on the pond floor, is that the sludge removal potential is greatly enhanced, as there are fewer obstructions like roots. The floating matrix can be removed entirely and processed externally, while the pond is drained, without excessive harm to the macrophytes, increasing the ease of harvesting of the macrophytic products. The macrophyte bioreactor system is more accessible if the ponds are designed in channels, but this also introduces the greatest weakness of the system: the large capital cost in channel construction as compared to conventional wetland pond systems.

Floating Treatment Wetlands (FTWs) form another type of macrophyte bioreactor, first developed about 20 years ago in Japan (Dodkins & Mendzil, 2014a). There are several FTW in operation, using a variety of methods to bind the matrix and allow it to float, including bamboo, empty plastic bottles, etc. A commercial design, marketed by Floating Islands International (Floating Island International, 2016), makes use of post-consumer polymer fibres (Reinsel, n.d.). It is possible under some circumstances for FTWs to be more efficient than conventional constructed wetlands (Dodkins & Mendzil, 2014a).

3.4.4 Solids bioreactor

A major objective for WWBR is the decoupling of solid and liquid residence time; it is expected that a large amount of wet solids be separated from the incoming liquid stream early in the process, with additional solids separated out in each reactor train.

The solids bioreactor specified for use in a WWBR uses solid state fermentation. Solid-state (substrate) fermentation (SSF) is generally defined as the growth of micro-organisms on (moist) solid material in the absence or near absence of free water (Pandey, et al., 2010).

In mixed solid-state fermentation, the microorganisms are various and not fully characterised. Consequently, microbial community characteristics may be used to realise and control the culture conditions and metabolic processes. Aerobic mixed solid-state fermentation can be divided into co-culture and mixed-culture processes (Pandey, et al., 2010). Co-culture is a process in which a small number of selected and known micro-organisms co-exist and drive the process in a concerted manner. Mixed-culture cultivation uses a variety of known or partially known microorganisms grown under conditions not requiring sterilisation such that the microbial community is dynamic, altering to meet the conditions within the system through an ecological approach.

There are several designs of SSF reactors, namely:

1. Static beds without forced aeration (tray bioreactors, Koji type)
2. Static beds with forced aeration (packed beds)
3. Pulsed mixing without forced aeration (discontinuously rotating drum)
4. Pulsed mixing with forced aeration (intermittently stirred beds)
5. Continuously mixed without forced aeration (continuously rotating drums)
6. Continuously mixed with forced aeration - (a) the rocking drum bioreactor, b) the gas-solid fluidised bed, c) the continuously stirred aerated bed
7. Other designs such as the patented periodic air-forced pressure oscillation and the immersion bioreactor, based on intermittent immersion in a liquid medium (Couto, et al., 2002; Couto & Sanromán, 2006).

The scale up of SSF reactors is a bottleneck in the application of SSF. In the bioreactor reaction system, activity is controlled by three major sub-processes: thermodynamics, biokinetics and heat and mass transfer. The transfer process (mainly mass and heat transfer) is the most important and is a core issue for scale-up (Mitchell, et al., 2010).

It is still uncertain whether the “non-biodegradable organics” in wastewater are biodegradable under the right conditions. Fungal metabolism is different and complementary to bacterial metabolism, and has been shown to degrade recalcitrant chemicals (Chen, et al., 2015; Gouma, et al., 2014). One hypothesis is that a dedicated solid substrate bioreactor orientated towards non-biodegradable organics could improve the characteristics of this fraction, and possibly produce valuable products. Existing research on solid substrate fermentation on municipal sludges is scarce; improved research in this field is strongly recommended.

3.4.5 Downstream processing in the WWBR

Downstream processing and fractional separations are generally well developed for the biotechnology and chemical engineering industries. In the WWBR context, a major cost component is expected to be the product recovery costs. The WWBR needs to be designed with product recovery in mind. This in turn needs integration with the appropriate reactor design (Chapter 6). Conventionally reactor design is focused on maximisation of productivity, and seldom cognisant of a need for reduction in downstream processing costs.

There is limited work available on downstream processing specifically for dilute streams. Approaches used in wastewater treatment as well as in mining of specifically low-grade ores give some indication of the requirements. While the processes listed in Tchobanoglous, et al. (2003) are focused on constituent removal, they are already adapted for the wastewater context. The processes need to be adapted to focus on product recovery as well. Unit operations and processes used to remove constituents found in wastewater (adapted from Tchobanoglous, et al. (2003)) include:

- Suspended solids: screening, grit removal, sedimentation, high-rate clarification, flotation, chemical precipitation, deep filtration, surface filtration,
- Biodegradable organics: membrane filtration
- Nitrogen removal: air stripping, ion exchange
- Pathogen removal: chlorine compounds, chlorine dioxide, ozone; ultraviolet radiation
- Colloidal and dissolved solids: membranes, carbon adsorption, ion exchange
- Volatile organic compounds: air stripping, carbon adsorption, advanced oxidation
- Odours: chemical scrubbers, carbon adsorption, biofilters, compost filters

Separation and purification processes play a critical role in biorefineries and their optimal selection, design and operation to maximise product yields and improve overall process efficiency. Separations and purifications are necessary for upstream processes as well as in maximising and improving product recovery in downstream processes (Ramaswamy, et al., 2013).

The first consideration is to increase the product concentration and reduce the total volume by orders of magnitude i.e. to recover the product. If the product is biomass-associated and the biomass can be recovered in high concentration, the biomass can be processed through conventional biotechnological processes. An overview of biomass conversion processes and separation and purification technologies in biomass biorefineries (adapted from Ramaswamy, et al. (2013)), and their suitability to the WWBR is given below:

- Distillation: Large energy requirement, is not suitable to the bulk stream, may be suitable after initial processing to reduce the total volume.
- Liquid-liquid extraction (LLE): Large solvent use, may reduce the quality of the water. Not recommended on bulk stream, can be feasible for final processing.
- Supercritical fluid extraction: Large energy requirement, may only be suitable for final processing
- Adsorption: Complex streams may foul the adsorption media, chemically or physically. It may be difficult to find and optimise a suitable adsorption method.
- Ion exchange chromatography: Complex streams may foul the exchange media, chemically or physically. It may be difficult to find and optimise a suitable chromatographic method.
- Simulated moving-bed technology for biorefinery applications: May be too technologically complex and difficult to maintain or operate optimally.
- Microfiltration, ultrafiltration and diafiltration: Maintenance may be expensive, need upstream processes to reduce fouling potential.
- Reactive absorption: The complex nature of the wastewater may foul/destroy the absorption surfaces.

Other processes which should be evaluated according to Ramaswamy, et al. (2013) include:

- Nanofiltration

- Membrane pervaporation
- Membrane distillation
- Filtration-based separations in the biorefinery
- Solid-liquid extraction in biorefinery
- Membrane bioreactors for biofuel production
- Extraction-fermentation hybrid (extractive fermentation)
- Reactive distillation for the biorefinery
- Pressure swing adsorption

The challenge of DSP for WWBR process streams is a complex combination of the wastewater and bioprocess situations with some unique additional issues predicated on the particular feedstock. Thus, for example, waste streams with a high complexity can present particular difficulties in terms of physical interference in filters and pumps from elements of the waste, such as feathers in poultry abattoir waste or cotton buds in municipal waste. Another example would be the difficulty of flow for high viscosity waste “waters” such as vinasse. A particular consideration is toxic compounds like heavy metals that bind, for example, to chromatographic columns irreversibly.

3.4.6 Other process considerations for the WWBR

Wastewater is usually a receptacle, meaning the composition and flowrates cannot be controlled, including seasonal variability and changing characteristics over time. This can to some extent be addressed through holding tanks and pre-treatment. Ideally, mitigation of this challenge will happen through partnerships and adequate communication with the industries creating the wastewater.

Anaerobic bioreactors have not been directly considered in this report, as this limits the production to the lower value biofuel and bioenergy products. Moreover, energy from wastewater is already in relatively common use (Section 2.7) and the technology is well developed. However, this is acknowledged to be an important component of wastewater treatment/valorisation, and will be suitable as a pre- or post-treatment step in the context of the WWBR.

Fundamental thermodynamic laws mean that the diffuse nature typical of wastewater remains a challenge that needs consideration. WWBR will not work in all cases, and frequently the compromise for producing product is one of time. Products take longer to be produced.

3.5 Considerations for Integration into the WWBR

In moving toward the WWBR, the considerations outlined in this chapter must be explored further with awareness of the impact of the interrelationship of unit operations. The principles of industrial ecology dictate that the components of an industrial system are optimised to function as an integrated system, rather than maximised with respect to individual unit productivities (Graedel & Allenby, 2010). These principles are followed with, and within, the WWBR as well. The integration and optimisation of the WWBR into the wider industrial ecosystem has two main aspects from an operational perspective: finding complementary streams to supplement the main wastewater stream for optimal operation and commercial production, and optimising the supporting units to optimise the unit producing the commercially relevant product.

3.5.1 Supplementary raw materials

The successful integration of processes into a WWBR is largely dependent on the availability of appropriate biomass feedstock. Special attention needs to be given to potential seasonality of wastes such as agricultural and food processing by-products. Feedstocks may need to be stored and managed to ensure efficient use of the equipment and controlled and stable deliverables to the market (Fava, 2012). Furthermore, multiple feedstocks may need to be processed on the same plant to enable all-year processing, owing to its major impact on economics.

While conventional wastewater treatment attempts to limit the use of supplementary substrates to reduce cost of treatment, it is well established practice to add reagents to obtain better treatment performance in biotechnology. In the treatment and resource recovery of mine wastewater, sewage sludge is used as electron donor in the BioSure™ process for treatment of acid mine drainage through biological sulphate reduction. Similarly, excess VFAs (Van Hille, et al., 2015), ethanol and molasses have been used (Buisman, 1995). Crude glycerol, a waste product from biodiesel production has been investigated at length as a supplementary, cheap substrate for bioprocesses (Dobson, et al., 2011). A typical supplementary substrate is methanol (Henze, et al., 2008). The methanol contaminants, methoxide and high pH limits its use for some applications, but it has promise for wastewater addition (Pagliaro & Rossi, 2008) in which these inhibitory components are diluted.

With the growth of the bioeconomy, more biologically suitable waste streams from industrial bioprocesses may become available. While this is currently viewed as a potential limitation of the bioeconomy in terms of efficient resource use, the biological nature of the wastes may contribute to a well-functioning bio-industrial ecosystem (Prasad, 2015).

While the most common additive to wastewater streams is with regards to the electron donor (or an organic source), the wastewater biorefinery may need more sophisticated additives (Ferry & Giljova, 2015; Olguín, 2012), possibly nutrient streams for a more appropriate C:N:P ratio, as would be required for intensive bioproduct formation in bacterial reactors or algal production, or addition of vitamins, co-factors, or specialised substrates like amino acids for biopolymer production. From a cost and complexity perspective, the need for such additives should be minimised, but from a WWBR perspective this should nonetheless be considered as an option. In particular, the sourcing of complex waste streams rich in these supplements may be appropriate.

It is tempting to design an eco-industrial park to tailor the waste streams' effective use. From an industrial ecology perspective, however, designing co-placement of industries to provide complementary waste streams (greenfield development) have proven to be less successful than shaping processes (and products) in response to the existing streams and potential synergies (brownfield development) (Desrochers & Sautet, 2008).

3.5.2 Optimising for the main economic unit

Overall process optimisation is a key factor with focus on both the economic product and the water product. The range of unit operations, type of microorganisms, catalysts, conversion efficiency, yield and productivity, amongst others, significantly affect the overall sustainability and economic aspects of a WWBR. The WWBR has the dual objective of water treatment and bioproduction. While the WWBR differs on a case by case basis, it is likely that one unit will be more intensively optimised for bioproduction. The other unit(s) will either contribute products to improve the operation of the economically relevant unit or provide secondary products. In either case, these supplementary units will have water treatment as their main optimisation criterion.

This approach already exists in bioproduction. For example the bacterial production of volatile fatty acids (VFA) to improve algal biomass growth where the algal unit is the main focus (Rose, et al., 2007), or the use of anaerobic digestion to provide VFAs for biological sulphate reduction – sulphide oxidation to yield a sulphur product (van Hille et al. 2015). In the WWTW, a similar interactive effect is obtained at the Johannesburg Water Northern Works detailed in Section 2.7.3. (Franks, et al., n.d.) where the heat energy from the CHP units is used to optimise biogas production by preheating the sludge entering the AD units. This has the knock-on effect of improving the quality (and therefore value) of the digestate. Several of the biogas production units installed by municipalities in the Western Cape (see Table 2-18) (Ferry & Giljova, 2015) combine waste streams (most frequently municipal solid waste and sewage) in order to optimise the feedstock for the AD units.

Within the WWBR, numerous possible synergies exist between products and processes. AD can be used as pre-treatment to hydrolyse complex molecules. The macrophyte biomass, in particular the fibres, could be used for support of fungal growth in the solid substrate reactor. Algal and macrophyte reactors can be used to scavenge N and P. Of course, energy (heat and/or electricity) can be used to fuel the WWBR. It is imperative that the dual focus of economic and environmental perspectives is always maintained.

3.5.3 The wider perspective

From a wider social perspective, several factors need to be in place for WWBR to be a viable option. These include a policy of treating wastewaters to recover nutrients simultaneously with producing clean water for reuse, as well as public approval of products-from-waste together with reuse of water.

Recognition within the industrial sector of the environmental need for and economic possibilities of the WWBR is beneficial. For wastewater to be used in a WWBR, the volume, composition and complexity must be known, as must its geographic location and seasonality. Due to South Africa's aging infrastructure and lack of investment in the water and wastewater sector, public-private partnerships to boost innovation in this space hold potential. Relationships between new and old technologies can be created with a variety of role-players in this field. Particularly, the re-definition of facilities to derive economic benefit while meeting water quality standards is expected to encourage investment. Evaluation of the potential products obtained from wastewater, their position in the value chain and their relevance within the South African economy is essential.

The process considerations of a WWBR in terms of the social and ecological niche, unit operations and downstream processing must have a synergetic relationship for considering the integration into a WWBR. According to the Brazilian Bioethanol Science and Technology Laboratory (CTBE, n.d.), integrated evaluations of biorefineries should include: optimisation of concepts and processes, consideration of the different facets of sustainability and analysis of the status of developing technologies. The integration of these factors is demonstrated in Figure 3-4.

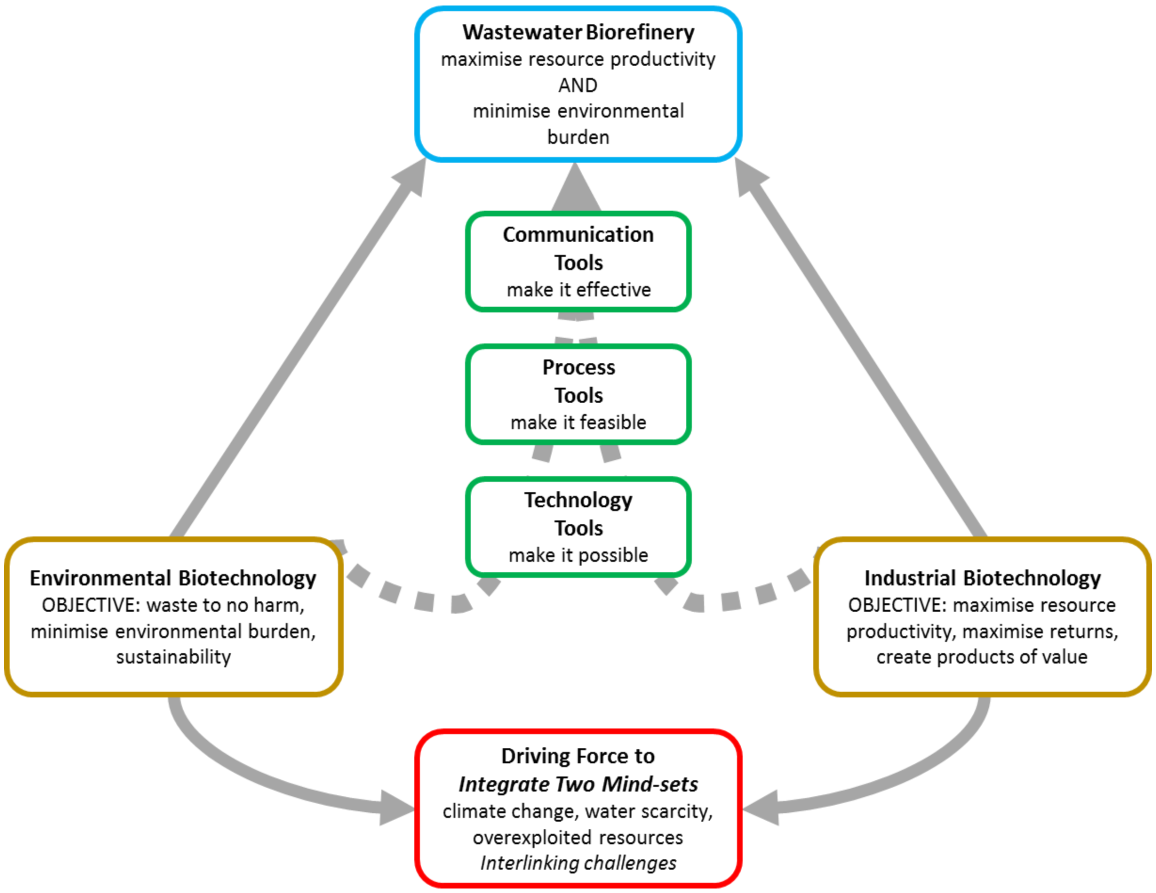


Figure 3-4: Integration of industrial and environmental technologies for emerging WWBRs

4 REVIEW OF POTENTIAL WASTEWATER BIOREFINERY FEEDSTOCK: SOUTH AFRICAN WASTEWATER STREAMS

In this chapter, wastewater burdens and resources within South Africa are reviewed. Both wastewaters from industrial sources and municipal wastewater are considered. Multiple sources were used to compile the data presented and these are noted. Where data could not be found, estimations were used and noted. Where data was unavailable, even for estimations, the stream is listed without data. Supplementary data, sources and calculations are presented in Appendix C.

The source data exists in a variety of forms. Mostly, these have been given in terms which translate easily to environmental impact rather than measures of suitability for valorisation. In this section, the data available are used to determine annual volumes and regional distribution. The carbon, nitrogen and phosphorus composition of these wastewater types allow assessment of suitability for use as feedstock for wastewater biorefineries. Where possible, known complexities have been mentioned. A number of wastewaters have been examined in more detail, presented in Section 4.2.

This chapter, supported by the accompanying appendix, is intended to inform the consideration of the potential of wastewater in South Africa as a source of valuable nutrients for production of bio-based products by drawing on specific wastewater examples. This consideration should be combined with concern for the potential within the wastewaters for remediation to clean water which complies with legislation. The data presented here is therefore seen as offering a first order estimation of WWBR potential.

4.1 Reviewing Previous Studies on Wastewater in South Africa

The major sources of information used for this report are Burton et al. (2009), Cloete et al. (2010) and several other WRC reports, including the "NatSurv" reports (WRC SA, 2015b), together with personal communications with staff at the WRC. Other information is obtained from a selection of journal articles, South African institutions and South African academic theses.

The feasibility study compiled by Burton et al. (2009) centred on the potential for energy from wastewater. From the analyses conducted, the volumes and COD content of wastewaters from several industries and municipal WWTWs was provided. Cloete et al. (2010) created a first order inventory of water use and effluent production by the South African industrial, mining and electricity generation sectors. Unfortunately the data used to complete these reports were not all recent at the time of publication and therefore much of it is now outdated.

The NatSurv reports published by the WRC are summarised in Table 4-1; it can be seen that these data are largely outdated. A new cycle of NatSurv reports are currently in preparation or under review for publication. Information from the WRC obtained through correspondence suggests that the following updated NatSurv reports will be published: Metal Finishing Industry in February 2016, Dairy Industry in March 2016, Brewery Industry in May 2016, Steel Industry and Pulp and Paper Industry in September 2016. These were not available sufficiently early for inclusion in this report. In 2017, reports for Laundry, Edible Oil and Abattoir/Red Meat are expected.

The Green Drop initiative of the Department of Water and Sanitation has reported the performance of municipal, public and private WWTWs. It is an incentive-based model to identify, reward and rectify non-compliance in the water sector. It supplies information pertaining to the volumes of WW entering the WWTWs nationally and gives an indication of the sizes of these WWTWs (DWS SA, 2014).

Table 4-1: NatSurv reports from the WRC

Report	Report number	Year of publication	Title	Revised report number	Year of new report publication
NatSurv 1	TT 29/87	1986	Water and wastewater management in the malt brewing industry	-	May 2016
NatSurv 2	TT 34/87	1987	Water and wastewater management in the metal finishing industry	TT 644/15	January 2016
NatSurv 3	TT 35/87	1987	Water and wastewater management in the soft drink industry	TT 640/15	October 2015
NatSurv 4	TT38/89	1989	Water and wastewater management in the dairy industry		March 2016
NatSurv 5	TT 39/89	1989	Water and wastewater management in the sorghum malt and beer industry		May 2016
NatSurv 6	TT 40/89	1989	Water and wastewater management in the edible oil industry		2017
NatSurv 7	TT 41/89	1989	Water and wastewater management in the red meat industry		2017
NatSurv 8	TT42/89	1989	Water and wastewater management in the laundry industry		2017
NatSurv 9	TT 43/89	1989	Water and wastewater management in the poultry industry		2017
NatSurv 10	TT 44/90	1989	Water and wastewater management in the tanning and leather finishing industry		
NatSurv 11	TT 47/90	1990	Water and wastewater management in the sugar industry		
NatSurv 12	TT 49/90	1990	Water and wastewater management in the paper and pulp industry		September 2016
NatSurv 13	TT 50/90	1993	Water and wastewater management in the textile industry		
NatSurv 14	TT 51/90	1993	Water and wastewater management in the wine industry		
NatSurv 15	TT 180/05	2005	Water and wastewater management in the oil refining and re-refining industry		
NatSurv 16	TT 240/05	2005	Water and wastewater management in the power generating industry		

4.1.1 Compiling data on wastewater in South Africa

As a quick reference for readers, the relationships found in the literature to calculate the approximate amount of wastewater that was generated for several industries in South Africa are shown in Table 4-2. This information was used together with other information mentioned to calculate effluent volumes when it was not readily available.

Table 4-2: Examples of relationships calculating the amount of wastewater for different industries

Industry	Relationship	Reference
Brewing	6 m^3 water consumed/m^3 beer produced 4-8 ℓ water consumed/ℓ beer produced 3 – 5 ℓ wastewater generated/ℓ beer produced	(CSIR SA, 2010) (IWA, 2009)
Dairy	15 -51 ℓ wastewater /cow/ day Pasteurised milk: wastewater 85-90% of water intake Butter and cheese: wastewater 90-95% of water intake Milk powder and condensed milk: wastewater >100% of water intake	(Du Preez, 2010) (Steffan, Robertson and Kirsten Inc, 1989a)
Fishery	11 m^3 H_2O consumed/t fish processed Large salmon processing: 3.12 ℓ wastewater generated/ kg fish Small salmon processing: 9.90 ℓ/kg fish Canning of tuna and sardines: 14 – 22 ℓ/kg	(Quiroz, et al., 2013) (Chowdhury, et al., 2010)
Petroleum	0.1 – 5 m^3 wastewater generated/t crude oil	(Burton, et al., 2009)
Poultry abattoir	15 – 20 ℓ/bird influent wastewater 80-85% of water intake	(CSIR SA, 2010) (Bremner & Johnston, 1996)
Pulp and paper	33 – 136 m^3/t integrated plant 1 – 49 m^3/t pulp and paper products 150 t wastewater/ 1 t paper produced wastewater 85% of water intake	(CSIR SA, 2010) (Hagelqvist, 2013) (Mac Donald, 2004)
Red meat abattoir	m^3/wrcu (1) 818 ℓ wastewater per slaughter unit (2) wastewater 85% of water intake	(CSIR SA, 2010) (Neethling, 2014) (DWA SA, 2001)
Soft drink	2.7 m^3 water/m^3 soft drink influent Carbonated drinks: 1.6 ℓ wastewater/ℓ product Fruit juice: 2.2 ℓ/ℓ product	(CSIR SA, 2010) (Pollution Research Group, 2015)
Sugar	30 – 100 m^3 water consumed/100 t of cane processed (average of 60 m^3/100 t) 18 m^3 wastewater generated/100 t of sugarcane processed 0.75 – 1.5 Mℓ wastewater/ day – is approximately 30% of the water intake	(Steffen, Robertson and Kirsten Inc, 1990) (CSIR SA, 2010) 30 -100 m^3/ t cane processed
Textiles	Specific water intake: 95 – 400 ℓ/kg 70-80% of H_2O consumed is expelled as wastewater	(Steffen, Robertson and Kirsten Inc, 1993)
Wine making	700 – 3800 ℓ/t of grapes 1.8 – 6.2 ℓ/ℓ absolute alcohol – spirit distillation 1-4 ℓ wastewater/ ℓ wine	(CSIR SA, 2010) (Welz, et al., 2015))

(1) wrcu – the number of non-bovine species equivalent to one bovine cattle unit in terms of water usage during processing.. One bovine cattle is equivalent to 2 calves or 6 sheep or 6 goats or 2.5 pigs (NatSurv 4, 1989)

(2) The waste per slaughter unit according to Neethling (2014) is 818 L of effluent and 31 kg of solid waste. A slaughter unit is based on weight and may be equivalent to 1 cow, bull or ox; 2 calves; 1 horse; 6 sheep or goats; 4 porkers; 2 baconers or 1 sausage pig

The data collected on wastewaters offered significant challenges in developing a complete and consistent dataset. Data available was presented in different units, collected by different methods and, in many cases, a range of parameters were not measured. In this study, first-order approximations were used to provide estimates where data could not be sourced. The aim is to provide a uniform approach to present relevant data (Section 3.2.2), including:

- annual volumes produced with site specific data, in terms of volumes per day, indicating the distribution of available streams
- concentrations of C, N and P present in the wastewater indicating potential for recovery
- indicators of handling issues: pH and conductivity
- noted complexities: solids, toxic compounds, metals, complex organics and other valuable components

One potential approach is to find specific effluent volume (SEV) produced per unit, as well as the annual production (either per site or per region). These can be used to estimate expected annual production, while also being used to analyse discrepancies in data. Such discrepancies may result from outdated data or be due to cleaning and other periodic, non-unit specific operations. Where large discrepancy exists, the source of the difference must be found. The data presented in this report provides a sample of the data required to develop the requisite water database as well as an approach to its collation. In addition to the development of a database for the water professionals, it will be useful to develop a tool (such as an excel sheet or visual aid) to allow the greater community to contribute to the data collection.

In addition, validation of the data as well as its analysis on a national, provincial and local basis is essential to assess the potential to derive value from the wastewaters where the regional and local distribution of the resource is critical in determining practicality of its beneficiation.

The composition data of major wastewater sources in terms of COD, NO^{3-} or NO^{2-} or NH^{4+} or TKN or TN and $PO4^{3-}$ has been compiled from a selection of references, this appears in summary form in Appendix section C.2. In cases where the COD, N and P content of a particular stream is not given, an approximation has been used based on literature findings. From the capacity and composition data, the amount of carbon, nitrogen and phosphorous that can be recovered from these industries is given.

This report does not deal with the specifics of the manufacturing process but focused only on the wastewater effluent that is generated from the process before it is either treated or disposed of in the municipal sewers. It is essentially a black box considering only water input to process and wastewater generation. Cleaning agents that are used in these industries and form part of the composition of the effluents are not considered. It is assumed that the cleaning agents used in the food and beverage industries are biodegradable.

4.1.2 Approach to data standardisation

Most volumes are given on an annual basis. In order to classify these flows according to the capacity of WWT in terms of volume per day, it was assumed that 365 days are used. All flows are reported as Mℓ/day. Using the categories that are specified in the Green Drop report (DWS SA, 2014) for municipal wastewater, the capacity of each wastewater stream is further classified as micro (<0.5 Mℓ/day), small (0.5 to 2 Mℓ/day), medium (2 to 10 Mℓ/day), large (10 to 25 Mℓ/day) and macro (>25 Mℓ/day). Further, the number of these streams or plants in operation in each industry is useful data. In some cases, the data collected was detailed and gave a good indication of the industry. In several cases only average data could be used.

In order to standardise to concentrations of C, N and P, the conversions from the COD, TKN/ ammonia/ nitrate/ nitrites and PO_4^{3-} found in literature were calculated as follows (details in Appendix section C.1):

- Concentration of C (mg/ℓ) = 3 x COD (mg/ℓ)
- Concentration of N (mg/ℓ)
  = (14/62) x NO_3^--N (mg/ℓ) or (14/46) x NO_2^--N (mg/ℓ) or (14/18) x NH_4^+-N (mg/ℓ).
  The Total Kjeldahl nitrogen (TKN) is the sum of organic nitrogen, ammonia (NH_3), and ammonium (NH_4^+) in the sample. Organic nitrogen consists of protein, urea and nucleic acids.
  The Total nitrogen (TN) is the sum of TKN, nitrate (NO_3^-)-N and nitrite (NO_2^-)-N.
- Concentration of P (mg/ℓ)= (31/95) x PO_4^{3-} (mg/ℓ)

4.1.3 Identifying the valorisation potential of South African wastewater

In South Africa, agriculture receives about 60 %, environmental use 18 %, urban and domestic use 11.5 %, mining and industrial use 10.5 % of water supply (Rand Water, n.d.). In this report, we have focussed be on the municipal and industrial effluents produced in South Africa.

The annual effluent production volumes from these industries, collected from the same literature sources, as well as their potential C, N, P contributions as calculated by the authors, are summarised in Table 4-3. This is a summary of the data that is presented later in this chapter where a number of these wastewater categories are discussed and characterised in more detail (Section 4.2 Overview of Municipal Wastewater in South Africa and Section 4.3 Overview of Industry-Specific Wastewaters in South Africa).

Table 4-3: Annual effluent production and the potential C, N and P contribution in several South African industries (detailed data are provided in Appendix section **Error! Reference source not found.**)

Industry Sector	Mℓ effluent per year	Estimated ton C / year	Estimated ton N / year	Estimated ton P / year	Comment	Reference
Municipal	1825000	4653750	118625	28288		(Henze, et al., 2008)
Abattoir (poultry)	5400	71280	945	308	Blood, skin, fat, viscera, faeces, significant solid waste	(Molapo, 2009)
Abattoir (red meat)	8188	139057	101	nl	Blood, skin, fat, viscera, faeces, significant solid waste	(DWA SA, 2001)
Brewing	8334	100008	438	250		(Burton, et al., 2009) (Brito, et al., 2007)
Canning	1074	11599	nl	nl		(Binnie and Partners, 1987)
Cleaning and Cosmetics	314.3	5003	11.7	5.64		(Cloete, et al., 2010)
Dairy	86393	3.9 million	30238	3456	Fats, protein, faeces, grit	(Du Preez, 2010)
Distillery (alcoholic beverages)	386.8 (#)	128	428	nl		(Melamane, et al., 2007)
Dyeing and Colouring	645	2137	nl	nl	Alkaline pH, toxic organic residues, high NaCl concentration (1590 mg/l)	(Cloete, et al., 2010)
Edible oil	1361	543039	42.2	3409	Pollutants such as fats, oils and grease, sodium, sulphates and phosphates	(Roux-Van der Merwe, et al., 2005) (Surujlal, et al., 2004) (Steffen, Robertson & Kirsten Inc, 1989d)
Fishery	1760	30624	62	nl	Flesh, scales, blood	(Chowdhury, et al., 2010) (Quiroz, et al., 2013)
Laundry	218.6	564	0.07	2	solvents, surfactants	(Cloete, et al., 2010)
Petroleum	77380	1.83 million	3691	101	Oil and grease, phenols	(Gasim, et al., 2012)
Pulp and Paper	339300	967005	3068	443	AOX, dioxin, chlorinated organics	(Cloete, et al., 2010)
Soft drinks	4070	74326	nl	nl		(Pollution Research Group, 2015)
Sugar	411	2158	nl	nl	Fibres, sand	(Mooij, et al., 2015)
Textiles	0.03 million	0.454 million	15	196	Azo dyes	(Cloete, et al., 2010) (Steffen, Robertson and Kirsten Inc, 1993)
Winery	2421	49388	266	126	Polyphenols, inorganics such as sodium and potassium	(Welz, et al., 2015) (Cai, et al., 2013) (Brito, et al., 2007)

Assumed from distillery production data (SAWIS, 2016) and assuming an SEV of 2.5 L effluent/ L wine produced
nl not listed

4.2 Overview of Municipal Wastewater in South Africa

Municipal wastewater usually includes considerable amounts of discharged industrial effluent. Examples of the top industrial effluent producers within some metropolitan areas are shown Table 4-4. Due to confidentiality no company names were mentioned in the Cloete, et al. (2010) report.

Table 4-4: Examples of top industrial effluent discharge into municipal wastewater (Cloete, et al., 2010)

Amathole	44% automotive	41% food manufacture	15% textiles			
Cape Town	30% brewery *	29% textiles	18% paper and paper products	13% food manufacture	10% beverage	
Johannesburg	34% yeast	17% beverage	15% electroplating	13% dairy	12% food manufacture	9% automotive
eThekwini	58% paper and paper products	18% petroleum and petroleum products	15% textiles	9% beverages		
Nelson Mandela	42% brewery	21% automotive	12% textiles	9% food manufacture	8% dairy	8% tannery
Tshwane	81% brewery	11% food manufacture	8% textiles			

* stand-alone WWTW installed

From the Green Drop report (2014), the status of municipal WWT in South Africa a total of 152 municipalities and 824 plants were assessed, as shown in Appendix section C.3. The total amount of WW entering these works is approximately 5 000 Mℓ/day or 1 825 000 Mℓ/year (365 day operation). There are also five privately owned WWTW that have a total treatment capacity of 106 Mℓ/day. This combined value (5 106 Mℓ/day) of WW going into the WWTW is comparable to the estimate obtained by Burton et al (2009) of 7 600 Mℓ/day. The volume, concentration and complexity data for the South African municipal WWTW is shown in Table 4-5.

Municipal wastewater is very different in terms of complexity and variability as well as being more dilute than most industrial wastewaters. The range of concentrations of COD, TN and TP reported by Henze, et al. (2008) was used as a first estimate. The COD ranged from 500 to 1200 mg/ℓ, the TN from 30 to 100 mg/ℓ and TP from 6 to 25 mg/ℓ. This can be converted to 1 500-3 600 mg-C/ℓ, 30-100 mg-N/ℓ and 6-25 mg-P/ℓ. The pH ranged between 7 and 8 and the total suspended solids (TSS) between 250 and 600 mg/ℓ (Appendix section C.3).

Table 4-5: Volume, concentration and complexity data for the South African municipal WWT industry (detailed data and references of data sources are provided in Appendix section C.3)

effluent volume in South Africa	**total estimated effluent volume in South Africa**	*ML/year*	1 825 000
	Days of operation	*days*	365
	total estimated effluent volume in South Africa	*ML/day*	5 000
cross-reference	to worksheet with primary data and calculations.		Appendix C.3
distribution: number of plants	**TOTAL**		**828**
	micro	*<0.5 ML/day*	168
	small	*0.5-2 ML/day*	269
	medium	*2-10 ML/day*	232
	large	*10-25 ML/day*	65
	macro	*>25 ML/day*	62
concentration	**estimated average carbon content**	*mg/L*	2550
	estimated average nitrogen content	*mg/L*	65
	estimated average phosphorus content	*mg/L*	15.5
	pH		7-8
	conductivity	*mS/m*	70-120
complexities **Present. The compounds will differ per municipal WWTW*	**solids component**		Present*
	toxic compounds		Present
	metals		Present
	complex organics		Present
	other valuable components		Present

4.3 Overview of Industry-Specific Wastewaters in South Africa

In terms of effluent production processed outside of municipal water treatment plant, a total industrial effluent of 69 Mm^3/year (69 000 Mℓ/year) is generated according to Cloete, et al. (2010). These effluents are summarised in Table 4-6. The largest industrial wastewater producers in South Africa are the pulp and paper (42%) and petroleum (25%) industries, with mining (10%) and power generation (7%) as the other major consolidated wastewater producers. Although mining wastewater poses the greatest potential risk of all industrial sectors (Cloete, et al., 2010) and has been studied in terms of both bioremediation and valorisation (Harrison, et al., 2014), this report excludes this wastewater because it is examined in detail in numerous reports published in the WRC “Mine Water” category (WRC SA, 2016b) and is typically low in C, N and P, the resources on which this study is focussed. Similarly, the wastewater from the power generation industry, which Cloete, et al. (2010) place third in terms of risk, has not been included in the evaluations in this report because the components and likely remediation and valorisation pathways differ from the generalised wastewaters considered here.

Table 4-6: Proportion of industrial wastewater by industry sector (Cloete, et al., 2010)

Sector	Effluent Volume %	Comments
Power Generation	7%	*not evaluated*
Mining Industry	10%	*not evaluated*
Pulp and Paper Industry	42.0%	
Petroleum Industry	25.5%	
Food and Beverage Industry	8%	Animal-based & Plant-based
Other Industries	7.5%	Organics-based & Non-organics-based

For the implementation of WWBR, the specific site or regional information is more important than the national values. The value that can be expected in terms of WWBRs differs for different scales of industry. Large, standardised plants gain from economy of scale, and usually have to comply with industry water-use standards. Small "backyard" industries are not regulated and do not have significant water savings practices in place. The smaller industries are valuable, however, and have high potential in the wastewater biorefinery space as they may be much more flexible in catering to a niche industry market need. The values examined in the following summaries are for the larger industries for which information is more readily available. It is anticipated that the smaller industries will have larger effluent values per unit, but the nutrient concentrations may be more dilute.

The pulp and paper industry and the petroleum industry are both large centralised industries and together produce nearly 70% of the industrial wastewater in South Africa. These are therefore high priority in terms of evaluating WWBR potential. The food and beverages industry, given by Cloete et al. (2010) as second after mining in terms of effluent risk, is evaluated in two subdivisions of animal-based and plant-based food and beverages because of the very different components present. Wastewaters of other industries can be divided into organic-based and non-organic-based, and the latter division is not considered for this report.

4.3.1 Pulp and paper industry

According to Hagelqvist (2013) an estimated 400 million tonnes of paper and paperboard was produced globally in 2012 with an estimated 30 to 90 billion tonnes of wastewater produced concomitantly. This equates to 150 tonnes (or 0.15 Mℓ) wastewater generated for every tonne of paper produced. From the CSIR (2010) report, the specific water intake is given as 33 – 136 m^3/ tonne (0.033 – 0.136 Mℓ/tonne) for an integrated plant and as 1 – 49 m^3/ tonne (0.001 – 0.049 Mℓ/tonne) pulp and paper products. This wastewater is deficient in phosphorous and nitrogen in terms of use as substrate for microorganisms, hence supplementation of these components may be needed in biological treatment.

The major producers in the pulp and paper sector are Kimberly-Clark, Mondi South Africa, Mpact, Nampak and Sappi (PAMSA, 2012). In 2014, the total pulp and paper production in South Africa was 1 967 000 tonnes and 2 262 000 tonnes respectively (PAMSA, 2015). Therefore, to produce 2.3 million tonnes of paper, it may be calculated that approximately 0.34 million Mℓ/year of wastewater is generated from the relationship of 0.15 Mℓ WW/ tonne paper. Data used in Burton et al (2009) (Appendix section C.4.1) and Cloete et al. (2010) reported 111 971 Mℓ/year (0.11 million Mℓ/year) and 39 488 Mℓ/year (0.039 million Mℓ/year) of effluent respectively produced in this sector. According the study done by MacDonald (2004) approximately 85% of water consumed in the pulp and paper industry is expelled as wastewater.

The COD values reported ranged from 700 mg per litre to 1200 mg per litre (2 100 – 3 600 mg C/ℓ) (Cloete, et al., 2010) while Burton et al. (2009) reported an average of 700 mg/ℓ COD (2 100 mg C/ℓ) (Appendix section C.4.1). The ammonia and nitrite/nitrate concentrations of the pulp and paper effluent in Tshwane in mg/ℓ are 8.7 (8.7 mg-N/ℓ) and 1.52 (0.343 mg-N/ℓ) respectively (total nitrogen is the sum of these values, and is 9.04 mg N/ℓ) while the phosphate is 4 mg/ℓ (1.305 mg-P/ℓ) (Cloete, et al., 2010) which is less than the general limits for wastewater treatment standards of South Africa effluent according to the General Authorisation Standards (DWA SA, 2013) listed in Table 3-2. The average pH ranges between 6 and 8 and does not pose a serious threat to the environment. The total suspended solids do pose a threat with levels as high as 6 000 mg/ℓ. Table 4-7 illustrates the volume, concentration and complexity data for the wastewater of the South African pulp and paper industry.

The pulp and paper sector utilises large amounts of lignocellulosic material and water during the manufacturing process. The process releases chlorinated lignosulphonic acids, chlorinated resin acids, chlorinated phenols and chlorinated hydrocarbons in the effluent. Approximately 500 different chlorinated organic compounds have been identified such as chloroform, chlorate, phenols, catechols, guaiacols, furans, dioxins, syringols, vanillins to name a few (IWA, 2009). These compounds are formed as a result of reaction between residual lignin from wood fibres and chlorine/chlorine compounds used for bleaching. Coloured compounds and adsorbable organic halogen (AOX) released from pulp and paper mills into the environment pose serious threats to aquatic organisms (IWA, 2009).

Table 4-7: Volume, concentration and complexity data for the South African pulp and paper industry (summarised from Appendix section C.4.1)

effluent volume in South Africa	**total estimated effluent volume in South Africa**	*ML/year*	339 300
	Days of operation	*days*	365
	total estimated effluent volume in South Africa	*ML/day*	929.6
cross-reference	to worksheet with primary data and calculations.		Appendix C.4.1 and Section 4.3.1
distribution: number of plants (data obtained from Burton et al (2009))	**TOTAL**		**18**
	micro	*<0.5 ML/day*	0
	small	*0.5-2 ML/day*	8
	medium	*2-10 ML/day*	3
	large	*10-25 ML/day*	2
	macro	*>25 ML/day*	5
concentration	**estimated average carbon content**	*mg/L*	2 850
	estimated average nitrogen content	*mg/L*	9.04
	estimated average phosphorus content	*mg/L*	1.30
	pH		6-8
	conductivity	*mS/m*	105 - 348
complexities	**solids component (TSS)**	*mg/ℓ*	6000
	toxic compounds		adsorbable organic halogen (AOX).
	metals		-
	complex organics		chlorinated lignosulphonic acids, chlorinated resin acids, chlorinated phenols and chlorinated hydrocarbons Chlorinated organics such as chloroform, chlorate, phenols, catechols, guaiacols, furans, dioxins, syringols, vanillins
	other valuable components		cellulose

4.3.2 Petroleum refineries and petroleum products industry

South Africa has four crude oil refineries (Engen, Sapref, Natref and Chevron), as well as the coal-to-liquid (Sasol) and gas-to-liquid operations (PetroSA, Sasol). Combined they process the crude-oil-equivalent of 703 000 bbℓ/day (83 165 tonnes/day). The production capacity of each refinery is shown in Appendix section **Error! Reference source not found.**. It is assumed that approximately 0.1 to 5 m^3 o f wastewater is generated per tonne of crude oil processed (Burton, et al., 2009). Using this relationship it can be calculated that the average wastewater produced is approximately 212 Mℓ/day (0.077 million Mℓ/year). All six of these refineries have macroscale WWTW (<25 Mℓ/day). This wastewater is generated from several sources in the refinery such as during the desalting of crude oil, stream stripping, product fractionators, reflux drum drains, hydro-skimming, hydro-cracking, sourwater, condensate, boiler blowdown and other sources during the process not directly involved in processing such as water runoff and sewage from the site ((Burton et al., 2009; Diya'uddeen et al., 2011). The volume, concentration and complexity of wastewater in the South African petrochemical industry is shown in Table 4-8.

In Burton et al (2009) only the COD value was reported from crude oil refineries and values given as 236 to 800 mg/ℓ (708 – 2400 mg C/ℓ). A COD value of 2036 – 7052 mg/ℓ (6108 – 21156 mg C/ℓ) and a ammonia value of between 303-834 mg/ℓ (236 - 649 mg N/ℓ) was reported by Pearce and Whyte (2005). The COD value according to Gasim, et al. (2012) of a petroleum refinery wastewater is 7896 mg/ℓ (23688 mg C/ℓ). The ammonia, nitrate and TKN concentrations are 13.5, 2.23 and 40.6 mg/ℓ (10.5, 0.50 and 40.6 mg N/ℓ) respectively, while the phosphate is 10.2 mg/ℓ (3.33 mg P/ℓ) (Gasim, et al., 2012). The pH value ranged from 4.2 to 9.1 (Pearce & Whyte, 2005). The oil content in petroleum wastewater was determined to be between 124 and 171 mg/ℓ (Pearce & Whyte, 2005). Due to the large variability in the COD and nitrogen concentrations further information is required from the industry in terms of how these compositions differ from crude oil refineries, coal-to-liquid and gas-to liquid operations.

Table 4-8: Volume, concentration and complexity data for the South African petroleum industry (summarised from Appendix section C.4.2)

effluent volume in South Africa	**total estimated effluent volume in South Africa**	*ML/year*	77 380
	Days of operation	*days*	365
	total estimated effluent volume in South Africa	*ML/day*	212
cross-reference	to worksheet with primary data and calculations.		Appendix C.4.2
distribution: number of plants	**TOTAL**		**6**
	micro	*<0.5 ML/day*	
	small	*0.5-2 ML/day*	
	medium	*2-10 ML/day*	
	large	*10-25 ML/day*	
	macro	*>25 Mℓ/day*	6
concentration	**estimated average carbon content**	*mg/L*	23688
	estimated average nitrogen content	*mg/L*	47.7
	estimated average phosphorus content	*mg/L*	1.30
	pH		4.2-9.1
	conductivity	*mS/m*	63-1364
complexities	**solids component**		oils and grease
	toxic compounds		phenols, sulphides
	metals		heavy metals
	complex organics		solvents
	other valuable components		

4.3.3 Animal-based food industry

Using the industrial categories reported across several sources (Burton, et al., 2009; Cloete, et al., 2010; WRC SA, 2016a), this subsector includes poultry abattoirs, red meat abattoirs, fisheries and dairies. These are each considered in some detail in the following subsections. While substantial wastewater is generated in some divisions of animal husbandry, especially where animals are raised in high density conditions within structures, such as piggeries, we do not consider this area here.

The animal-based food subsector uses large quantities of water because of the stringent cleanliness requirements. The wastewater for all divisions contains high-complexity organics, fats and oils and a considerable amount of solids. Cleaning and sterilisation is an important part of the processing and these products appear in the wastewater but are not considered in this report.

Poultry abattoirs

Abattoir wastewaters are highly complex and fairly variable, with a nutrient rich composition. They also pose a high health risk (Steffen, Robertson and Kristen Inc, 1989b). In South Africa approximately 46% of the high-throughput poultry abattoirs render blood waste into several kinds of by-products (carcass meal, feather meal, poultry oil and blood meal) as opposed to direct disposal. The most commonly identified blood waste disposal methods include land application (3.8%), municipal sewer (7.6%), sold to contractors (11.5%), burial (34.6%), and rendering (46.1%). Rendering is a heating process for meat industry waste products through which fats are separated from water and protein residues for the production of edible lards and dried protein residues. Commonly it includes the production of a range of products of meat meal, meat-cum-bone meal, bone meal and fat from animal tissues (FAO UN, 1996). Although rendering produces by-products, it is also classified as a disposal method. Effluent from rendering plants contains very high loads of organic matter, therefore it is regarded as a further source of contaminating effluent (Appendix section C.4.3) (Molapo, 2009). An estimated 15 to 20 ℓ of water is required per bird in poultry abattoirs (Steffen, Robertson and Kristen Inc, 1989b). The volume of water discharged as wastewater may amount to between 80 and 85% of the waste load (Bremner & Johnston, 1996). The slaughtering and operational status of these plants (26 abattoirs) is given in Appendix section C.4.3 along with the composition of poultry abattoir effluent characteristics found in literature and the volume of wastewater generated (Molopo (2009). This is summarised into Table 4-9. As poultry abattoir wastewater is contaminated with fat, viscera, blood, feathers and faeces, it can be characterized and distinguished from other industrial wastewater by its high organic matter, oil and grease and solid content.

Table 4-9: Volume, concentration and complexity data for the South African poultry abattoir industry (summarised from Appendix section C.4.3)

effluent volume in South Africa	**total estimated effluent volume in South Africa**	*ML/year*	5400
	Days of operation	*days*	365
	total estimated effluent volume in South Africa	*ML/day*	14.8
cross-reference	to worksheet with primary data and calculations.		Appendix C.4.3
distribution: number of plants	**TOTAL**		**26**
	micro	*<0.5 ML/day*	1
	small	*0.5-2 ML/day*	16
	medium	*2-10 ML/day*	3
	large	*10-25 ML/day*	6
	macro	*>25 ML/day*	0
concentration	**estimated average carbon content**	*mg/L*	13200
	estimated average nitrogen content	*mg/L*	175
	estimated average phosphorus content	*mg/L*	57.1
	pH		7.0-7.2
	conductivity	*mS/m*	nl
complexities	**solids component**		fat, viscera, blood, feathers and faeces
	toxic compounds		-
	metals		-
	complex organics		fats, oils, protein
	other valuable components		feathers (keratin)

Valorising wastewater from abattoirs needs to take advantage of the high fat content. Fungal products may be particularly well suited here, integrated with energy recovery in the form of biodiesel. Biogas production through anaerobic digestion may be less effective due to the high fat content; however, recently AD for waste treatment at poultry abattoirs has been reported (Molapo, 2009). An installation at RCL Foods Worcester Poultry Processing in the Western Cape is being constructed and commissioned during 2016 for concomitant biogas production for electricity generation for the RCL facilities and remediation of wastewater to reduce the COD load by 80% (Worcester Standard, 2016).

Red meat abattoirs

The "Guidelines for the Handling Treatment and Disposal of Abattoir Waste" (DWA SA, 2001) reported that the red meat industry was comprised of 285 abattoirs and that the annual water consumption as recorded in 1989 was approximately 5 800 Mℓ. Approximately 85% of this water was discharged as effluent (4 872 Mℓ/year) containing high organic loads and suspended matter. The COD ranged from 2 380 to 8 942 mg/ℓ (7140 – 26826 mg C/ℓ) and the TKN was between 0.71 to 24 mg/ℓ (0.71 – 24 mg N/ℓ) (DWA SA, 2001). The number of abattoirs has increased to approximately 479 in 2014 (Neethling, 2014). By using a linear correlation, the effluent was estimated to be 8 188 Mℓ/year for 2014. Table 4-10 gives the estimated volume, concentration and complexity of the red meat abattoir wastewater (see also Appendix section C.4.4. In addition to its organic complexity, this wastewater is contaminated by antibiotics and growth hormones as well as pesticides to control external parasites that were administered to the animals during their life time (IWA, 2009).

Table 4-10: Volume, concentration and complexity data for the South African red meat abattoir industry (summarised from Appendix section C.4.4)

effluent volume in South Africa	**total estimated effluent volume in South Africa**	*ML/year*	8 188 (estimate by extrapolation)
	Days of operation	*days*	365
	total estimated effluent volume in South Africa	*ML/day*	22.4
cross-reference	to worksheet with primary data and calculations.		Appendix C.4.4
distribution: number of plants	**TOTAL**		**480** (estimate)
	micro	*<0.5 ML/day*	
	small	*0.5-2 ML/day*	
	medium	*2-10 ML/day*	
	large	*10-25 ML/day*	
	macro	*>25 ML/day*	
concentration	**estimated average carbon content**	*mg/L*	16 983
	estimated average nitrogen content	*mg/L*	12.36
	estimated average phosphorus content	*mg/L*	
	pH		5.7 – 8.4
	conductivity	*mS/m*	
complexities	**solids component**		fat, viscera, blood, skin, hair, flesh, faeces, manure, grit and undigested feed
	toxic compounds		antibiotics, growth hormones, pesticides
	metals		
	complex organics		fats, oils and protein
	other valuable components		skin for leather products

Dairy industry

The South African dairy industry produced approximately 230 million litres of milk for the month of February 2016 (MPO, 2016). Milk production is seasonal, with the lowest milk yields from April to July and 30 to 40 percent more from September to November. To reduce seasonality dairy processors encourage farmers to produce more milk between April and July by paying highest prices during these months. Throughout the year cows are averaging about 19 litres milk per day per cow (Lassen, 2012).

Primary dairy industry: milking parlours

A study by Du Preez (2010) on the treatment of typical South African milking parlour wastewater (i.e. primary diary industry) by means of anaerobic sequencing batch reactor technology estimated the water usage in five typical South African milking parlours. For the five dairy factories an annual wastewater production ranged from 15 to 51 $\ell.cow^{-1}.day^{-1}$ (average 33 $\ell.cow^{-1}.day^{-1}$). The water used for the cleaning in place (CIP) washing of the milking equipment was similar in all five milking parlours and ranged between 4.9 and 6.4 $\ell.cow^{-1}.day^{-1}$.

Depending on the literature there are either approximately 4 000 milk producers in South Africa (Brand South Africa, 2008) or between 2 200 and 2 700 milk producers (DAFF SA, 2013; Erasmus, 2012; GCIS SA, 2013). From the Dairy Industry Review of 2014 (SAMPRO, 2014) in 2010 2 638 Mℓ of milk was produced, and in 2013 it increased to 2 817 Mℓ. Working on the assumption that there are 2500 milk producers in South Africa and if each milk producer has an average of 151 cows (Du Preez, 2010) and the average milk production per cow is 19 ℓ/cow, then this equates to 2 618 Mℓ milk that was produced annually in 2010 (assuming 365 days of operation), and an average of 1.74 ℓ wastewater per ℓ milk produced. This then equates to an average of 4 547 Mℓ/year of wastewater is produced. Information regarding the water usage and effluent production from the five milking parlours in the Free State and the Western is summarized in Appendix section **Error! Reference source not found.**.

Information from Burton et al (2009) suggested an average of 5.3 g-COD /ℓ (15 900 mg-C/ℓ) while (Du Preez, 2010) reported 20 g-COD/ℓ (60 000 mg-C/ℓ) (unfiltered) and 10 g/ℓ COD (30 000 mg-C/ℓ) (filtered), 350 mg-N/ℓ total nitrogen and 40 mg-P/ℓ total phosphorous. Using an average of 15 000 mg/ℓ COD (45 000 mg-C/ℓ) in Table 4-11: volume, concentration and complexity for the South African primary dairy industry is summarised.

Table 4-11: Volume, concentration and complexity data for the South African primary dairy industry (summarised from Appendix section C.4.5

effluent volume in South Africa	**total estimated effluent volume in South Africa**	*ML/year*	4 547
	Days of operation	*days*	365
	total estimated effluent volume in South Africa	*ML/day*	237
cross-reference	to worksheet with primary data and calculations.		Appendix C.4.5
distribution: number of plants	**TOTAL**		**2 500** (estimated)
	micro	*<0.5 ML/day*	
	small	*0.5-2 ML/day*	
	medium	*2-10 ML/day*	
	large	*10-25 ML/day*	
	macro	*>25 ML/day*	
concentration	**estimated average carbon content**	*mg/L*	45 000
	estimated average nitrogen content	*mg/L*	350
	estimated average phosphorus content	*mg/L*	40
	pH		8.2
	conductivity	*mS/m*	
complexities	**solids component**		faeces, grit
	toxic compounds		
	metals		
	complex organics		fats, proteins
	other valuable components		

Secondary dairy industry: milk processing

Apart from the primary dairy (milking) industry, the secondary dairy industry processes all milk, producing products such as long-life milk, cheese, butter, yoghurt, milk powder, whey powder and condensed milk. Water use and the effluent discharged vary with the type of produce and size of the company (Steffan, Robertson and Kirsten Inc, 1989a). In this NatSurv 4 report it was estimated that the dairy processing industry in 1986 used approximately 4.5 million m^3 water. In 1986 there were more than 150 factories producing a wide range of products such as fresh milk, butter, cheese, yogurt, milk powder, ice cream, condensed milk and various milk-based desserts (Steffan, Robertson and Kirsten Inc, 1989a). In 2012 there were 131 companies in South Africa processing raw milk that they produced themselves to secondary products such as pasteurised milk, yogurt and cheese and 163 companies that buy raw milk and process it to products such as pasteurised milk, yoghurt, sour milk, buttermilk, milk powder, buttermilk powder, whey powder and cheese (World Dairy Summit, 2012). These factories discharged large quantities of effluent from the processing and cleaning processes, the ratio being dependent on the particular products made. In the case of pasteurized milk, the effluent discharge was often 85 to 90% of water intake, for butter and cheese 90 to 95%, whereas for milk powder and condensed milk sometimes more than 100% (Strydom, et al., 1997; Steffan, Robertson and Kirsten Inc, 1989a). The revised NatSurv report on dairies has not been published and should available later in 2016. A preliminary table of the volume, concentration and complexity data for the South African secondary dairy industry is shown in Appendix section C.4.5.

Fishery industry

Information on the South African fisheries wastewater was difficult to source and there is no NatSurv report on this. There are more than a 100 processing factories in South Africa according to the Status of the South African Marine Fishery Resources report for 2014 (DAFF SA, 2014).

The information used in this section was collected from Brazil (Quiroz, et al., 2013) and Canada (Chowdhury, et al., 2010).The wastewater that originates from fish processing units depend on the composition of the raw fish or shellfish, the unit processes used, the quality of the processing water and additives such as brine and oil used for canning processes (Chowdhury, et al., 2010). Generally, 11 m^3 water is consumed per tonne fish processed (11 ℓ/kg), resulting in a significant volume of wastewater (Quiroz, et al., 2013). For a large salmon processing plant in Canada, the wastewater discharge is 3.12 ℓ/kg and for small salmon plants 9.90 ℓ/kg, while for the canning of tuna and sardines it is between 14 and 22 ℓ/kg (Chowdhury, et al., 2010). In South Africa, the hake catch is approximately 145 000 tonnes per year and is included in the total catch (including other species such as monk, kingklip and horse mackerel) of approximately 160 000 tonnes per year (SADISTA, 2013). Using these data for the South African catch and the wastewater estimation of 11 ℓ/kg, the annual wastewater generated can be estimated as 1 760 Mℓ/year (Table 4-12).

Fish processing wastewater contains high soluble, colloidal and particulate organic content (TSS range from 200-10 000 mg/ℓ), with COD concentrations ranging from 3000-10 000 mg/ℓ (9 000-30 000 mg-C/ℓ) for herring processing or even as low as 1 600 mg/ℓ (4800 mg-C/ℓ) for tuna processing (Chowdhury, et al., 2010). The ammonia concentration ranged from 0.7 to 69.7 mg/ℓ for a few fish processing plants with 42 mg/ℓ for salmon processing and 20 mg/ℓ for groundfish processing. For fish condensate the ammonia concentration can be as high as 2 000 mg/ℓ (Chowdhury, et al., 2010). Phosphorous partially originates from the fish during processing but can also be introduced with cleaning agents (Chowdhury, et al., 2010). The pH value ranges from 6.4 to 10.

Fat, oil and grease (FOG) are also important parameters of fish processing wastewater, approximately 60% of the oil and grease originates from the butchering process while the remaining 40% is from the fish canning and fish processing operations (Chowdhury, et al., 2010). Approximate FOG concentrations are between 60 and 800 mg/ℓ.

Table 4-12: Volume, concentration and complexity data for the South African fishery industry

effluent volume in South Africa	**total estimated effluent volume in South Africa**	*ML/year*	1 760
	Days of operation	*days*	365
	total estimated effluent volume in South Africa	*ML/day*	4.8
distribution: number of plants	**TOTAL**		**100 (estimated)**
	micro	*<0.5 ML/day*	
	small	*0.5-2 ML/day*	
	medium	*2-10 ML/day*	
	large	*10-25 ML/day*	
	macro	*>25 ML/day*	
concentration	**estimated average carbon content**	*mg/L*	17 400 (4 859 – 30 000)
	estimated average nitrogen content	*mg/L*	35.2 (7 – 69)
	estimated average phosphorus content	*mg/L*	-
	pH		6.4-10
	conductivity	*mS/m*	-
complexities	**solids component**		scales, flesh, blood, bones
	toxic compounds		-
	metals		-
	complex organics		oils, protein fats, oils and grease (FOG): 60-800 mg/ℓ
	other valuable components		

4.3.4 Plant-based food industry

Putting together the industrial categories used in several sources (Burton, et al., 2009; Cloete, et al., 2010; WRC SA, 2016a) this subsector includes a considerable number of industries. These can be subdivided into the following rough groupings: (i) *Raw plant food handling* consists of fruit, vegetables and grains with possible freezing, milling and packing. (ii) *Processed plant food* encompasses canning (and bottling and tetra-packs), sugar, yeast and edible oils. (iii) *Cooked plant food* comprises bakery, confectionery and snack production. (iv) *Alcoholic beverages* includes brewing, wine making and distilling. (v) *Soft drinks* incorporates sodas (fizzy drinks), fruit juices and concentrates.

Processing of raw plant food requires large volumes of potable water. The wastewater that is generated in food operations is non-toxic but has a high BOD and suspended solids in the form of sugars, hemicellulose, cellulose and lignin as well as surfactants (used in washing the produce). Processed plant-based food wastewater can also contain amounts of salt, flavourings, colouring material, acids, alkali and oil or fats (IWA, 2009).

Cloete et al. (2010) identified soft drinks, brewing and sugar as the three highest wastewater producers in the plant-based food subsector. These and the edible oil industry are considered here. This is followed by a section commenting on several other industries in this sub-sector. Details are included in the data in Appendix section C.4.6.

Soft drink industry

South Africa produced approximately 3 700 Mℓ of soft drinks in 2012 (Pollution Research Group, 2015). The effluent generated from the soft drink industry contains wasted soft drink and syrup, wash water from bottle and crate washing, caustic soda (NaOH), detergent and machine lubricant. For the NatSurv 3 edition 2 (Pollution Research Group, 2015) 67 production sites were identified in the soft drinks industry, and these all were approached, however data were only obtained from 16 of these. These 16 companies ranged in annual production volume from three at < 5 Mℓ, thorough six at 10 to 100 Mℓ, 6 at 100 to 340 Mℓ, to one producing > 500 Mℓ per annum.

The amount of wastewater generated at the production sites varies enormously, depending not only on the annual production volume, but also on whether it produces carbonated drinks, bottled water or fruit juices, and which parts of the entire process are included on site. The average specific water intake (SWI) is 1.6 ℓ/ℓ for carbonate drinks, 1.4 ℓ/ℓ for bottled water and 2.2 ℓ/ℓ for fruit drinks. The reported specific effluent volume (SEV) has an extremely wide range even within each subgroup of factories (Pollution Research Group, 2015) (Appendix section **Error! Reference source not found.**). An a verage SEV for these soft drink can be calculated as 1.1 ℓ/ℓ and using the amount of soft drinks produced (3 700 Mℓ) then the equivalent effluent is approximately 4 070 Mℓ/year (Table 4-13).

Wastewater is generally high in COD and TDS and contains nitrates, phosphates, sodium and potassium. An average COD was calculated from the extremely varied values for COD which were given in NatSurv 3 (Pollution Research Group, 2015)(see also Appendix section C.4.6); this COD value is 6 087 mg/ℓ (18 262 mg-C/ℓ). The total carbon that can be recovered nationally from the soft drink effluent streams would then be approximately 74 326 tonnes/year. The TDS also appears to vary considerably even within the two categories of carbonated and fruit juice drinks. The pH fluctuates widely with different stages of the process, and for carbonated drinks can vary between 2.8 and 12.2 and for fruit drinks between 6.1 and 11. The higher pH values result from cleaning with caustic soda (NaOH) (Pollution Research Group, 2015). The values of nitrogen and phosphates were not measured in the survey.

Table 4-13: Volume, concentration and complexity data for the South African softdrink industry (summarised from Appendix section C.4.6)

effluent volume in South Africa	**total estimated effluent volume in South Africa**	*ML/year*	4 070
	Days of operation	*days*	365
	total estimated effluent volume in South Africa	*ML/day*	11.2
cross-reference	to worksheet with primary data and calculations.		Appendix C.4.6
distribution: number of plants	**TOTAL**		**67**
	micro	*<0.5 ML/day*	
	small	*0.5-2 ML/day*	
	medium	*2-10 ML/day*	
	large	*10-25 ML/day*	
	macro	*>25 ML/day*	
concentration	**estimated average carbon content**	*mg/L*	18 262
	estimated average nitrogen content	*mg/L*	
	estimated average phosphorus content	*mg/L*	
	pH		2.8-12.2
	conductivity	*mS/m*	
complexities	**solids component**		in fruit juice effluent
	toxic compounds		
	metals		
	complex organics		
	other valuable components		

Alcoholic beverage industry

In South Africa, a total of 3 946 Mℓ of alcoholic beverages were produced in the year from July 2014 to June 2015; with the market share by volume of beer 77.7% compared to that of spirits 2.8%, ready to drink (RTD) alcoholic beverages 10.6%, wine 8.2%, and fortified wine 0.7% (Holtzkampf, 2016). The amount of wastewater produced depends on the production process, with details on breweries, wineries and distilleries given below.

Breweries

From the NatSurv 1 report (Binnie and Partners, 1987) an average of 89.8 Mℓ/month of beer was produced i.e. 1 077.6 Mℓ/year and an annual brewery effluent volume in South Africa of 5.9 million m^3/year (5 900 Mℓ/year) was generated, which relates to an SEV of 5.5 ℓ/ℓ. Burton et al. (2009) reported that a total of 2 604.6 Mℓ/year of beer was produced in 2008 and 8 334 Mℓ/year wastewater generated with a SEV of approximately 3.2 ℓ-effluent/ℓ-beer produced at the brewery considered. Typical SEV for breweries is between 3 and 5 ℓ/ℓ beer according to information from the IWA Water WIki (IWA, 2009). The typical composition of untreated wastewater effluents can be seen in Appendix section C.4.7.

The effluents from the individual steps of the brewery process are variable with effluents from the fermentation and filtering processes containing high COD and BOD but low volume (about 3% of the total wastewater volume), while the bottle washing produces large volumes of effluent with low organic content. The COD values between the different breweries ranged between 700 and 20 000 mg/ℓ, while the TDS ranged between 5 600 and 9 900 mg/ℓ (Binnie and Partners, 1987). Typical COD, nitrogen and phosphorous concentrations for brewery wastewaters are 2 000-6 000 mg/ℓ (6000–18 000 mg-C/ℓ), 25-80 mg/ℓ and 10-50 mg/ℓ respectively (Brito, et al., 2007). The total brewery effluent has an average pH of 7, but the pH value fluctuates from 4.5 to 12 depending on the cleaning process (Brito, et al., 2007).

Table 4-14: Volume, concentration and complexity data for the South African brewing industry (summarised from Appendix section C.4.7)

effluent volume in South Africa	**total estimated effluent volume in South Africa**	*ML/year*	8 334
	Days of operation	*days*	365
	total estimated effluent volume in South Africa	*ML/day*	22.8
cross-reference	to worksheet with primary data and calculations.		Appendix C.4.7
distribution: number of plants	**TOTAL**		**7**
	micro	*<0.5 ML/day*	-
	small	*0.5-2 ML/day*	-
	medium	*2-10 ML/day*	-
	large	*10-25 ML/day*	-
	macro	*>25 ML/day*	-
concentration	**estimated average carbon content (range)**	*mg/L*	12 000
	estimated average nitrogen content (range)	*mg/L*	52.5
	estimated average phosphorus content	*mg/L*	30
	pH		7
	conductivity	*mS/m*	
complexities	**solids component**		
	toxic compounds		
	metals		
	complex organics		
	other valuable components		

Wineries

From the SAWIS report (2016) the annual production of wine in 2015 was 968.4 Mℓ, while 959 Mℓ/year for 2015 was noted for another source (Froud, 2016) . Each litre of wine accounts for the generation of 1 to 4 ℓ of winery wastewater, which is regarded as the most significant environmental risk from wine cellars (Welz, et al., 2015). If an average SEV of 2.5 ℓ/ℓ is used for 968.4 Mℓ wine produced then the effluent generated was calculated as 2 421 Mℓ. Effluent COD values typically range from 800 to 12 800 mg/ℓ, but peaks greater than 25 000 mg/ℓ have been reported (Saadi, et al., 2007; Malandra, et al., 2003). Inorganics, including sodium and potassium are often encountered in high concentrations (Welz et al., 2015) while the total Kjeldahl nitrogen is approximately 110 mg/ℓ and the total orthophosphates is 52 mg/ℓ (Cai, et al., 2013). The winery effluent consists of varying ratios of readily biodegradable sugars, moderately biodegradable alcohols and slowly biodegradable recalcitrant phenolics (Welz et al., 2015). The phenol concentration in some effluents can range from 29 to 474 mg/ℓ and, due to its antimicrobial activity, it is responsible for the strong inhibitory effects on microbial activity in WWTW, hence should be removed (Melamane, et al., 2007). The pH value ranged from 4.0 to 5.7 according to data from Brito et al. (2007). (See also Appendix section C.4.7)

Distilleries

A review on the wastewaters created in the distillery industry with the emphasis on using anaerobic membrane reactors to remediate it was presented by Melamane et al. (2007). Appendix section C.4.7 contains a summary the chemical characteristics of the distillery wastewaters (Melamane et al., 2007). Distilleries can be divided into two groups, the one for human consumption and the other for fuel ethanol production.

Sugar industry

The sugar industry is a major example of a processed plant-based food industry in South Africa, supporting the livelihood of approximately 1 million South Africans. Some 2.3 million tonnes of sugar were produced in the 2014/2015 season (SASA, 2016). An average SWI of 0.6 m^3/tonne (0.0006 Mℓ/t) sugarcane is used (Steffen, Robertson and Kirsten Inc, 1990). Typically, 18 m^3 wastewater is generated per 100 t (0.00018 Mℓ/t) of sugar cane processed, or 0.75 to 1.5 Mℓ/day wastewater (roughly 30% of the water requirement). Sugarcane is processed continuously from April to December. Plant maintenance occurs over the remainder of the year (Steffen, Robertson and Kirsten Inc, 1990). The annual wastewater generated per season (9 months or 274 days) was calculated to be 411 Mℓ/year. The organic content in the wastewater is high with a COD of 1 500 to 2 000 mg/ℓ; however, the wastewater is deficient in nitrogen and phosphorous (Steffen, Robertson and Kirsten Inc, 1990). Using these data, the average estimated carbon that can be recovered from the wastewater is calculated to be 2 156 t/year (Table 4-15). The major liquid by-product from a sugar processing plant is molasses; this can be fermented to produce fuel ethanol or sold on to the bioprocessing and animal feed industries as a nutrient source. The wastewater from the ethanol fermentation is called vinasse. For every litre of ethanol produced 10 to 15 ℓ of vinasse is generated (Christofoletti et al., 2013).

Table 4-15: Volume, concentration and complexity data for the South African sugar industry

effluent volume in South Africa	**total estimated effluent volume in South Africa**	*ML/year*	411
	Days of operation	*days*	274
	total estimated effluent volume in South Africa	*ML/day*	1.5
cross-reference	to worksheet with primary data and calculations.		
distribution: number of plants	**TOTAL**		**14**
	micro	*<0.5 ML/day*	
	small	*0.5-2 ML/day*	
	medium	*2-10 ML/day*	
	large	*10-25 ML/day*	
	macro	*>25 ML/day*	
concentration	**estimated average carbon content**	*mg/L*	5 250
	estimated average nitrogen content	*mg/L*	
	estimated average phosphorus content	*mg/L*	
	pH		
	conductivity	*mS/m*	
complexities	**solids component**		
	toxic compounds		
	metals		
	complex organics		
	other valuable components		

Edible oil industry

In 1989, there were 16 edible oil plants in South Africa. The NatSurv 6 (Steffen, Robertson & Kirsten Inc, 1989d) report represents data obtained from 11 of these. From these, it was estimated that the edible oil industry consumed approximately 1.75 million m^3 water/year (1 700 million Mℓ/year). An oil plant typically discharged about 35% of the incoming water to the sewer (Steffen, Robertson & Kirsten Inc, 1989d). The total quantity of edible oil produced in 1989 was 250 000 t (0.25 Mt/year) and was expected to increase by 3% per annum. Using the principle of compound interest, the total quantity of edible oil was estimated to be 0.56 Mt/year in 2016 (Steffen, Robertson & Kirsten Inc, 1989d). The water used and wastewater generated for 2016 was calculated from the 1989 data using the same ratios (Appendix section C.4.8) i.e. no improved efficiencies were taken into account although these would be expected to have occurred. The water used and wastewater generated for 2016 were thus estimated as 3 776 and 1 361 Mℓ/year respectively. A study by Roux-Van der Merwe, et al. (2005) on fungal treatment of edible oil containing industrial effluent found the COD to range from 16 000 and 250 000 mg/ℓ (48 000 – 750 000 mg-C/ℓ), the conductivity between 88.2 and 268/m and the TKN ranged from 16.1 to 45.9 mg/ℓ. No quantitative information was given on phosphate other than its content being significant (Roux-Van der Merwe, et al., 2005; Steffen, Robertson & Kirsten Inc, 1989d). Surujlal et al (2004) reported average phosphate data ranging from 500 to 4 510 mg/ℓ (163.2-1 471.6 mg P/ℓ). These and other data are shown in Appendix section C.4.8. Replacement of phosphoric acid with citric acid in the process resulted in reduction of the total phosphate concentration of the effluent to meet discharge standards (Surujlal, et al., 2004). The revised NatSurv report on edible oils with updated data will be available in 2017.

Table 4-16: Volume, concentration and complexity data for the South African edible oil industry (summarised from Appendix section **Error! Reference source not found.**)

effluent volume in South Africa	**total estimated effluent volume in South Africa**	*ML/year*	1 361 (estimate)
	Days of operation	*days*	365
	total estimated effluent volume in South Africa	*ML/day*	3.73
cross-reference	to worksheet with primary data and calculations.		Appendix C.4.8
distribution: number of plants	**TOTAL**		
	micro	*<0.5 ML/day*	
	small	*0.5-2 ML/day*	
	medium	*2-10 ML/day*	
	large	*10-25 ML/day*	
	macro	*>25 ML/day*	
concentration	**estimated average carbon content**	*mg/L*	399 000
	estimated average nitrogen content	*mg/L*	31
	estimated average phosphorus content	*mg/L*	2505
	pH		4.6-10.6
	conductivity	*mS/m*	98-388
complexities	**solids component**		
	toxic compounds		
	metals		
	complex organics		
	other valuable components		

Other plant-based food industries

Canning industry

The canning of fruit and vegetables is to preserve perishable foods such that they can be stored for prolonged periods of time. A study by Binnie and Partners (1987) investigated the process of canning of certain fruits (apples, apricots, guava and peaches) and vegetables (beans in tomato, beetroot, corn and green beans). The raw materials processed, water intake, effluent produced, COD and TSS values of these are summarised in Table C-31 in Appendix C. The total amount of effluent produced was 1 074 Mℓ/year for the canning of 0.2 million tonnes of raw material. The COD values ranged from 700 to 6500 mg/ℓ (2 100–19 500 mg-C/ℓ) and the TSS from 195 – 400 mg/ℓ. No values for nitrogen and phosphorous content were given and only a pH range of 4.4 to 11.7 for peach canning was given. Since the 1970's the industrial practices and optimisation of these have changed, yet no comprehensive evaluation has been performed since 1987, according to the review of Khan et al. (2015) of the fruit waste streams of South Africa. (Appendix section C.4.9)

Fruit processing industry

Several studies have investigated the valorisation of food processing wastewater through production of methane by anaerobic digestion. Some examples are given in Appendix section C.4.9

The study by Khan et al (2015) reviewed the fruit waste streams of South Africa in terms of the solid waste and wastewater produced. Fruit processing in South African includes canning, juicing, winemaking and fruit drying. The water consumption that occurs during these processes is reported as between 7 -10.7 m^3/tonne (0.007- 0.0107 Mℓ/t) of raw produce. The wastewaters generally contain particulate organics, suspended solids, various cleaning solutions and softening or surface-additives (Khan, et al., 2015). The focus in this report was on olive oil processing, citrus, grapes and apples.

Confectionery industry

South Africa is said to be one of the largest and most well established confectionery markets in the African continent with consumption of 1.3 kg of chocolate per capita per year and 2.1 kg of sugar confectionery per year, as recorded in 2010 (Food Stuff South Africa, 2011). The confectionary industry is divided into three segments, namely chocolate, flour (starch) and sugar confectionary. Chocolate confectionary comprises mainly chocolate bars, chocolate blocks, boxed chocolates and other chocolate products. Flour confectionary includes items made from the flour or starch, mainly as bakery products. Sugar confectionary includes the rest of the products in the confectionary industry. More recently, sugar-free confectionary contains no sugar or sugar alternatives (Ersahin, et al., 2011).

The confectionery industry can generate large amounts of wastewater that contain high concentrations of readily biodegradable organic materials characterized with high chemical oxygen demand (COD) and biological oxygen demand (BOD) (Beal & Raman, 2000; Diwani, et al., 2000; Ersahin, et al., 2011). The range of COD reported in confectionery wastewater lies between 2 840 and 19 900 mg/ℓ COD and 1 840 and 4910 mg/ℓ BOD (Ersahin, et al., 2011). (See also Appendix section C.4.10.)

4.3.5 Other organics-based industries

From industrial categories used in several sources (Burton, et al., 2009; Cloete, et al., 2010; WRC SA, 2016a), the remaining industrial sectors can be subdivided into organic and inorganic sectors. In this report on wastewater biorefineries we focus on the organic subsector. These organic wastewaters include those from the cleaning and cosmetics, dyeing and colouring, laundry, pharmaceutical, paint, plastic, tanning and leather, and textiles industries. Cloete et al. (2010) recorded the textile industry as the highest wastewater producer of these sectors, although it is noted that this data was from before the reduction in production volume of the South African textile industry. The wastewater from these industries varies from site to site and contains unusual components specific to the manufacturing process. It may contain reactive or hazardous components, like unreacted catalyst, extreme pH, salt concentration, heavy metals, or toxic solvents used in chemical synthesis. The complexity will vary from simple, predictable, consistent streams to highly varied streams of undisclosed composition. As the company producing the stream has the most information about the wastewater, the most experience with the components in the wastewater and may be unwilling to share the information due to proprietary concerns, substantive chemical production wastewater treatment and beneficiation are best considered as a point-source solution, and not mixed with other streams before beneficiation. Two example industries, textiles and cleaning agents manufacture, have been considered in Appendix sections C.4.11 and C.4.12.

4.4 Potential of South African Wastewaters as Feedstocks for Wastewater Biorefineries

In this section, the potential for generation of value-added products from wastewater in an industry-tailored fashion has been illustrated. This is not intended to provide a comprehensive inventory but rather as a starting point for conceptualising WWBRs in South Africa and defining future research. Challenges associated with the compiling of a comprehensive inventory of South African wastewaters include the lack of availability of up-to-date information, data available having been generated from small sample sizes owing to a limited number of industries being willing to disclose these numbers, and discrepancies in data collected from the same industry. Hence a rudimentary inventory of a number of important contributing industries to South Africa's wastewaters has been compiled in this chapter. This provides a basis on which to investigate the potential of wastewater as a source of valuable nutrients for the production of bio-based products, as a source of clean water and as a source of revenue with the aiming of facilitating its implementation in the near future.

The products from wastewaters, however, do not typically find favour for use in the food and beverage industry itself, due to health (and religious) concerns. However, there is potential for products used downstream of the food and beverage production, e.g. in the wastewater treatment as bioflocculants. Thus for example, the polylactic acid produced from an organic waste stream could not be recommended for use as food packaging, but could be used for paper coating for advertising billboards or plastic wrapping of sealed beverage containers. In valorising these industrial wastewaters, a partnership between the industry generating the waste stream, the biorefinery and the industry using the product is desired, preferably in close physical proximity.

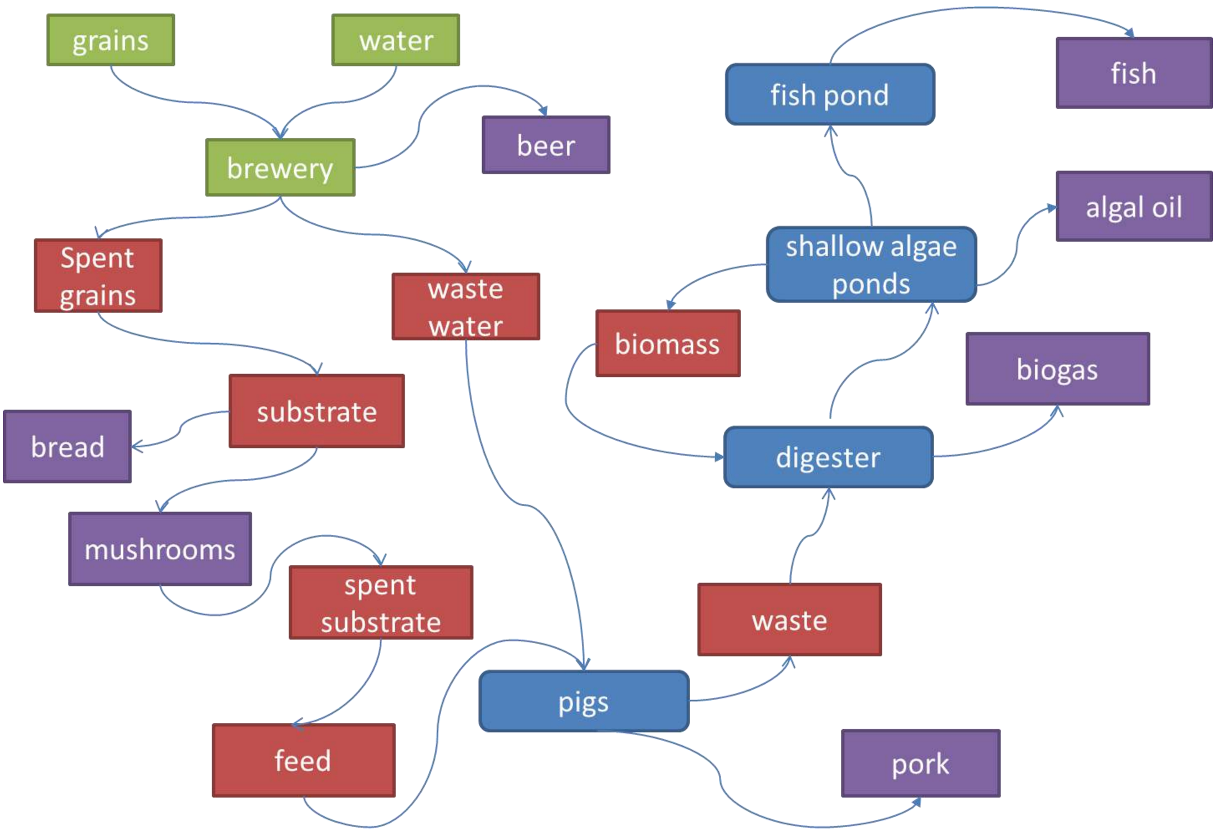


Figure 4-1: Brewery process flow diagram (adapted from (ZERI, n.d.)

The brewing industry has been recognised as favourable for the WWBR as these wastewaters tend to be readily biodegradable, do not contain biohazardous material like heavy metals and may contain microbial consortia already adapted to their environment. Thus, in principle, these wastewaters would be well suited to bioconversion.

Two examples of using brewery waste have been reported as part of the ZERI brewery process (ZERI, n.d.). In the first example, the spent grain from the brewery process was used to grow mushrooms. The spent substrate from mushroom production was then used as animal feed (Figure 4-1) (Zhang, et al., 2007). In the second example, bioflocculant produced from brewery wastewater was used to treat indigotin printing and dyeing wastewater with a maximum removal of the COD and the chroma of 79.2% and 86.5%, respectively (Zhang, et al., 2007).

5 REVIEW OF POTENTIAL BACTERIAL PRODUCTS IN THE SOUTH AFRICAN CONTEXT

The key to the concept of the WWBR is the production of multiple value-added products, simultaneously with improvement in water quality. Dependent on the composition of the feed stream to the WWBR, the process train used may have different groups of products associated with them. As has already been noted (Section 3.3.2), not all bioproducts are suitable for production in WWBRs due to the unique constraints presented by wastewater streams, especially dilute streams, and the environment in which they typically occur. This means that potential products must be carefully assessed and a selection made from the most viable alternatives.

Various options for bio-based products microbially produced using predominantly the organic, carbon-rich components of the wastewater are assessed here. A broad overview of bio-based products is given in Section 5.1. Following this biopolymers (Section 5.2) and bio-based building blocks (Section 5.3) are reviewed as potential products, with a final focus on two biopolymers, the biodegradable bioplastic poly-β-hydroxyalkanoates (PHA) (Section 5.4.1) and the water-retaining poly-glutamic acid (PGA) (Section 5.4.2). Finally (Section 5.5) product-related factors are considered specifically in the context integration of units into a WWBR.

5.1 Bio-Based Products

A European Commission-sponsored study on promoting the implementation of bio-based products (European Commission, 2009) described bio-based products as non-food crops which can be derived from biomass (plants, algae, crops, trees, marine organisms and biological waste from households, animals and food production). These products ranged from high value added chemicals such as pharmaceuticals or food additives to high volume products such as bio-polymers or chemical feedstocks (European Commission, 2009). Table 5-1 presents an overview of common bio-based products and their corresponding characteristics.

Table 5-1: An overview of common bio-based products and their corresponding characteristics (excluding food, energy and fuel products) (European Commission, 2009)

Product type	Characteristics or functionalities
Chemical and chemical building blocks Various chemicals made from renewable raw materials	Sustainable chemical production, lower GHG and other emissions in production, lower resource use in terms of energy and water with less waste depending on production process, typically better biodegradability, potentially less toxic
Bio-based plastics, biopolymers and biomaterials e.g. polyhydroxyalkanoate (PHA), polyethylene (PE), polylactic acid (PLA) and propanediol-based plastics from biotransformation of glucose, sucrose, plant-derived carbohydrates or starch	Sometimes biodegradable and/or compostable, savings in GHG emissions, potentially less toxic, materials with new qualities (composite materials, textiles, boards etc)
Renewable construction materials and composite materials from natural fibres e.g. flax, hemp, jute, wood used in building construction and automotive components etc.	Good mechanical properties (impact resistance, acoustic qualities, strongly reduced weight/lightweight concrete), better waste recycling (easier to recycle or burn than fiberglass)
Surfactants Surfactants lower surface tension of liquids and are used in soaps, detergents, pharmaceuticals, food additives, etc. and for the production of emulsions and foams. Chemical surfactants are produced largely from oils. Next generation "biosurfactants" can be produced using algae, fungi or bacteria	Low eco-toxicity, offers biodegradability and compostability. Enzyme-based detergents are used in household washing machines and offer environmental advantages (lower temperature, energy savings, more efficient washing, have replaced phosphorus)
Biosolvents Solvents are used in paints, inks, varnishes, adhesives etc.	Bio-based solvents do not emit volatile organic compounds (VOC) which are harmful to human health and the ozone layer. Some 23% of VOCs emitted into the air are from petrochemical solvents
Biolubricants Lubricants made from vegetable oils and their direct derivatives for engines, gearboxes, chains, etc.	Biodegradable, lower toxicity, can be used in sensitive environments, may reduce pollution from non-biodegradable or otherwise environmentally unacceptable lubricants from machines and vehicles
Enzymes, amino acids and organic acids These types of molecules can be used e.g. to enhance industrial processes to produce food and feed supplements and as building blocks for bio-polymers, cosmetics and pharmaceuticals	Economic value-added when used as inputs in various industries. Constitute technological advances that improve products or processes. Environmental benefits, e.g enzymes can replace several steps in chemical synthesis, save energy and avoid toxic chemicals (e.g. acid, alkali)

Bio-based products may be classified into two groupings:

1. Bio-based products that are chemically identical to their petrochemical counterparts (so-called 'drop-ins') can be used directly in the current industrial infrastructure. These can make an otherwise petroleum-based material partly or completely bio-based.
2. Bio-based chemicals and materials from renewable raw materials may provide products with unique characteristics that have not yet been produced from or are too costly to produce from petrochemical raw materials. These are termed novel bio-based products. Table 5-2 presents a SWOT analysis on the two different kinds of bio-products, according to Higson (2013).

Table 5-2: SWOT analysis of drop-in and novel bio-products (Higson, 2013)

Strengths	Drop-in: known targets and downstream products Novel: exploit attributes of biomass or biological processing
Weaknesses	Drop-in: number of unit operations required Novel: requirement for product development
Opportunities	Drop-in: rapid route to market through existing infrastructure and know how Novel: provides new or improved functionality
Threats	Drop-in: challenge to achieve cost competitiveness Novel: immature supply chain and market awareness

Bio-based products spread over a large spectrum of product types as shown in Table 5-1. However, owing to the large organic resource contained in wastewater, commodity products with a non-food use have been considered as the most relevant products of wastewater biorefineries. In this chapter, biopolymers form the focus as examples of relevant commodity products. An overview of bio-based chemical building blocks is also presented as the basis for production of biopolymers.

5.2 Microbial Polymer Production

5.2.1 Bio-based polymers

A polymer is a chemical compound which is made up of repeating structural units (monomers) that can be synthesised in a polymerisation or fermentation process (Dammer, et al., 2013) . Table 5-3 gives an overview of different bio-based polymers and their production methods.

Biopolymers are naturally occurring polymers produced during the growth cycles of all microorganisms. They are usually synthesised by enzyme-catalysed reactions and chain growth polymerisation reactions of activated monomers through complex metabolic cellular processes (Ghanbarzadeh & Almasi, 2013). Based on the different monomer units, biopolymers can be classified into three main groups:(i) polynucleotides (RNA and DNA) consisting of 13 or more nucleotide monomers (ii) polypeptides and proteins which are polymers of amino acids (iii) polysaccharides, which are linear bonded polymeric carbohydrate structures (Mohanty, et al., 2005; Kumar, et al., 2007).

Table 5-3: Overview of different bio-based polymers and their production methods (Weidmann-Marscheider, et al., 2005)

Bio-based polymer (group)	Type of polymer	Structure/Production method
Starch polymers	Polysaccharides	Modified natural polymer
Polylactic acid (PLA)	Polyester	Bio-based monomer (lactic acid) by fermentation, followed by polymerization
Other polyesters from bio-based intermediates i) Polytrimethyleneterephthalate (PTT) (ii) Polybutyleneterephthalate (PBT) (iii) Polybutylene succinate (PBS)	Polyester	i) Bio-based 1,3-propanediol by fermentation plus petrochemical terephthalic acid (or DMT) ii) Bio-based 1,4-butanediol by fermentation plus petrochemical terephthalic acid (or DMT) iii) Bio-based succinic acid by fermentation plus petrochemical terephthalic acid (or DMT)
Polyhydroxyalkanoates (PHAs)	Polyester	Direct production of polymer by fermentation or in a crop (wild type or genetically engineered bacteria; genetically engineered plants)
Polyurethanes (PURs)	Polyurethanes	Bio-based polyol by fermentation or chemical purification plus petrochemical isocyanate
Nylon i) Nylon 6 ii) Nylon 66 (iii) Nylon 69	Polyamide	i) Bio-based caprolactam by fermentation (ii) Bio-based adipic acid by fermentation (iii) Bio-based monomer obtained from a conventional chemical transformation from oleic acid via azelaic (di) acid
Cellulose polymers	Polysaccharides	(i) Modified natural polymer (ii) Bacterial cellulose by fermentation

5.2.2 What are bioplastics and how are they classified?

A plastic material is essentially a blend of one or more polymers and additives (Dammer, et al., 2013). (Haughn, 2015). The term “bio-plastic” can be defined in a variety of ways which can lead to ambiguity (bio-plastics.org, 2013):

i. Bio-based plastics: reference is made to the source of the raw materials
ii. Biodegradable plastics: reference is made to their functionality and fate
iii. Biocompatible plastics: reference is made to their functionality in terms of their compatibility with human or animal bodies

The first two categories are usually used to classify a bio-plastic, thus a bio-plastic can be either bio-based or biodegradable or both. Bio-based plastics are produced from bio-based raw materials while biodegradable plastics can be produced from both bio-based feedstock and petrochemical raw materials (Shen, et al., 2009). This can be represented in the material coordinate system given in Figure 5-1.

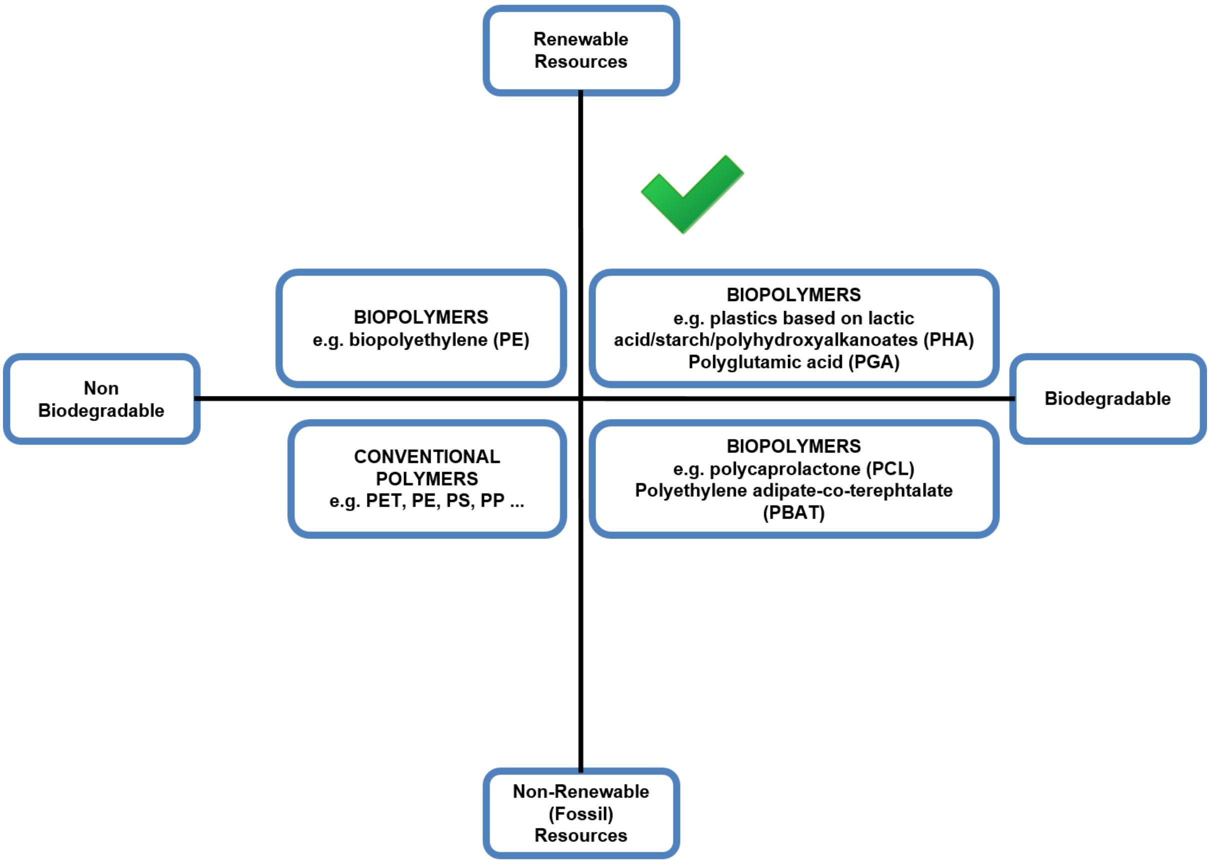


Figure 5-1: Material coordinate system for bioplastics (Scharathow, 2012)

The most attractive group in Figure 5-1 is the bio-based and bio-degradable bio-plastics group, whereby the bio-plastics can be fully degraded by microorganisms, thus having a closed carbon cycle (e.g. polylactides, aliphatic polyesters, polysaccharides and polyhydroxyalkanoanates) (Reddy, et al., 2003).

Starch- and cellulose-based plastics are the most common bio-based and bio-degradable plastics and have been used for decades (Shen, et al., 2009).

PLA was discovered in 1932 but was only commercialised in the early 1990’s owing to its similar properties to hydrocarbon polymers such as PET (Babu, et al., 2013). PLA is mainly used in food packaging applications but is not suitable for use in electronic devices and engineering applications (Babu, et al., 2013). PHAs are biologically synthesised polyesters which occur naturally in a variety of microorganisms. They were first discovered as bacterial storage products in the 1920's and commercialisation started in the 1990's (DiGregorio, 2009).

5.2.3 Bioplastics market trends

The current bioplastics market is growing strongly every year. The European Bioplastics is an association dedicated to help bioplastics industry in Europe achieve commercial success by providing unique networking possibilities with stakeholder groups (European Bioplastics, 2016).This association has conducted intensive market data research, some of which are publicly available and will presented in this section.

Bioplastics currently represent less than one percent of the 300 million tonnes of plastics produced globally (European Bioplastics, 2016) . However, there are numerous driving factors promoting the growth of the bioplastics industry, such as high consumer acceptance, climate change concerns, and depletion of fossil resources. The increasing rate of market penetration is also driven by the move of bioplastics from niche markets to mass markets with bioplastics being integrated into the packaging and automobile industries by existing companies such as Coca-Cola, Heinz, Mercedes and Toyota.

The latest market data published by European Bioplastics (2016) indicates that the production of bioplastics is expected to quadruple from around 1.7 million tonnes in 2014 to approximately 7.8 million tonnes in 2019.

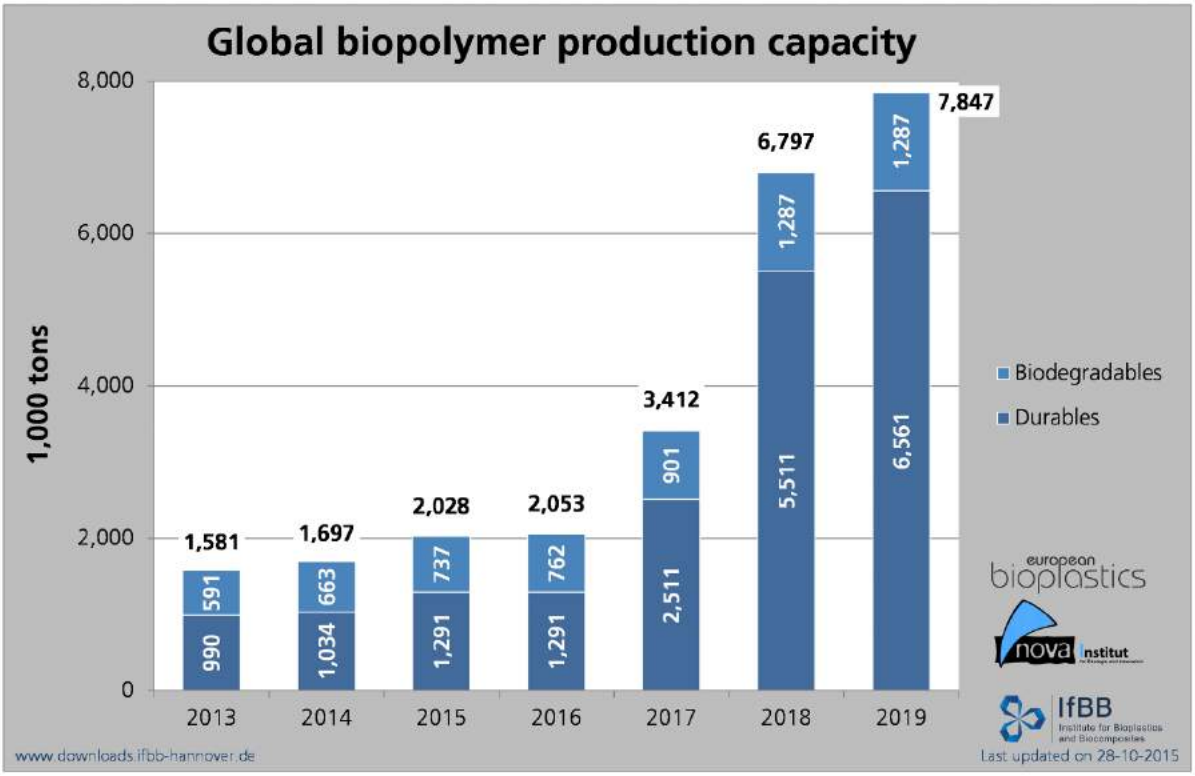


Figure 5-2: Global biopolymer production capacity (IfBB, 2016)

The growth rate of the biopolymer market is affected by state policy, technology, feedstock cost, competition (biomass versus fossil fuels), crude oil prices, and consumer acceptance amongst others (Dammer, et al., 2013). It has been interesting to note, however, the continued growth in the bioplastics market in spite of decreasing fossil fuel price (European Bioplastics, 2015).

The production growth is dominated by bio-based and non-biodegradable products such as biobased PE and biobased PET due to the aforementioned advantages in Table 5-2 available for these 'drop-in' bio-based products.

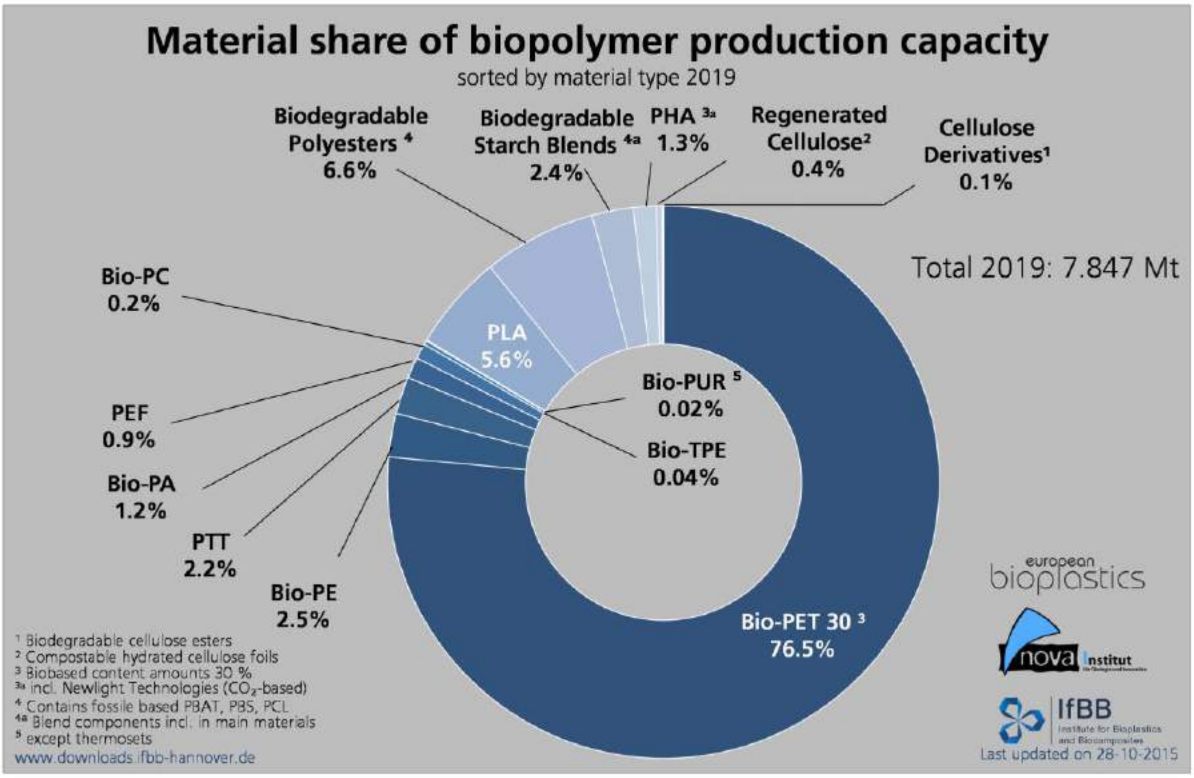


Figure 5-3: Forecast material share of biopolymer production capacity by material grade 2019 (IfBB, 2016)

However the production of bio-based and bio-degradable plastics such as PLA, PHA and starch blends is expected to double from 0.7 million tonnes in 2014 to over 1.2 million tonnes 2019 (European Bioplastics, 2016).

The data research done in conjunction with the research institutes IfBB – Institute for Bioplastics and Biocomposites (University of Applied Sciences and Arts Hannover, Germany) – and nova-Institute (Hurth, Germany) concluded that the major market sector for bioplastics remains the packaging industry accounting for over 70 % (1.2 million tonnes) of the overall bioplastics markets (European Bioplastics, 2016).

5.3 Bio-Based Building Blocks

Chemical building blocks (CBB) consist of a range of molecules that can be converted into secondary chemicals and intermediates that, in turn, can be used in manufacturing industries. Interest in bio-based chemical building blocks mostly lies in the production of bio-based polymers, lubricants and solvents. 'Drop-in' bio-based chemicals can be used in an already established spectrum of products derived from petrochemicals and their associated value chains, thus they carry less financial and technological risk than novel bio-based chemicals (BIO-TIC, 2014). However, they are more susceptible to competition than new compounds with novel properties.

5.3.1 Bio-based chemical platforms

Owing to their ability to replace petroleum derivatives, bio-based production of platform chemicals has been gaining more and more interest. However, this necessitates the development of efficient cost-effective production strategies and optimization of downstream processes along with the possibility of retrofitting within existing industrial infrastructure.

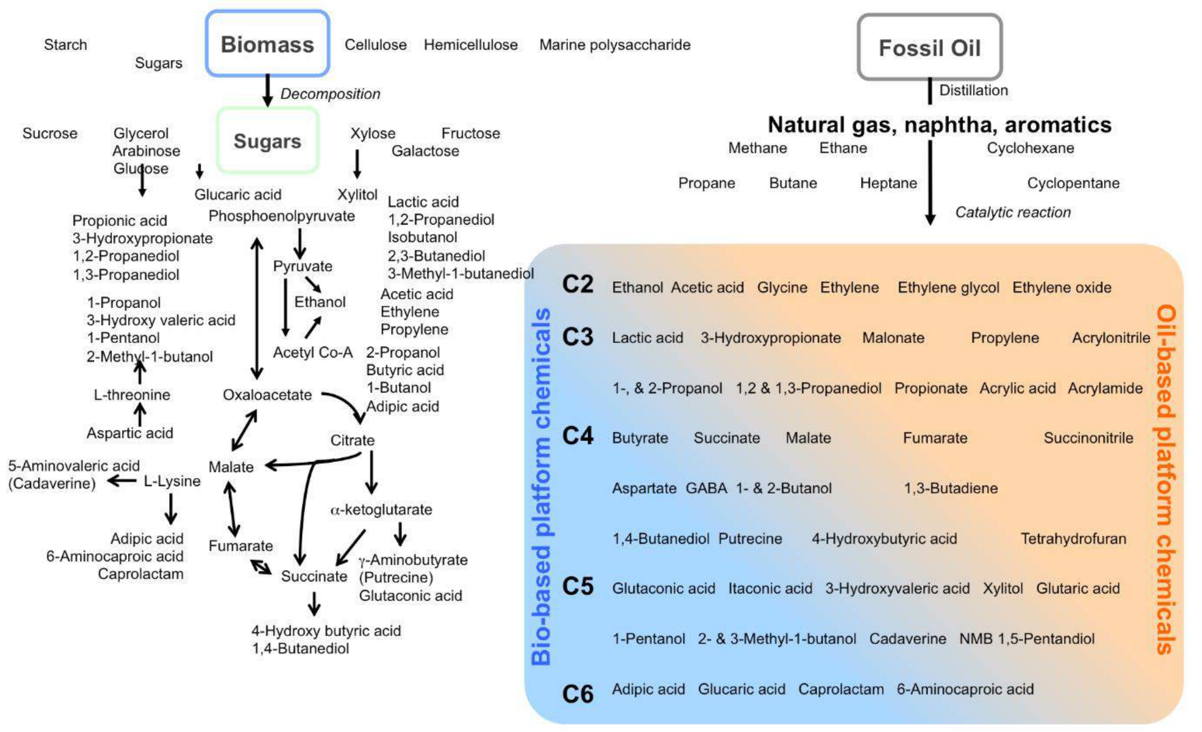


Figure 5-4: Replacing oil-based platform chemicals by bio-based platform chemicals production (Jang, et al., 2012)

Figure 5-4 illustrates the potential of the bio-based chemical platforms which can essentially deliver the same or equivalent petroleum derivatives or building blocks produced from the petrochemical production platform. The orange shading shows the various petroleum derived chemicals that are used as precursors for producing platform chemicals. The blue shading shows the different platform chemicals that can be produced from bio-based production. The blue shade replacing the red shade is indicative of the possibility of replacing petroleum derived chemicals by their bio-based counterparts. On the left panel of the diagram, the network of arrows indicates simplified biosynthetic networks that can result in the production of bio-based platform or intermediate chemicals in microorganisms (Jang, et al., 2012).

5.3.2 Building blocks and monomers as a precursor of polymers

Based on the characteristics of bio-based building blocks and platform chemicals and hence the nature of the bond governing polymerisation (e.g. amide-, ester- and C=C bonds), a number of classes of polymer compounds are available with individual polymers within these classes providing a diverse range of properties. This is shown in Figure 5-5.

The most common building blocks used in condensation polymerisation reactions are dicarboxylic acids (oxalic acids, malonic, succinic, glucaric, adipic, fumaric and malic acids), diamines (ethylenediamine, cadaverine and putrescine), diols (ethylene glycol, propanediols and butandediols) and aldehydes (formaldehyde) (Jang, et al., 2012). Amines and carboxylic groups from diamines and dicarboxylic acids can form amide bonds during the polymerisation of polyamide production. Ethylene which possesses a carbon-carbon double bond can polymerise to make polyethylene (PE).

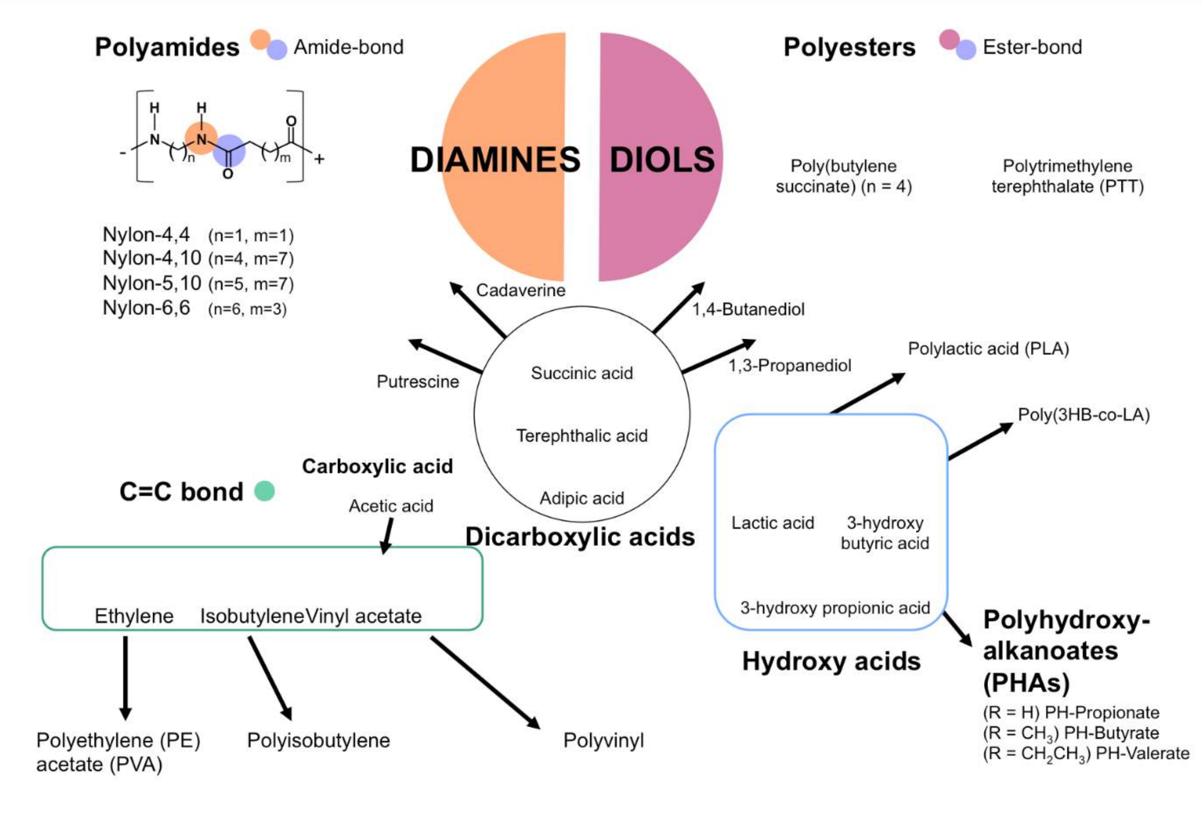


Figure 5-5: Various polymerisation schemes for generating platform and chemical block chemicals (Jang, et al., 2012)

5.4 Biopolymers from Wastewater and their Significance in Industry

Industrial and agricultural wastewater usually contains a plentitude of possible substrates for fermentation by microorganisms, such as sugars and organic acids, which can fit in the metabolic pathways shown in Figure 5-4. Polymers produced from wastewater may not be acceptable in the food and pharmaceutical industry but are suitable for the packaging, fittings, apparel and automobile industry. In all cases, using a waste stream as feedstock has a number of challenges. Wastewaters are often considered as receptacles for varied waste which may lead to the presence of noxious pollutants or inhibitors compromising functionality of the microorganisms. Further variability in the flowrate and composition of waste streams may lead to difficulty in reproducing and controlling the process. Among the keys to successful implementation of WWBRs is the selection biopolymers that can be readily produced from wastewater and bioreactor designs that facilitate process robustness. As explained in Section 2.3, the wastewater biorefinery is a relatively new concept that is only starting to gain momentum globally with the vision of closing resource cycles, exploiting the value of wastewater components along with the production of bio-products and recovery of clean water.

The market analysis demonstrates that bio-based counterparts of petroleum plastics (‘drop-in’ biopolymers) are currently the front runners in terms of bioplastics development and large scale production. While it is recognised that bio-based plastics such as bioPET that “drop in” to current value chains may more easily be marketed, these are not considered here. Referring to the material coordinate in Figure 5-1, preferred biopolymers that adhere to the WWBR vision are bio-based and biodegradable, such as PLA and PHAs. Compared to PLA, PHAs are considered more versatile with a range of applications in almost all areas of conventional plastics since there are at least 150 monomers of PHA as opposed to the monomeric (D and L-lactic acids) structure in PLA (Chen, 2009). Section 5.4.1 expands on PHAs as fulfilling these criteria and explores producing them from wastewater as an example of a potential WWBR product. Polyglutamic acid (PGA) is considered in Section 5.4.2

as another example of a polymer in this category, with certain differences from PHAs in terms of DSP as well as the option for use as-is.

5.4.1 Polyhydroxyalkanoates (PHAs)

PHAs are a group of bio-based and biodegradable polymers that have a wide variety of physical and chemical properties resembling petroleum plastics. PHAs can be tailored to meet end needs through incorporation of different monomers. This gives PHAs the potential to replace petroleum plastics in various applications (Chen 2009). Table 5-4 shows various applications of PHAs; they are among the most sought after bio-based and biodegradable polymers.

Table 5-4: Applications of PHAs in various industries (Chen, 2009)

Applications	Examples
Packaging industry	All packaging materials that are used for a short period of time, including food utensils, films, daily consumables, electronic appliances etc.
Printing & photographic industry	PHAs are polyesters that can be easily stained
Other bulk chemicals	Heat adhesives. Latex, smart gels. PHA nonwoven matrices can be used to remove facial oils
Block copolymerisation	PHA can be changed into PHA diols for block copolymerisation with other polymers
Plastic processing	PHA can be used as processing aids for plastic processing
Textile industry	Like nylons, PHA can be used as processing aids
Fine chemical industry	PHA monomers are chiral R-forms and can be used as chiral starting materials for the synthesis of antibiotics and other fine chemicals
Medical implant biomaterials	PHAs are biodegradable and biocompatible and can be developed into medical implant materials. PHA can also be turned into drug controlled-release matrices
Medical	PHA monomers, especially R3HB have therapeutic effects on Alzheimer's and Parkinson's diseases, osteoporosis and even memory improvement etc.
Healthy food additives	PHA oligomers can be used as food supplements for obtaining ketone bodies
Industrial microbiology	The PHA synthesis operon can be used as a metabolic regulator or resistance enhancer to improve the performance of industrial microbial strains
Biofuels or fuels additives	PHA can be hydrolysed to form hydroxyl-alkanoate methyl esters that are combustible
Protein purification	PHA granule binding proteins phasin or PhaP are used to purify recombinant proteins
Specific drug delivery	Coexpression of PhaP and specific ligands can help achieve targeting to diseased tissues

To date there are about 150 different variations of PHAs produced by using different monomers (Braunegg *et al.* 1998; Chee *et al.* 2010a; Bernard 2014). PHAs come from a family of optically active biological polyesters which contain hydroxyalkanoic units in the R configuration because of the stereospecificity of the enzymes involved in synthesis (Garate 2014). Most PHAs are aliphatic polyesters of carbon, oxygen and hydrogen as shown in Figure 5-6 (Braunegg *et al.* 1998)

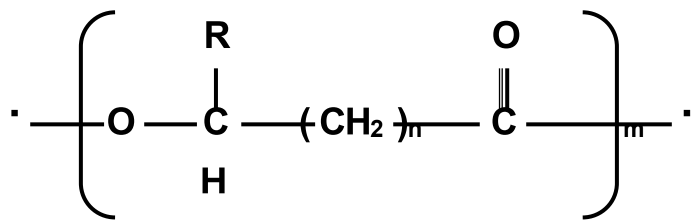


Figure 5-6: General structure of polyhydroxyalkanoates (Ebnesajjad, 2013)

where, R is the side chain on the monomer, n defines the length of the monomer and m is the number of monomeric units in the polymer chain. Both n and R determine the type of HA monomer unit. PHAs have been studied extensively due to their close resemblance to conventional plastics (Loo & Sudesh, 2007).

An imbalanced growth condition in the form of a limiting nutrient like nitrogen, phosphorus, sulphur, oxygen or magnesium in the presence of excess carbon triggers the polymerisation of soluble carbon intermediates into water-insoluble molecules like PHAs (Annuar, et al., 2008). By accumulating PHAs, microorganisms have a natural reserve of carbon and energy. On restoring the limiting nutrient, the PHAs can be degraded by intracellular enzymes and used as a carbon or energy source (Lee, 1996). PHA synthesis relies on important biochemical pathways such as the tricarboxylic acid (TCA) cycle, fatty acid degradation (β-oxidation) and fatty acid biosynthesis. Numerous studies have shown that PHAs can be readily produced from activated sludge biomass using volatile fatty acids (VFAs) as carbon substrates, as well as from simple sugars, oils and a variety of waste feedstocks such as molasses, milk waste and others (Verlinden, et al., 2007). PHAs are produced intracellularly and serve as storage compounds in microorganisms which can often also provide biological phosphorus removal, making PHAs interesting candidates in wastewater treatment (Satoh, et al., 1999). By enriching the activated sludge with PHA producing microorganisms and having adequate carbon substrate and oxygen concentration in the presence of a limiting nutrient, PHA production can be exploited. Chua, et al. (2003) investigated the feasibility of PHA production using activated sludge and concluded that with the required process optimisation, PHA production was an added benefit to waste treatment in the form of waste conversion to a valuable product. .

Potential wastewaters for PHA production are VFA mixtures (acetate, propionate), food waste, olive and palm oil mill effluents, sugarcane molasses, diary effluents, paper mill effluents, fruit and tomato cannery effluents, brewery effluents and municipal wastewaters. Regarding process optimisation, where wastewaters are rich in organic loading, more conventional approaches to PHA production can be utilised. Under these conditions, it is essential to utilise a microorganism giving a high PHA productivity to sustain its economic production i.e. a cheap or free carbon source alone is insufficient (Theobald, 2015).

5.4.2 Polyglutamic acid (PGA)

Polyglutamic acid (PGA), an extracellular biopolymer, is produced by many *Bacillus* species and was discovered as a capsule surrounding *Bacillus anthratis* by Ivanovics and co-workers in 1937. In 1942, Bovarnick discovered that it can be produced as an extracellular by-product of fermentation by *Bacillus subtilis*. (Goto & Kunioka, 1992). It is a biodegradable anionic substance that consists of D- and L-glutamic monomers held together by γ-amide linkages between the carboxylic groups, as shown in Figure 5-7: .

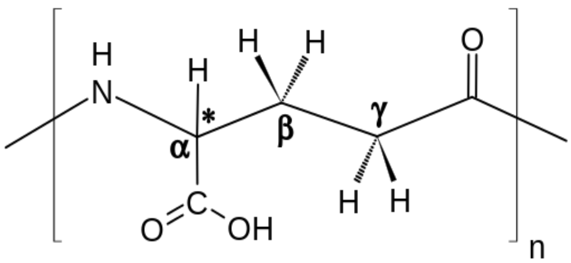


Figure 5-7: Chemical structure of PGA (Wikimedia, n.d.)

This water soluble, non-toxic polyamino acid has potential for a diverse set of industrial applications because it has a wide range of functions (Shih & Wu, 2009). It has been successfully used in the food, wastewater and medical industries (Ogunleye, et al., 2014) as shown in Table 5-5. It is currently expensive to produce, with the main costs associated with purification (Kumar, et al., 2014). In less pure form, it can be used as a flocculant (Carvajal-Zarrabal, et al., 2011) and soil conditioner (Shih & Wu, 2009).

Table 5-5: Applications of PGA in various industries (Ogunleye, et al., 2014)

Applications	Function	Reference
Biopolymer flocculant	PGA supplemented with cations show a high flocculating activity.	(Bajaj & Singhal, 2011)
Heavy metal removal	The covalent incorporation of PGA on to a microfiltration membrane results in a high metal sorption ability.	(Bhattacharyya, et al., 1998)
Dye removal	PGA could be used to remove basic dyes from solution. At a pH of 1, the dyes can be removed from the PGA, making the PGA available for re-use.	(Inbaraj, et al., 2006)
Medical metal chelator	PGA-coated super paramagnetic iron oxide NPs demonstrated high heavy metal removal efficiency from simulated gastrointestinal fluid and a metal solution.	(Inbaraj & Chen, 2012)
Medical biological adhesive	An aqueous solution of PGA and gelatine can be used for the formation of hydrogels in the presence of water-soluble carbodiimide. This can be used as a tissue adhesive.	(Otani, et al., 1999)
Medical calcium absorber	The presence of PGA in in the intestine increases calcium absorption by inhibiting the formation of an insoluble calcium complex with phosphate.	(Tanimoto, et al., 2007)
Food texture enhancer	Wheat bread supplemented with PGA enhances the thermal and rheological properties of the dough.	(Shyu, et al., 2008)
Food oil-reducing agent	Food supplemented with PGA reduces oil uptake in deep frying.	(Lim, et al., 2012)
Biodegradable plastic	Esterified PGA has shown to be a good thermoplastic. PGA's ester derivatives have the ability to form fibres and films.	(Kubota, et al., 1995) (Shih & Wu, 2009)
Bio-control agent	A combination of lipopeptides and PGA increase nutrient consumption in seedlings.	(Wang, et al., 2008)
Glucose sensor	PGA film helps with the immobilisation of glucose for glucose sensor preparation.	(Yasuzawa, et al., 2011)
Antibacterial activity	PGA has demonstrated activity against *Samonella enteritidis* SEM 01 and was compared with commercial antibiotics linezolid and ceflaclor and cytocompatible.	(Inbaraj, et al., 2011)
Treatment of xerostomi (dry mouth)	The presence of PGA in the mouth aids with salivary secretion.	(Uotani, et al., 2011)

The *Bacillus* species is a well-known robust workhorse that is used in many industrial applications such as production of heterologous proteins, antibiotics, nucleotides, biosurfactants, biofuels and biopolymers (Meissner, et al., 2015) They produce PGA under starvation as a glutamate (Ogunleye, et al., 2014) source as well as for protection under harsh conditions (McLean, et al., 1990). The industrial production of PGA is traditionally by running fermentation in a classic continuous stirred tank reactor (CSTR) with a steady nitrogen source (Bending, et al., 2014). PGA producing bacteria can be grouped into two categories: (i) L-Glutamic acid dependent microorganisms, where PGA cannot be synthesised without the presence of this amino acid in the cultivation medium and (ii) L-glutamic independent bacteria where they are able to synthesise the polymer in the absence of L-glutamic acid in the medium because of the de novo pathway of L-glutamic acid synthesis (Xu, et al., 2005). PGA biosynthesis takes place in two steps. The first step involves the synthesis of L- and D- glutamic acid monomers. The second step joins these monomers into a polymer. The size of these polymers differ from organism to organism and is also dependent on the nutrients in the cultivation medium (Bajaj & Singhal, 2009).

PGA is produced mainly from citric acid and ammonium sulphate found in the tricarboxylic (TCA) cycle as shown in Figure 5-8 in the mitochondria of the cells. Citric acid is metabolised to isocritic acid and then α-ketoglutaric acid which is a glutamate precursor (Moraes, et al., 2013).

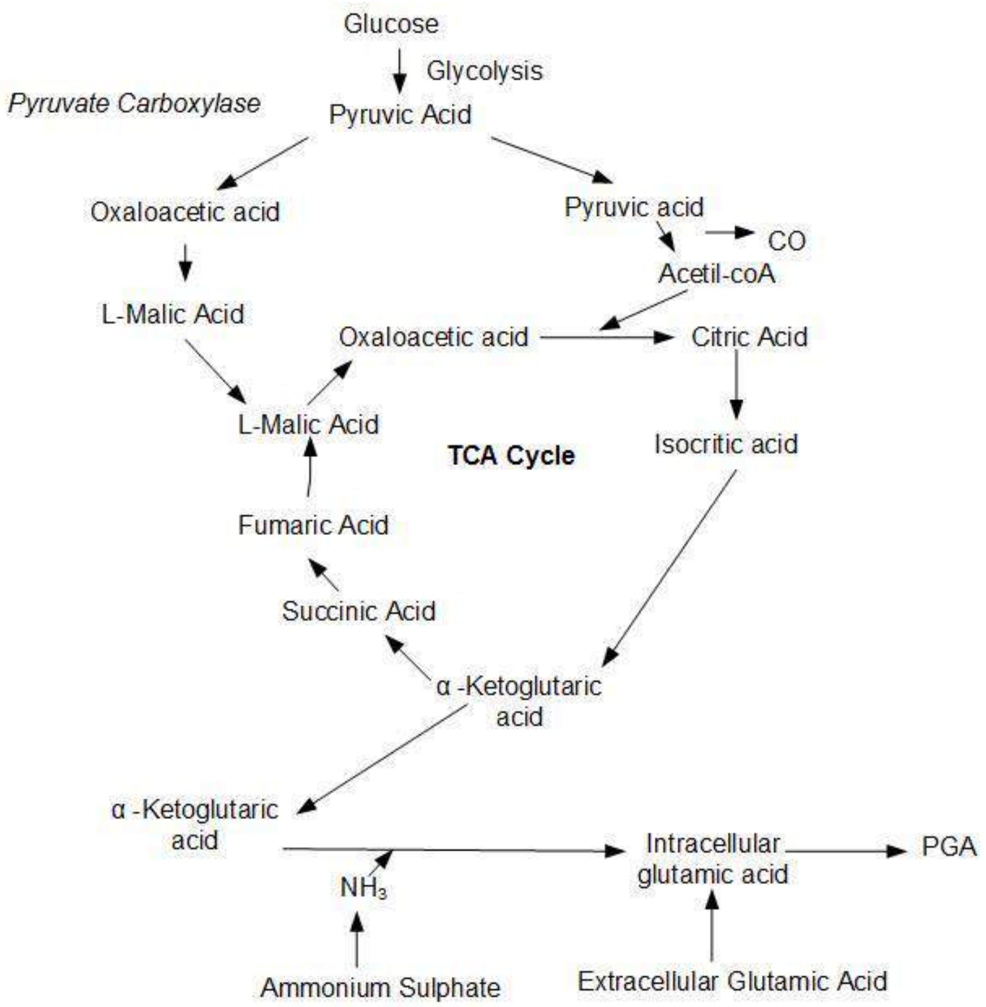


Figure 5-8: Pathway for PGA production (image redrawn from Moraes, et al. (2013))

Due to its potential for wastewater treatment and range of other possible uses, producing this polymer in the wastewater biorefinery will be beneficial. The polymer's properties (Margaritis, 2003) and protective function towards the bacteria producing it makes it likely that its production from wastewater by a mixed microbial consortium could be successful.

Research on PGA has largely been focused on sterile bioprocesses at laboratory scale (Cromwick, et al., 1995). Some research has investigated production from waste solids, notable swine manure (Chen, et al., 2005), cow manure (Yong, et al., 2011) and solid substrate fermentation using soybean powder and wheat (Xu, et al., 2005). One study used untreated cane molasses, at laboratory scale (Zhang, et al., 2012) but to date no publications have been found on production of PGA from wastewater. This was extensively investigated in a previous WRC project K5/2000 (Verster, et al., 2013) and (Madonsela, 2013).

In order to further the ability to select the correct bioproduct for each WWBR, more experimental work is needed investigating production of these products using local microbial cultures with wastewater feedstock. The start of an experimental study in the production of PGA using local microbial cultures and moving towards testing on wastewater as growth medium is reported in Appendix D.

5.5 Bio-Based Products for the Integrated WWBR

In an integrated WWBR the whole range of potential products must be assessed so that the entire process produces an adequate range of bio-based products, while simultaneously breaking down and consuming the nutrients available in the feedstock to produce the compliant effluent water. This chapter examined the potential of various bio-based products focussing particularly on biopolymers likely to be associated with the functioning of the bacterial bioreactor, positioned to deplete the organic loading of the wastewater.

The process followed here demonstrates how, beginning with a broad overview of common products (Section 5.1), the most suitable suite of product types can be selected for each bioreactor. This selection can take account of the potential for use within the WWBR or the parent process, as well as ensuring a good spread of output from the biorefinery as a whole.

Within each product type selected, a procedure can be employed similar to that used here, enabling the choice of specific product. This will include a careful analysis of the potential products within that category, detailing the various methods of grouping the potential products.

Some additional assessments will then have to be made within each grouping of products. The specific products will have to be evaluated in terms of market trends (global, national and local). The technological position of each must then be appraised in terms of both the availability of commercial scale technology for production and the technical readiness of the potential market for absorption of the product. For some products the sociological positioning of the product as produced from wastewater will also have to be considered.

The way this consideration of product options is incorporated within the whole conceptualisation of a particular WWBR forms part of the exploration of the way forward in Chapter 9.

6 REVIEW OF POTENTIAL BACTERIAL BIOREACTORS: CRITERIA FOR SELECTION

Owing to the typically dilute nature of wastewater streams, their variability, the impracticalities of sterilisation, and the need to handle large effluent volumes, the selection of appropriate bioreactors for the WWBR application depends on meeting multiple process criteria. The particular technologies available for each of the different types of bioreactor, bacterial, algal, macrophytic and fungal (outlined in Section 3.4), must be assessed with the constraints of the WWBR in mind. A detailed assessment of bacterial bioreactors is presented in this chapter as a paradigm for bioreactor selection.

To aid in this selection, the necessary criteria for WWBR reactors are developed in Section 6.1. Current bacterial bioreactor technology used in South Africa's WWTWs is reviewed in Section 6.2. The various technologies are then assessed in Section 6.3 against the criteria specified and suitable bioreactor technologies for application in WWBRs are listed and reviewed. The bacterial bioreactors selected as suitable for application in WWBRs are detailed in Section 6.4.

6.1 Challenges for Bioproduction from Wastewater

Current wastewater bioreactors are well designed to achieve nutrient removal from the wastewater with limited design towards product recovery. The main focus is the delivery of clean water. From a bioprocess engineering perspective, using wastewater streams presents unique challenges in terms of product recovery. Traditional product-focused bioreactor optimisation aims to reduce the bioreactor volume in order to reduce the energy invested per unit product. It also aims to achieve a high biomass concentration which results in lower DSP cost per unit product (Richardson, 2011). Using wastewater as raw material is counter-intuitive as it combines wastewater treatment and bioprocess approaches. Intentionally innovative bioreactor design contributes to the viability of using wastewater as a low cost and highly available raw material.

WWBRs are not suited to all types of product. The chosen products are required to meet commodity market needs, be suited to the utilization of organics from large stream flows and serve an ecological function for the microorganism to drive its competitive advantage (Kleerebezem & van Loosdrecht, 2007; Verster, et al., 2014). Bioreactor design needs to enhance this ecological niche in order to produce the desired product.

These challenges are listed in Table 6-1 along with design and operational requirements needed to address them. These are investigated further in the following sections.

Table 6-1: Wastewater biorefinery bioreactor design requirements

#	Requirement	Comply?
Large Volume		
1	Decouple hydraulic and solid retention times	✓
2	Continuous or semi-continuous (cannot store flows)	✓
3	Think big! Commodity rather than niche	✓
Complex, Variable		
4	Influence microbial community, non-sterile	✓
5	Give advantage to product: create ecological niche	✓
Environment		
6	Water released into environment eventually	✓
Down-Stream Processing		
7	Product formation in different phase?	✓
8	Can product be recovered?	✓
9	Reactor design conducive to reducing DSP load?	✓

6.1.1 Large volumes of wastewater

Very low concentration of valuable product

One significant challenge of bioprocesses is the dilute nature of the medium, with both substrates and products present at very low concentration, typically less than 5% of the total dissolved solids. When using waste streams like municipal wastewater which can be a thousand fold more dilute, this aspect is even more challenging. The apparent biocatalyst concentration must be increased to enhance process intensity over the current approach of huge dilute vats of water by allowing a reduction in residence time. In addition, adequate nutrient provision to the cells must be ensured without compromising the ability to recover the product. This defines the mass and energy transfer needs. Aeration and heat transfer in dilute media is inefficient and energy intensive. By using biomass retention, these requirements can be better managed.

With respect to the product, for cost and energy efficient downstream processes, localising product in an accessible location with high apparent concentration is preferred. Many processes currently use standard bioreactor setups and optimise the downstream processing (DSP) subsequent to production. Bioreactor design has scope to facilitate DSP and can have a greater impact on overall process optimisation (Richardson, 2011). The entire process needs integrated optimisation, cognisant of the performance at the level of unit operation, process operation and systems operation (including aspects outside of the process).

Aeration

Oxygen is sparingly soluble in water. In the typical high-volume low-concentration bioprocesses energy for aeration is the biggest burden in terms of economics and sustainability. In wastewater treatment, aeration can be up to 70% of the operating costs (Tchobanoglous, et al., 2003). Oxygenation is often controls stoichiometric limitation, and frequently also governs the reaction rate (Bailey & Ollis, 1986). Aeration in biofilms presents a special challenge due to the additional barrier that the thickness of the biofilm layer poses to oxygen diffusing through to the deeper biomass.

Aeration can also be used as a mixing device. With biofilms, the shear associated with aggressive airflow can be used for the sloughing off of biomass as a rudimentary type of downstream processing. Types of aeration include separate aeration of the flow of recycle, aeration in the support medium itself and aeration of the biofilm. (Henze, et al., 2002):

6.1.2 The need for biomass retention

When the substrate concentration in the feed is high (> 10 g-COD/ℓ) and rapidly growing organisms (growth rate > 0.1 /h) are used, there is no need for biomass retention from a biomass concentration perspective (Figure 6-1), (Nicolella, et al., 2000). In dilute WWT, biomass retention is advantageous as conversion is limited by the amount of biomass present and retention allows the necessary increase in biomass concentration (Nicolella, et al., 2000). This may be applied to the retention of an inoculated or a natural mixed microbial community. Biomass retention also facilitates the effective decoupling of the hydraulic and solid retention time which may be used to improve bioreactor volumetric conversion capacity.

A majority of WWTW employ activated sludge. The resultant flocs require large settling ponds. The two approaches that are most promising for WWBR bioreactor design are to generate conditions suitable for static biofilms with slightly higher flowrates, and particle biofilms occurring at slightly higher substrate concentrations. At high substrate concentrations, sufficient biomass or product may be formed to justify conventional bioprocess approaches using single cells.

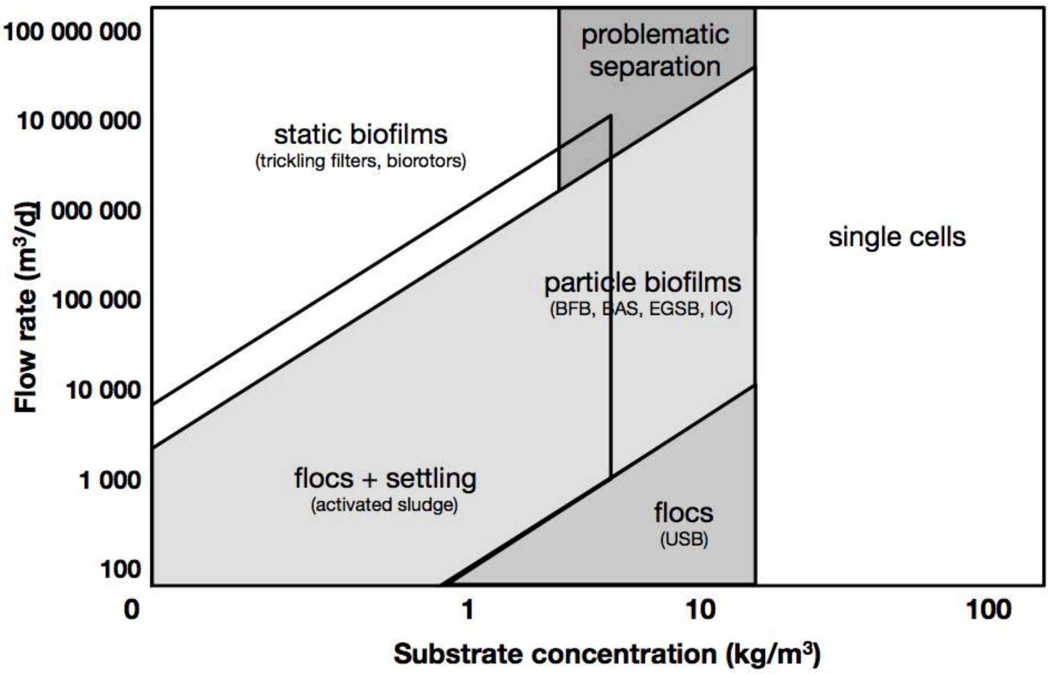


Figure 6-1: Concentration-flowrate phase diagram for application of floc and biofilm bioreactors (adapted from (Nicolella, et al., 2000)).

If a bioprocess is designed to produce and isolate a product from a low substrate stream as well as treat the water, retention of the biomass and product recovery are essential. This involves decoupling hydraulic residence time and biomass residence time. Biomass retention is used to increase the apparent biocatalyst concentration and ensure separation of biomass from the liquid stream. Accumulation of the product into a phase other than the dilute liquid phase may also be used to concentrate the product.

Biomass retention can be established by using recycle loops, immobilisation through biofilm formation, granulation, retaining the biomass in suspended form through selective membranes, or a combination of these. In wastewater treatment, immobilisation typically relies on the controlled growth of a biofilm or the formation of flocs or aggregates of biomass. Cell entrapment in immobilisation matrices is more common in bioprocess applications. Flocs are included as a form of biofilm without solid support in this review to allow inclusion and comparison of the granular sludge with other biofilm techniques. Filters may require less maintenance if the biomass is not suspended, for example through combining cell immobilisation with filtering or by including a settling stage prior to filtering.

If the product is cell-associated, retention of the biomass forms the first stage of product concentration and the retention medium needs to be designed to be suitable for biomass recovery.

6.1.3 Design for downstream processing

A fundamental consideration in the feasibility of bioprocess from dilute streams from both an economic and environmental point of view is in the approach to DSP. In these dilute systems, recovery of both the product and the water is essential. The latter may be recycled back to the process upstream of the WWTW or recovered as water of useable quality, 'fit for purpose'. In a systems approach, the recovery and quality of both water and co-product need to be considered. There are three main requirements to realise effective product formation and recovery from dilute (waste) streams:

- Decouple hydraulic residence time and biomass residence time. Biomass retention to increase the apparent biocatalyst concentration can also contribute to concentrating the product into a different phase.
- Ensure adequate nutrient provision to the cells without excessive energy requirement for mass transfer, and without compromising the ability to recover the product.
- Design for DSP. Bioreactor design and choice of the biological system used affects the cost of DSP significantly. In dilute waste streams, many DSP methods are not cost effective, as the combination of volume processed and energy requirement per unit volume is too great. The need for centrifugation, for example, is a challenge that cannot be addressed at DSP level, but needs to be prevented through choice of system and bioreactor design. Concentration of the product into a separate phase, either the gas phase or settleable biomass phase is proposed to facilitate product recovery.

Design of cell retention and recovery can be used in combination for improved productivity and facilitation of DSP. If the product is soluble, separation of the biomass from the liquid usually precedes purification steps such as precipitation, ultra-filtration and chromatography, unless an affinity step can be implemented. However, the solid-liquid separation is still needed for water purification. Where chemicals are added to precipitate product, biomass should be removed first to prevent its contamination of the product. It should be noted that the need for addition of chemical reagents such as precipitation agents is not a preferred route for the recovery of products from dilute suspension.

While biomass retention is important for reasons outlined in this review, it serves different functions depending on where the product is located, and whether the biomass itself is recovered or not. This determines bioreactor selection. Figure 6-2 is an initial guideline for wastewater biorefinery bioreactor selection.

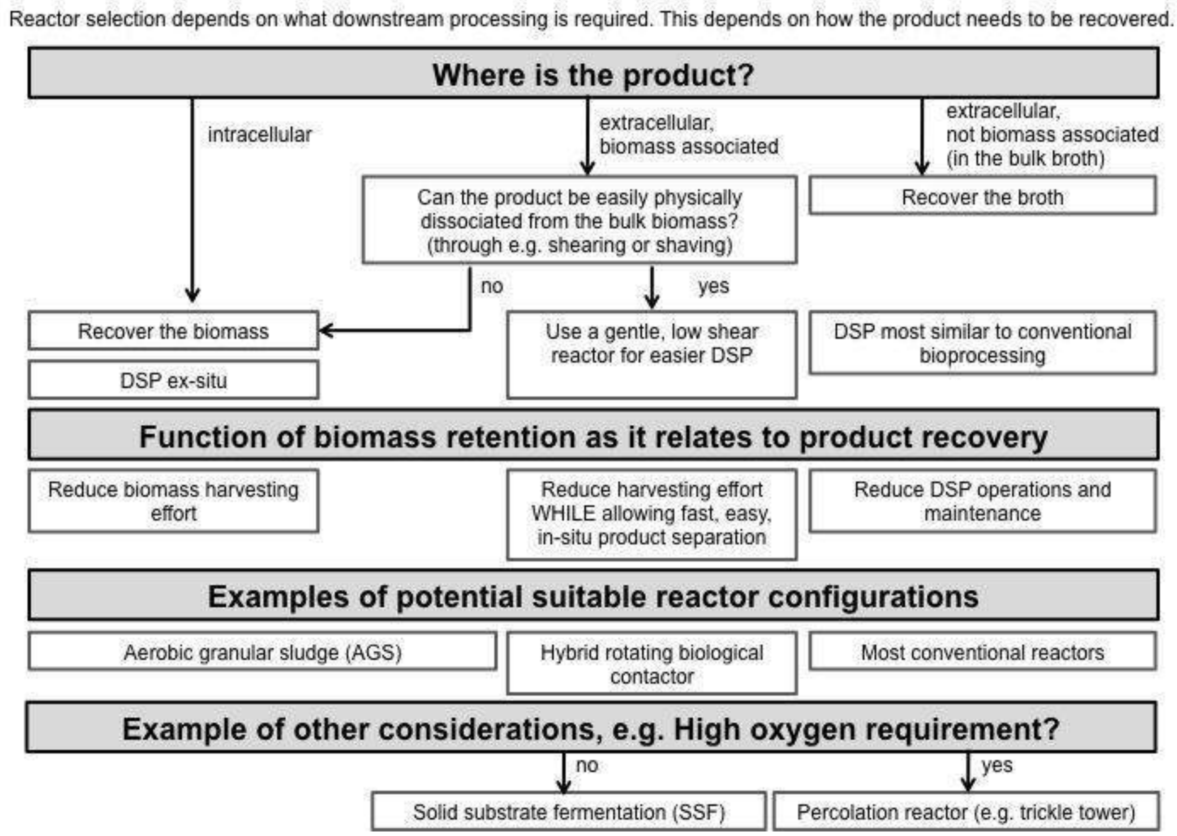


Figure 6-2: Suggested guideline for wastewater biorefinery bioreactor selection (Verster et al. 2013)

6.1.4 Release into the wider environment

Conventional bioprocessing requires a homogeneous, highly controlled environment, but wastewaters tend to be more complex and heterogeneous. As the water is destined for discharge to the environment, any additives to improve the characteristics of the stream need to be non-hazardous. The volume of the stream precludes extensive stream modification. Depending on the robustness of the organism, environmental regulation and social acceptability, the use of genetically modified organisms may also be precluded. Further, sterilisation is typically not practical. This results in limited scope for modification of the microbial community in the manner currently favoured for bioprocess applications. Instead, the most robust and resilient microorganisms make up a mixed community which is well adapted to the physicochemical environment in which it exists and is able to withstand shock loads and hostile environments (Chen, 2013).

Reducing the pathogen loading in the water that is released to the environment relies on the production and recovery of products. Although bioreactor design may be orientated towards reducing DSP costs through a product which can be recovered from a concentrated stream (Section 6.1.3), there can be significant advantage to bioproduction processes including downstream processing of the entire stream for product recovery because of the reduction of pathogens in the effluent (Stephenson, et al., 2000).

6.2 Reviewing and Assessing Bioreactors Currently Used in WWT in South Africa

Through the WRC projects K5/1732 (Brouckaert, et al., 2013) and K5/2000 (Verster, et al., 2013), it has become evident that the implementation of the WWBR concept benefits from adhering to key principles in the selection for each unit operation of the system and that bioreactor selection is a crucial element of this. The key principles, established in Section 6.1 are

- the selection of a product existing in a different phase to the aqueous nutrients to facilitate product recovery
- the selection of a microbial phase favouring retention in the system to allow the decoupling of the biomass and hydraulic residence times
- application of non-sterile bioproduction systems
- the utilisation of a multicomponent system allowing the integrated optimisation of the system rather than direct competition between water quality and product formation

These principles provide the framework for bioreactor selection for the conversion of organics to product. Through selected case study(s), the role of bioreactor design and configuration can be explored. The principles for integrated optimisation, including product recovery and product formation operations, should also be explored.

Table 6-2 provides an overview of technologies and bioreactor types used in current WWTW in South Africa, as well as their principle of operation. Their suitability for use in a WWBR, as defined by the selection criteria of Table 6-1, is assessed and the number of categories that each bioreactor type or technology fulfils is indicated. The bioreactors suitable for WWBRs are reviewed in detail in Section 6.3 to inform final bioreactor selection, detailed in Section 6.4.

The list of requirements rendering the bioreactor type and technology useful has been numbered in Table 6-1: In Table 6-2 the number of each category that the bioreactor or technology fulfils is shown and its relevance for application in WWBRs indicated based on the number of the categories fulfilled. The focus was on the principle of operation of the bioreactors, whether they would result in easier downstream processing (category 7 to 9) and their potential for retrofitting for use in the WWBR. Based on the findings of Verster, et al. (2013) in Section 6.1.3, the highest priority requirements were outlined to be the decoupling of hydraulic and solid residence times (1), and the downstream processing requirements (7 to 9).

The existing technologies used in South African WWTW which did not fulfil categories 1 and 7 to 9 were excluded from the shortlist. There is little sense in selecting a technology that increases the financial investment based on its principle of operation and reliance on the traditional energy-intensive downstream processing, known to be costly. With this in mind, Activated Sludge and Biological Nutrient Removal were not considered further.

Table 6-2: Summary of bioreactor types or technologies used in WWT and their suitability to be used in WWBRs

Bioreactor Type / Technology Used		Principal of Operation	Number of the nine requirements fulfilled	Suitable for use in wastewater biorefineries?
A	Activated Sludge (in CSTR)	This technology makes use of suspended growth bioreactor technology. It consists of flocculated slurry of microorganisms that are used to remove soluble and particulate biodegradable matter from the wastewater. The type of bioreactors used are typically CSTRs in various configurations depending on the conditions desired and or level of treatment.[1] It is one the most common forms of wastewater treatment technologies used in South African municipalities. [1. 2. 3]	2, 3, 4, 6 (4/9)	X
B	Biological Nutrient Removal (BNR) (in CSTR in series with recycle)	BNR is similar in operation to the activated sludge systems. These systems are some of the most complicated technologies used for WWT, and come in a variety of configurations. BNR processes are divided into different zones where the biological environments are different and allow for removal of nitrogen and/or phosphorus [1] BNR usually consists of CSTRs in series with recycles incorporated to achieve the different zones.	2, 3, 4, 6 (4/9)	X

Bioreactor Type / Technology Used		Principal of Operation	Number of the nine requirements fulfilled	Suitable for use in wastewater biorefineries?
C	Packed Bed Reactor (PBR)	PBRs fall under the category of submerged attached growth bioreactors. Granules used to create the packed bed are small in size and typically only a few millimetres in diameter. The particle carriers used can be plastic, rounded sand or fired clay. The packed bed acts as a physical filter for particulates, and can be used to oxidise soluble and particulate organic matter and achieve nitrification and denitrification. The flow within the packed bed can be either upward or downward.	1, 3, 4, 5, 6, 7, 8, 9 (8/9)	✓
D	Fluidized Bed Biological Reactors (FBBR)	FBBRs are also type of submerged attached growth bioreactor that has been largely used for the treatment of industrial wastewater. The upward flow of the influent wastewater creates drag forces that suspend the carrier particles upon which the biofilm grows. As the biomass grows, it results in the expansion of the bed height. To prevent the loss of carrier particles and uncontrolled bed expansion, separators are usually included in the process to return carrier particles to the FBBR and remove excess biomass. [1. 3]	1, 3, 4, 5, 6, 7, 8, 9 (8/9)	✓
E	Rotating Biological Contactor (RBC)	RBCs fall under the category of attached growth bioreactors. The microorganisms form biofilms on the disks that are attached to a shaft and rotate in the liquid (wastewater). The shaft and disks are oriented perpendicularly to the direction of the influent. More than one RBC is typically used, oriented in series to achieve the desired effluent quality. Oxygen transfer is created by the rotation of the disks that are only partially submerged. They are commonly used by WWTWs.[1]	1, 2, 3, 4, 6, 7, 8, 9 (8/9)	✓
F	Trickle Bed Reactor (TBR or TF)	The TBR, also known as a trickling filter, is a type of attached growth biofilm bioreactor in which the substrate is trickled over a fixed carrier. Air is passed counter-current up the bed where diffusion between the wastewater and biofilm occurs. The trickling filter bioreactors used in industrial applications consist of a recycle stream to improve nutrient removal, as well as a liquid-solid separation unit. It was one of the first technologies used to treat wastewater and is well-established and understood. [1. 2. 3]	1, 3, 4, 5, 6, 7, 8, 9 (8/9)	✓
G	Membrane Bioreactor (MBR)	This technology is a variation of the Activated Sludge process that includes a liquid-solid separation through the use of filtration membranes (flat sheet or tubular). It achieves a high quality of effluent and is increasingly being used in the WWTWs and in some industries in South Africa. [1. 2]	1, 2, 3, 4, 5, 6, 7, 8 (8/9)	✓
H	Moving Bed Bioreactor (MBBR)	The MBBR process is based on attached growth biofilm principles of biological WWT. The core of the process is the biofilm carrier particles. While the biofilm is fixed to the carrier particles, it is thoroughly mixed and retained within a bioreactor. Carrier particle circulation within the bioreactor is provided by the aeration system or by mixers (anaerobic conditions). [1. 3]	2, 3, 4, 5, 6, 8, 9 (7/9)	✓
I	Aerobic Granular Sludge (AGSR)	Dense granules of strong biomass structure are formed which are essentially aggregates of microorganisms that are densely packed with a much higher settling rate than the conventional sludge, that is so well known in biological WWT. [4]. Out of their unique characteristics, the most desirable attribute is their high biomass retention ability, which allows the smaller reactors and shorter hydraulic residence times. . Thus far, Sequentially Operated Batch Bioreactors (SBRs) are the only bioreactor type that has successfully been able to cultivate the granules according to Adav et al. [4]. The bioreactor is very simple in design and is fed discontinuously, although it can be manipulated to operate under continuous flow conditions. These characteristics of the bioreactor, along with the high settling rates of AGS make it an ideal niche to study the formation of products from wastewater in laboratory settings. [5])	1,3,4,5,6,7,8,9 (8/9)	✓

Bioreactor Type / Technology Used	Principal of Operation	Number of the nine requirements fulfilled	Suitable for use in wastewater biorefineries?
1. Grady, C. L., Daigger, G. T., Love, N. G., & Filipe, C. D. M. (2011). Biological Wastewater Treatment (3rd ed.). London: IWA Publishing. 2. DWA. (2008). Municipal Wastewater Treatment. Gauteng. Retrieved from https://www.dwa.gov.za/dir_ws/wsam/vdfileload/file.asp?val=14&tablename=AsetFiles&fld=ID 3. Merwe-Botha, M., & Quilling, G. (2012). Drivers For Wastewater Technology Selection Assessment of the Selection of Wastewater Treatment Technology. Retrieved from http://www.wrc.org.za/Knowledge Hub Documents/Research Reports/TT 543-12.pdf 4. Adav, S. S., Lee, D.-J., Show, K.-Y., & Tay, J.-H. (2008). Aerobic granular sludge: recent advances. Biotechnology Advances, 26(5), 411–23. doi:10.1016/j.biotechadv.2008.05.002 5. Johnson, K. (2010). PHA Production in aerobic mixed microbial cultures. Technische Univeriteit Delft, Kingdom of Netherlands.			

6.3 Detailed Review of Shortlisted Bioreactors

A detailed review of the bioreactors selected in Section 6.2 is presented. For each bioreactor type, its general description, physical characteristics, operating conditions, economic requirements and impact on downstream processing and recovery is considered. The selection of five bioreactors was made based on current technologies that are used by South African WWTWs, new technologies showing promise in large scale application for wastewater treatment and that also fulfil the requirements for application in the wastewater biorefineries space, and finally, that are suitable for large flowrates.

Figure 6-3 provides a visual representation of the reactors presented in Table 6-2, with respect to product recovery potential and degree of biomass retention. Both of these are important when selecting an appropriate bioreactor technology for the WWBR.

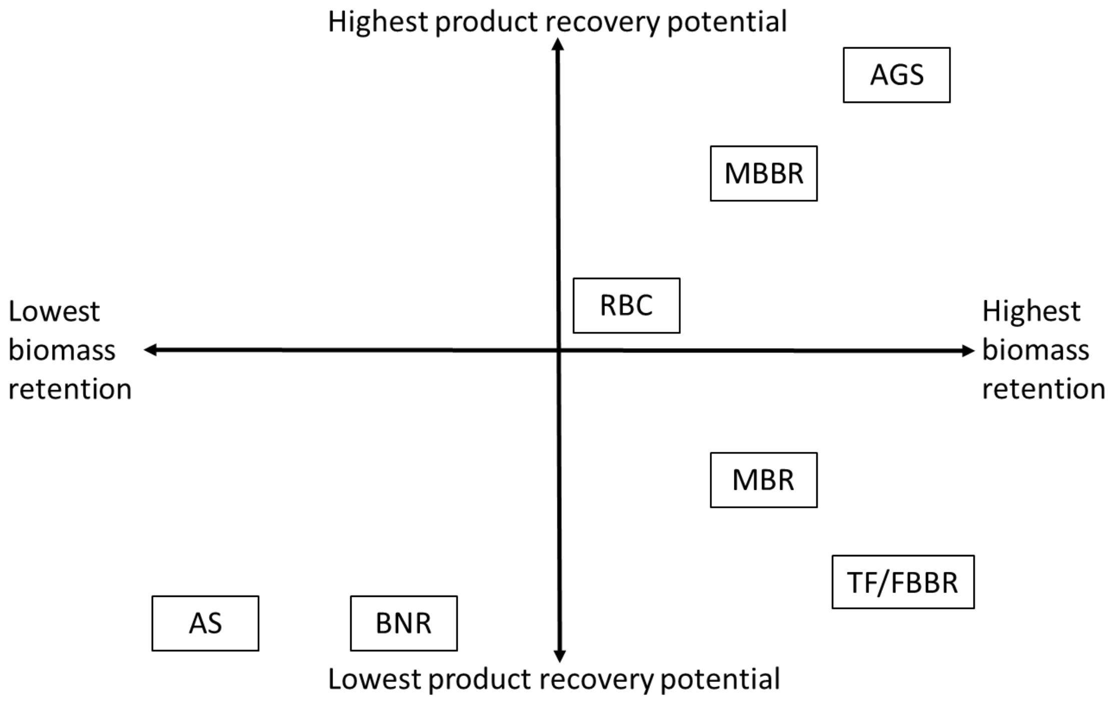
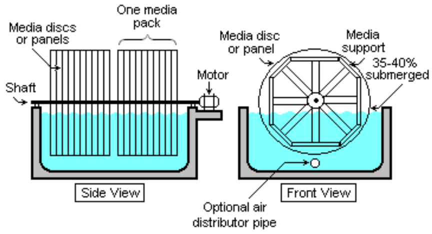


Figure 6-3: Summary chart of the bioreactor technologies in Table 6-2 and their compliance with important criteria for application in WWBRs
AGS: aerated granular sludge; AS: activated sludge; BNR: biological nutrient removal; FBBR: fluidised bed biological reactor; MBBR: moving bed bioreactor; MBR: membrane bioreactor; RBC: rotating bed contactor; TF: trickle filter (trickle bed reactor TBR)

The five bioreactors that were selected and assessed are the Rotating Biological Contactor (Table 6-3), Trickle Bed Reactor (Table 6-4), Aerated Granular Sludge Reactor (Table 6-5), Membrane Bioreactor (Table 6-6) and Moving Bed Bioreactor (Table 6-7). The packed bed reactor has similar principles of operation to the Trickle Bed Reactor, and detail is provided on the last of these only, as it known to be one of oldest and most well understood wastewater treatment technologies. The tables summarise the main characteristics of these bioreactors in WWTWs. Where possible, examples in use in South Africa are provided. The main advantages and disadvantages and physical and operational characteristics are also discussed. Associated approaches to use in a wastewater biorefinery have been considered as has the effect on downstream processing.

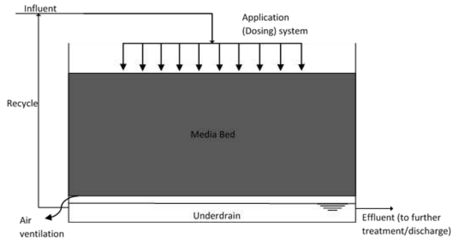
Table 6-3: Comparison of five bioreactors suitable for wastewater biorefineries – Rotating Biological Contactor

Rotating Biological Contactor	
Diagram	 [1]  [2]
Description	The RBC consists of closely packed circular disks with surface areas of approximately 9 300 m^3 to 13 900 m^3, made from polystyrene or polyvinyl chloride. The disks are mounted on a horizontal shaft and submerged (typically 40% of the rotating unit as shown above) in the holding tank containing the wastewater. Typical dimensions of the shaft are 1.52 m to 8.23 m with a thickness of 13 to 30 mm. The shaft rotates slowly at 1 to 2 revolutions per minute. The disks are typically made of high density polyethylene with UV inhibitors. [3]
When or Why Used in WWTWs	RBCs are a type of static biofilm reactor that uses attached-growth biological treatment. The RBC process has been used extensively and is a well-established process, for the pre-treatment of industrial wastewater, BOD removal as well as nitrification and denitrification [3]. This type of bioreactor is used in WWTWs with flows below 40 000 m^3/day as economies of scale are poor. They are typically used to nitrify municipal wastewaters with carbon oxidation and nitrification applications. They have also been used successfully in treating industrial wastewaters with low to moderate strengths of hydrogen sulphide. [4]
Advantages	Mechanically simple and reliable. Low energy usage (3.7 to 5.6 kW per shaft). Motion of shaft causes aeration by exposure to atmosphere and shear stress. Large-scale operations are successful and well implemented worldwide. Modifications are easy to apply and biomass can be easily removed. Able to handle lower substrate concentrations (preferable) [3. 4. 5].
Disadvantages	Requires good pre-treatment and primary clarification to avoid solids in the units. Algal growth has been noticed if units are not covered sufficiently. Lack of understanding of biological process causes system and structural failure. Limited process flexibility. Often more than one unit required, taking up valuable land space [3. 4. 5].
Physical Characteristics	
Reactor Size	Units are typically produced in standard dimensions [4]. Information on typical reactor sizes used in South Africa's Wastewater Treatment Plants could not be found.
Arrangement/ Configuration	Typical arrangement is in series with stages dependent on the degree of treatment required. Two to four stages has been used to achieve BOD removal, with six or more stages to achieve nitrification. Typical staging arrangements are flow parallel to shafts, flow perpendicular to shafts, step feed flow or tapered feed flow parallel to shafts [4].
Operating Conditions	
Hydraulic Retention Time	Variable. A function of each reactor design and the constraints. Also dependent on level of treatment desired. $\Lambda_{H,RBC} = \frac{F}{A_s} \ldots (1)$ where A_s is the media surface area and F is the influent flowrate. [4]
Organic Loading	Studies on full-scale RBC facilities indicate that oxygen limitations occur at COD Soluble Organic Loading of 20 to 35 g COD/(m^2day) (SOL value). [4]
Effluent Treatment	Effluent is of the South African general standards for discharge limits [6].
Aeration Requirements	Oxygen is supplied from the atmosphere into the attached biofilm on the portion of the RBC media exposed to the atmosphere. Oxygen enters the bulk liquid by turbulence created from the motion of the rotating disks.
Economic Requirements [7]	
Capex	Medium capital cost
Opex	Medium Operation Cost Medium power consumption Medium technology level

Downstream Processing and Product Recovery	
Associated Approaches to Product Recovery	For biomass associated products, biomass is removed from disks e.g. by low shear forces. Product in the supernatant is collected by DSP is similar to traditional, costlier bioprocessing
Potential Products	A bioproduct associated with the biomass would be ideal

http://dipgra-feder.es/proyectos/images/ecemed/actuaciones/ampliacion-edar/despues.jpg
https://upload.wikimedia.org/wikipedia/commons/3/3c/Rotating_Biological_Contactor.png
Satterfield, C. N. (1975). Trickle-bed reactors. AIChE Journal, 21(2), 209–228. doi:10.1002/aic.690210202
Grady, C. L., Daigger, G. T., Love, N. G., & Filipe, C. D. M. (2011). Biological Wastewater Treatment (3rd ed.). London: IWA Publishing
Adav, S. S., Lee, D.-J., Show, K.-Y., & Tay, J.-H. (2008). Aerobic granular sludge: recent advances. Biotechnology Advances, 26(5), 411–23. doi:10.1016/j.biotechadv.2008.05.002
De Kreuk, M., Krishida, N., & van Loosdrecht, M. (2007). Aerobic granular sludge - State of the Art. Water and Science Technology, 55(8-9), 75 – 81
Merwe-Botha, M., & Quilling, G. (2012). Drivers For Wastewater Technology Selection Assessment of the Selection of Wastewater Treatment Technology. Retrieved from http://www.wrc.org.za/Knowledge Hub Documents/Research Reports/TT 543-12.pdf

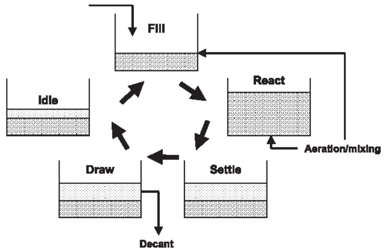
Table 6-4: Comparison of five bioreactors suitable for wastewater biorefineries – Trickle Bed Reactor

Trickle Bed Reactor	
Diagram	 [1]  [2]
Description	A typical TBR consists of five major components: namely, the carrier bed, containment structure, wastewater application system, underdrain system and the ventilation system. The carrier bed provides the surface on which the biomass grows. Medium bed materials vary in size, porosity and shape. Plastic (PVC or polypropylene) is typically used as the medium material. [3]
When or Why Used in WWTWs	The TBR, also known as the trickling filter has been in use for nearly 100 years to treat municipal and industrial wastewaters aerobically [4. 5. 6]. It is essentially a packed bed biofilm reactor in which the wastewater is trickled over a fixed carrier. Air is counter-currently passed up the media where diffusion between the wastewater and biofilm occurs. The TBRs used in industrial applications include a recycle stream to improve nutrient removal, as well as a liquid-solid separation unit
Advantages	Well-established and accepted treatment process. Easy to operate. Recycling of the unclarified effluent stream can re-inoculate the reactor with biomass producing bacteria. Low pressure drop across bed lowers the power requirements for ventilation. Able to handle low substrate concentrations
Disadvantages	Clogging of biofilm carrier due to excessive biomass or extracellular polymer growth, or poor pre-treatment of influent (presence of particulates). Harvesting of biomass could be difficult because biomass is attached growth and densely packed Recycling increases pumping duty and operating costs. A continual aeration requirement adds to operating costs
Physical Characteristics	
Reactor Size	Variable, depending on what the treatment objectives are. WEF reports that the depth of typical trickle bed reactors varies from 0.91 to 6.10 m if roughing is desired [7]. For carbon oxidation, BOD and nitrification, and pure nitrification, the bed depth is typically <12.2 m
Arrangement/ Configuration	TBRs are typically arranged in series, with primary clarifiers before the first stage, and secondary clarifiers after the final stage. Occasionally intermediate clarifiers are between stages
Operating Conditions	
Hydraulic Retention Time	It is not possible to determine the biomass concentration within a trickling bed reactor easily, thereby making it difficult to calculate a sludge retention time or a process-loading factor. Some values for the biomass concentration in a TBR have been reported, but no consensus has been reached on the appropriate manner of calculating this [3]. WEF reports that the Total Hydraulic Load (THL) can be calculated as follows: $THL = \frac{Q_{in} + Q_R}{A} \ldots (2)$ where Q_{in} is trickling filter influent, Q_R is recirculation stream A is media specific surface area [7]
Organic Loading	Roughing: 1.5 to 3.5 TOL kg BOD_5 (m^3day) Carbon Oxidation: 0.7 to 1.5 TOL kg BOD_5 (m^3day) Combined Carbon Oxidation and nitrification: <1.0 TOL kg BOD_5 (m^3day) [3]
Effluent Treatment	This depends entirely on the treatment objectives: roughing, carbon oxidation, combined carbon oxidation and nitrification, separate stage nitrification
Aeration Requirements	Oxygen or air is bubbled into bottom, counter current to flow of influent
Economic Requirements [8]	
Capex	Medium capital cost
Opex	Low operational cost

Downstream Processing and Product Recovery	
Associated Approaches to Product Recovery	If the product were biomass associated, the removal of the biomass from the media bed would be required. This would require multiple reactors to allow for downtime and removal of the biomass. If the product is loosely associated to the biomass and extracellular, low shear forces will separate it from the biomass. If product is in the bulk liquid, the broth will need to be collected and the DSP will be similar to traditional bioprocessing
Potential Products	A bioproduct that is associated loosely with the biomass, or extracellular products would be ideal

1. http://www.pallrings.co.uk/wp-content/uploads/2013/06/PR-Brochure_24_Pallpak.pdf
2. adapted from http://www.totalwatersolutions.co.za/rotorclear_package_plants.html#tab4
3. Grady, C. L., Daigger, G. T., Love, N. G., & Filipe, C. D. M. (2011). Biological Wastewater Treatment (3rd ed.). London: IWA Publishing.
4. Rusten, B., Eikebrokk, B., Ulgenes, Y., & Lygren, E. (2006). Design and operations of the Kaldnes moving bed biofilm reactors. Aquacultural Engineering, 34(5), 322–331. doi:10.1016/j.aquaeng.2005.04.002
5. Satterfield, C. N. (1975). Trickle-bed reactors. AIChE Journal, 21(2), 209–228. doi:10.1002/aic.690210202
6. Stephenson, T., Simon, J., Jeffereson, B., & Brindle, K. (2002). Membrane Bioreactors for Wastewater Treatment (p. 62). London: IWA Publishing
7. WEF. (2010). Biofilm Reactors by WEF. Mc Graw Hill.
8. Merwe-Botha, M., & Quilling, G. (2012). Drivers For Wastewater Technology Selection Assessment of the Selection of Wastewater Treatment Technology. Retrieved from http://www.wrc.org.za/Knowledge Hub Documents/Research Reports/TT 543-12.pdf

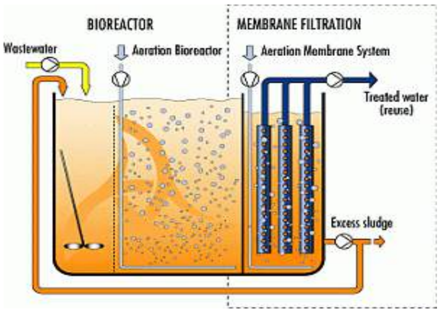
Table 6-5: Comparison of five bioreactors suitable for wastewater biorefineries – Aerobic Granular Sludge

Aerobic Granular Sludge in Sequencing Batch Reactor	
Diagram	 [1]  [2]
Description	Dense granules of strong biomass structure, larger than 0.2 mm in diameter, are formed which are essentially aggregates of microorganisms that are densely packed with a much higher settling rate than the conventional sludge in biological WWT [3]. Out of their unique characteristics, the most desirable attribute is their high biomass retention ability, which allows the smaller reactors with shorter hydraulic residence times Thus far, Sequentially Operated Batch Reactors are the only reactor type that has successfully been able to cultivate the granules according to Adav et al. [3]. The reactor is very simple in design and is fed discontinuously, although it can be manipulated to operate under continuous flow conditions
When or Why Used in WWTWs	Currently, more than 20 large scale Nereda WWTWs are in operation or under construction. In Wemmershoek and Gansbaai (Western Cape), two large scale AGS plants using Nereda technology have been successfully implemented to treat a combination of domestic and municipal wastewater. This treatment technology has shown great promise in replacing or for use in conjunction with Activated Sludge systems in WWTWs, to achieve desirable treatment objectives
Advantages	Strong, dense microbial structures are formed. High biomass retention and settleability Able to withstand high flow rates and organic loading rates Uniform and spherical in shape SBRs can be used as a continuous process No separate settling tank required, reducing plant footprint. Granules form aerobic and anoxic layers, resulting in COD removal and nitrification and the anoxic layer allows for denitrification to occur. Odour is controlled more effectively by having minimal open areas
Disadvantages	If incorrect HRT is employed, washout of fast settling granules will occur. Modifications/improvements required for streams with low COD Pre-screening and filtration to remove suspended solids required Whilst it is considered 'easy to operate', it is a new technology so is not without its challenges. Plant operators need to be trained in managing unexpected problems
Physical Characteristics	
Reactor Size	Typical reactor depths vary from 5.5 to 9 m. The reactors in Gansbaai are 18 m in diameter and 7 m in depth [4]. Morgenroth et al. [5] used an SBR with a volume of 31.4 ℓ and a diameter of 20 cm
Arrangement/ Configuration	Liu and Tay [6] reported that aerobic granules were formed in column-type upflow reactors. The AGS process in Gansbaai makes use of a three parallel reactor configuration that increases the flexibility of the plant during the low and peak seasons. This results in operating cost savings in the low seasons when one reactor can be decommissioned. Wemmershoek WWTW uses two 2.5 Ml/day reactors in parallel, with each reactor operating at a different stage (feeding, aeration, settling) at any time. Bruin et al. [4] states that the process configuration is flexible, depending on the desired process conditions

Operating Conditions	
Hydraulic Retention Time	The HRT should be short enough to waste the slow settling sludge but long enough to achieve the treatment objectives and retain faster settling granules. Liu and Tay [6] found that a short cycle time of four to six hours stimulates microbial activity and production of cell polysaccharides, which in turn favours the formation of granules. These findings would most likely need to be altered for application in WWBRs and large-scale application. Currently, the Wemmershoek WWTW operates at a retention time of 4 hours
Organic Loading	Aerobic granules can form across a wide range of organic loading rates from 2.5 to 15 kg/m^{3}day (TOL value).
Effluent Treatment	The 5-step sequence of events that occur in a SBR produce effluent that is suitable for environmental discharge [7]. Laboratory studies in an aerobic granular SBRs have shown 90% removal of organic matter and up to 55% ammonia removal [8]. Results from the Gansbaai plant indicated a 93.8% removal in COD, 99% removal in ammonia and 83.5% removal in phosphates [4]. Verbal communication with the plant manager at Wemmershoek, also confirmed that those reactors treat the wastewater to environmental standards
Aeration Requirements	Dissolved oxygen is an important variable and it has been noticed that granules have formed at DO concentrations ranging from 0.7 to > 2 mg/ℓ in an SBR. Submerged aeration is used in the Gansbaai AGS process in the form of flat panel diffusers [4]. Fine bubble aeration is used in the Wemmershoek reactors
Economic Requirements	
Capex	Comparatively speaking, it is difficult to make a direct comparison of this technology to the other technologies used in South Africa since there are only 2 large scale operational AGS plants. However, Bruin et al., [4] reported that the technology installed in Gansbaai reported significant reductions in CAPEX and OPEX. It saves land space since the entire granular activated sludge process takes place within the reactor, including settling. The Nereda technology system setup in Garmerwolde WWTW in the Netherlands showed a 48% reduction in energy usage [9]
Opex	
Downstream Processing and Product Recovery	
Associated Approaches to Product Recovery	Due to the flocculent nature of the aerated granular sludge, product recovery would be considerably more efficient if the products were intracellular or extracellular and biomass associated. It would merely require the removal of the granules and would improve the DSP time.
Potential Products	Due to the principal of operation of AGS, products associated with the biomass and not in the bulk liquid would improve the ease of DSP. The tendency for the granules to have a high settling rate would improve the efficiency of separating the product and biomass from treated effluent. The production of γ-PGA, which is an extracellular, biomass-associated product, is being investigated

1. Tayana Raper. 2015. Wemmershoek WWTW. Photographed with permission.
2. http://web.deu.edu.tr/atiksu/ana07/02-01.gif
3. Adav, S. S., Lee, D.-J., Show, K.-Y., & Tay, J.-H. (2008). Aerobic granular sludge: recent advances. Biotechnology Advances, 26(5), 411–23. doi:10.1016/j.biotechadv.2008.05.002
4. Bruin, B. De, Guideman, G., & Gaydon, P. (2008). Granular Aerobic Activated Sludge
5. Morgenroth, E., Sherden, T., van Loosdrecht, M., Heijen, J., & Wilderer, P. (1997). Aerobic Granular Sludge in a Sequencing Batch Reactor, 31(12), 3191–3194
6. Liu, Y., & Tay, J. H. (2004). State of the art of biogranulation technology for wastewater treatment. *Biotechnology advances*, *22*(7), 533-563.
7. Grady, C. L., Daigger, G. T., Love, N. G., & Filipe, C. D. M. (2011). Biological Wastewater Treatment (3rd ed.). London: IWA Publishing
8. Mosquera-Correl, A., Vazquez-Padin, J., Arrojo, B., Campos, J., & Mendez, R. (2005). Nitrifying granular sludge in a Sequencing Batch Reactor. In Water and Environmental Management Series: Aerobic Granular Sludge (pp. 63 – 70). London: IWA Publishing
9. Robertson, S. & Joana Doutor, A. v. B., 2015. Sustainable Wastewater Treatment using Aerobic Granular Sludge – the innovative Nereda® technology. South Africa, Royal HaskoningDHV

Table 6-6: Comparison of five bioreactors suitable for wastewater biorefineries – Membrane Bioreactor

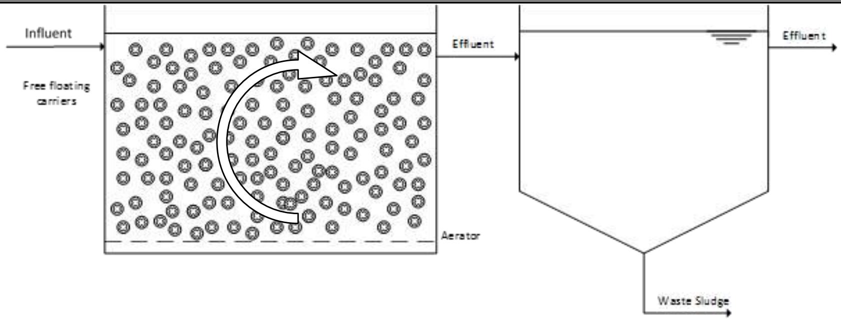
<table>
<tr><th colspan="2">Membrane Bioreactor</th></tr>
<tr><td>Diagram</td><td> [1] 

[2]</td></tr>
<tr><td>Description</td><td>Membrane bioreactors can be classified into three types: for separation of and retention of solids; for bubble-less aeration within the bioreactor and for the extraction of pollutants from industrial wastewaters. The membranes are used for the separation of biomass and treated effluent and are used for the extraction of pollutants that are usually difficult to treat using traditional biological wastewater treatment processes. [3]</td></tr>
<tr><td>When or Why Used in WWTWs</td><td>In the late 1970's, the first commercial scale aerobic MBR process emerged in North America. In South Africa, the equivalent anaerobic process entered the industrial WWT sector in the 1990s [3]. This technology is increasingly being used to treat wastewater as a key component of water reclamation and reuse systems. It is a variation of the activated sludge process in which a membrane system is used for liquid-solids separation. These reactors are typically used to achieve a high quality effluent. [3]
Illovo Sugar's MBR at Sezela's is a large open tank (4000 m^3) filled with effluent and is aerated from the base of the reactor. There is a bank of flat sheet membranes submerged in the tank through which the treated effluent passes. It was constructed as a 'pilot' plant and was designed to treat a third of the effluent. Practically, it has been found that it can satisfactorily treat 25% of the plant effluent. [4]</td></tr>
<tr><td>Advantages</td><td>Combined COD, solid and nutrient removal in one unit.
Fast start up.
Superior removal of particulate and colloidal matter.
Production of high quality effluent.
Excellent for pre-treatment if further treatment is required.
Reduction of land footprint.
High loading rate capability.
Capable of treating toxic industrial effluents.
[3]</td></tr>
<tr><td>Disadvantages</td><td>Replacement of membranes could be costly.
High capital cost.
Membrane fouling
New technology therefore full scale process requires skilled professionals
Does not handle high amounts of non-biodegradable settleable solids and pre-treatment is required
[3]</td></tr>
<tr><th colspan="2">Physical Characteristics</th></tr>
<tr><td>Reactor Size</td><td>Average sizes of these reactors could not be found, however they are usually produced in standard sizes. Various commercial technologies are available. Some of the more well known ones include the Kubota process, Zenon Municipal Systems (ZenoGem Process), The ZeeWeed Membrane, Pleide membrane module developed by Orelis & Mitsui Chemicals and there are numerious other listed in [3]. The table below summaries the key design information for one of the first full scale Kubota MRBs installed in Southern Affrica, at the Illovo Sugar Plant in Sezela, Kwa-Zulu Natal. [4]
<table>
<tr><td>Screen</td><td>1.5 mm wedgewire rundown screen</td></tr>
<tr><td>Design sludge age</td><td>30 days</td></tr>
<tr><td>MBR blower</td><td>2880 Nm^3/hr @ 500 mbar (61.5 kW)</td></tr>
<tr><td>FBDA Blower</td><td>2 no. Each 7060 Nm^3/hr @ 740 mbar (224 kW)</td></tr>
<tr><td>Reactor Dimensions</td><td>28 m. 7 m deep. Volume 4310 m^3</td></tr>
<tr><td>Membrane Unites</td><td>12 no. EK400</td></tr>
<tr><td>Total number of membrane panels</td><td>4800</td></tr>
<tr><td>Membrane type</td><td>Kubota Flat Sheet Membrane Panels</td></tr>
</table></td></tr>
<tr><td>Arrangement/ Configuration</td><td>The number of units required is dependent of the maximum influent flowrate. [3]
The Illovo Sugar Plants makes use of one MBR to reduce the footprint. [4]</td></tr>
</table>

Operating Conditions	
Hydraulic Retention Time	HRTs are reported for municipal wastewaters to be between 2 and 24 hours in Stephenson et al. [5]. However, they do state that for industrial applications, the HRTs are generally much longer and extend to days rather than hours [3] The Illovo Sugar Plant has a sludge age (HRT) of 30 days. [4]
Organic Loading	Reported organic loading rates of between 0.25 and 16 kg COD/m^{3}d .[3]
Effluent Treatment	Stephenson et al. [5] reported removal efficiencies of 90 to 99.8%. It also has a higher performance when compared with that of activated sludge [3]. Illovo Sugar MBR plant has recorded a 95% reduction in COD. [4]
Aeration Requirements	Sezela Plant: Two large blowers supply air via fine bubble diffusers along the floor of the tank. A third smaller blower supplies air as coarse bubbles used to scour and clean the membranes (in all membrane operations regular cleaning is necessary to prevent fouling of the membranes). [4]
Economic Requirements [6]	
Capex	High Capital Cost
Opex	High Operating Cost High Skills requirement
Downstream Processing and Product Recovery	
Associated Approaches to Product Recovery	Cell associated bioproducts would be removed from the section of the reactor that holds the biomass and influent wastewater. If the product was in the bulk liquid it would need to be determined if it is filtered through the membranes with the effluent, or retained with the biomass by the membranes, to determine the appropriate DSP approach
Potential Products	Products that are easily removed through liquid-solid separation and exploit this inherent principle of operation of the MBR would be ideal

1. Bahrudeen, A. (2014). Rotating Biological Contactor. Retrieved from http://www.thewatertreatments.com/wastewater-sewage-treatment/rotating-biological-contactor
2. http://www.lenntech.com/images/mbr_submerged_scheme.jpg
3. Stensel, H. D., & Burton, F. (2003). Wastewater Engineering: Treatment and Reuse. New York: Mc Graw Hill
4. Kennedy, S., & Young, T. (n.d.). Membrane Bioreactors for Water Re-Use in Southern Africa. Water (p. 62)
5. Stephenson, T., Simon, J., Jefferson, B., & Brindle, K. (2002). Membrane Bioreactors for Wastewater Treatment (p. 62). London: IWA Publishing
6. Merwe-Botha, M., & Quilling, G. (2012). Drivers For Wastewater Technology Selection Assessment of the Selection of Wastewater Treatment Technology. Retrieved from http://www.wrc.org.za/Knowledge Hub Documents/Research Reports/TT 543-12.pdf

Table 6-7: Comparison of five bioreactors suitable for wastewater biorefineries – Moving Bed Bioreactor

Moving bed Bioreactor	
Diagram	 [1]
Description	The Moving Bed Biofilm Reactor (MBBR) process is based on attached growth biofilm principles of WWT. The core of the process is the biofilm carrier particles. While the biofilm is fixed to the carriers, the medium is thoroughly mixed within a reactor and retained in the reactor. Carrier particle circulation within the bioreactor is provided by the aeration system or by mixers (anaerobic conditions). Biomass and carriers are retained in the bioreactor using effluent screens. Excess biomass sloughs off the carrier particles and passes into the process effluent where it must be separated in a downstream liquid-solids separation system [2]
When or Why Used in WWTWs	MBBR technology is a simple, robust, versatile and compact technology that has become well established in the past two decades. It is used in the WWT industry to achieve treatment objectives such as BOD removal, nitrification and ammonia oxidation. This technology helps to promote a specialised active biofilm that results in higher efficiencies and a more compact reactor. It is a continuous flow process, independent of the solid separation step due to the retention of active biomass within the reactor [3]. Rusten [4] reported over 400 MBBRs being used for wastewater treatment in 22 different countries.
Advantages	Uses conventional wastewater treatment equipment due to versatility of the technology A variety of liquid-solids separation approaches can be used Potentially easy adaptions/modifications to remove biomass Efficient nutrient removal to environmental specifications Self-sustaining technology, requiring minimal maintenance [3]
Disadvantages	Excess biological phosphorous removal not easily accomplished cycling biomass through anaerobic and aerobic zones is necessary for biomass to develop Requires separate liquid-solids separation step No filtration capability Volumetric loadings higher than purely suspended growth systems but lower than other attached growth systems [3]
Physical Characteristics	
Reactor Size	Due to the simplicity of this technology, the size of the reactor is entirely dependent on the treatment plant where it is being implemented. An MBBR is known to be used to treat process effluent containing phenol
Arrangement/ Configuration	Multiple reactors can be placed in a continuous flow through series arrangement to achieve various treatment objectives such as BOD removal, nitrification and denitrification, with each reactor designated to a specific treatment [3]

Operating Conditions	
Hydraulic Retention Time	HRT is dependent on the desired treatment objective. An example of one applied in South African municipalities was not found. $SRT = \left(\frac{VX}{Q_w X_w + Q X_e}\right)$ V is reactor volume (ℓ); X is the average biomass concentration (mgVSS/L), Q_w is the excess sludge (L/d); X_w is the concentration of the excel sludge (mgVSS/ℓ); Q is the wastewater flowrate (ℓ/d) and X_e is the effluent concentration (mgVSS/ℓ). [5]
Organic Loading	The Surface Area Organic Loading (SALR) depends on whether the MBBR is being used for high, normal or low rate treatment objectives: High Rate - >20 g/m^{2}d Normal Rate – 5 to 15 g/m^{2}d Low Rate – 5 g/m^{2}d [3]
Effluent Treatment	The level of treatment is dependent on the loading rate: High Rate - 75 to 80% removal of BOD Normal Rate – 80 to 90% removal of BOD Low Rate – preceding nitrification [3]
Aeration Requirements	Air is bubbled with a coarse bubbler into the reactor to help with suspension of the carriers and oxygen transfer
Economic Requirements	
Capex	There was no data in the WRC report resource for the MBBR, which was used for the other reactors
Opex	
Downstream Processing and Product Recovery	
Associated Approaches to Product Recovery	In this situation, a solid-liquid separation step would be required regardless of whether the bioproduct is in the medium or biomass associated, merely due to the nature of operation of this bioreactor
Potential Products	Products that are easily removed through liquid-solid separation and exploit this inherent principal of operation of the MBR would be ideal. The production of ɣ-PGA, which is an extracellular, biomass-associated product, is being investigated

1. Adapted from Grady, C. L., Daigger, G. T., Love, N. G., & Filipe, C. D. M. (2011). Biological Wastewater Treatment (3rd ed.). London: IWA Publishing
2. Grady, C. L., Daigger, G. T., Love, N. G., & Filipe, C. D. M. (2011). Biological Wastewater Treatment (3rd ed.). London: IWA Publishing
3. Stephenson, T., Simon, J., Jeffereson, B., & Brindle, K. (2002). Membrane Bioreactors for Wastewater Treatment (p. 62). London: IWA Publishing
4. Rusten, B., Eikebrokk, B., Ulgenes, Y., & Lygren, E. (2006). Design and operations of the Kaldnes moving bed biofilm reactors. Aquacultural Engineering, 34(5), 322–331. doi:10.1016/j.aquaeng.2005.04.002
5. Ahmadi, M., Izanloo, H., Mehr alian, A., Amiri, H., & Sepehr, M. N. (2011). Upgrading of Kish Island Markazi wastewater treatment plant by MBBR. Journal of Water Reuse and Desalination (Vol. 1, p. 243). doi:10.2166/wrd.2011.038

6.4 Final Selection of Bioreactors for WWBR

6.4.1 Refinement of the key criteria for selection

In order to select suitable bioreactors from Section 6.3, a prioritisation of these criteria is needed. Table 6-8 was constructed to represent this prioritisation by analysing each of the selection criteria critically and identifying whether they satisfy the two key requirements, based on the definition of a WWBR:

- Produce a product in a different phase that is easily removed and separated from the substrate and biomass to decrease the load on the downstream processing through the inherent bioreactor design
- Decouple hydraulic and solid residence times

Table 6-8: Bioreactor Design Requirements in order of priority

	#	Requirement
Design Priority	1	Decouples hydraulic and solid retention times
	2	Continuous or semi-continuous (can't store flows)
	3	Product formation in different phase
	4	Bioreactor design facilitates the recovery of the product
Operational Priority	5	Think big! Commodity rather than niche
	6	Influences microbial community, non-sterile
	7	Gives advantage to product: creates ecological niche
	8	Water released into environment eventually

The first four requirements have been labelled as 'Design Priority'. Requirement number 4 is a combination of two requirements in Table 6-1: #8 "Can product be recovered?" and #9 "Bioreactor design conducive to reducing DSP load?" These two points have been combined since the recovery of the product is a function of how the biofilm grows and attaches in the bioreactor and whether the bioreactor design facilitates this attachment process. This in turn affects how the product is removed and whether additional process units are required to separate product from the bulk liquid. If a bioreactor is unable to fulfil **all** four of the design priorities, then it is unlikely that it will be able to produce the desired bioproduct in a quantity and phase that makes the process economically feasible.

The other four categories have been labelled as "Operational Priority". This set of criteria refers to factors that are independent of the design and pertain to important operational factors of a WWBR that ensure its success. Should a bioreactor technology fail to comply with the "design priority" criteria, in spite of fulfilling the "operational priority" criteria, it remains unsuitable for the use in wastewater biorefinery applications.

The desired goal for these wastewater biorefineries is to incorporate the bioreactors into existing wastewater treatment plants. Thus, it is critical that they are able to handle continuous or semi-continuous flows that have seasonal variations in flowrates and composition. WWTWs cannot 'shut down' owing to the continuous flow of wastewater generated by industry and the population.

6.4.2 Criteria fulfilment of the selected bioreactors

In order to adequately justify the selection of three bioreactors for further study, a summary table has been compiled for each of the five bioreactors outlined in Section 6.3, to show the degree to which the bioreactors fulfil the criteria outlined in Table 6-8.

The following scale will be used to show the extent to which the bioreactors satisfy the requirements:

Completely complies	Mostly Complies	Marginally Complies	Does not comply
+++	++	±	x

Table 6-9: Composite table showing the degree to which the five bioreactor categories fulfil the selection criteria

		Criteria	AGS in an SBR	Rotating Biological Contactor	Membrane Bioreactor	Moving Bed Biofilm Reactor	Trickle Bed Reactor
Most Important	1	Decouples hydraulic and solid retention times	+++	+++	+++	+++	+++
	2	Continuous or semi-continuous (cannot store flows)	++	+++	++	+++	++
	3	Product formation in different phase	+++	+++	++	+++	+++
	4	Reactor design facilitates the recovery of the product	+++	++	±	+++	x
Least Important	5	Think big! Commodity rather than niche	++	+++	++	+++	+++
	6	Influences microbial community, non-sterile	+++	++	++	+++	++
	7	Gives advantage to product: creates ecological niche	+++	++	++	+++	++
	8	Water released into environment eventually	+++	++	+++	++	++
# of criteria that completely comply			6	4	2	7	3

From Table 6-9, it is evident that the reactor types that fulfil the critical requirements, are (in order of best compliance): Moving Bed Biofilm Reactor (MBBR), Aerobic Granular Sludge in a Sequencing Batch Reactor (AGS-SBR) and the Rotating Biological Contactor (RBC). The main reasons behind this analysis are outlined in the SWOT analysis in Section 6.4.3.

The Trickle Bed Reactor did not fulfil criterion number 4, due to the process down time that would be required to remove the packed material, separate the biomass and product and start-up the treatment procedure. This would require the storing of wastewater flows, or having multiple bioreactors, and the re-establishment of equilibrium in the system. Clogging is a known problem in TBRs used in regular wastewater treatment, resulting in channelling and poor treatment efficiencies (Antonie, 1976). Clogging may be aggravated with product formation, especially if the product is extracellular.

The Membrane Bioreactor also fell short in category number 4. While it is a continuous system, the membranes require replacement and maintenance to prevent clogging. Membrane bioreactors have also not yet been applied at full municipal scale. They have high CAPEX and OPEX and require skilled plant technicians. In the context of South Africa's existing WWTWs, this presents an additional obstacle (Henze, et al., 2002; Grady, et al., 2011).

6.4.3 SWOT analysis of the three reactors selected for use in WWBRs.

For the purpose of this comparison, the SWOT Analysis has been based on the ability of each of the outlined bioreactors to fulfil the top criteria that were outlined in Section 6.4.1. The following questions were asked when performing the SWOT analysis on these bioreactors:

Strengths: What characteristics of the bioreactor technology allow it to fulfil the requirements and render it suitable for applications in wastewater biorefineries?

Weaknesses: What are the major drawbacks about this technology, concerning process operation and treatment objectives?

Opportunities: Is there potential for retrofitting and adaption to South Africa's current wastewater treatment plants and infrastructure?

Threats: Does the bioreactor technology have risks associated with its operation, and implementation?

Table 6-10: SWOT analysis of selected bioreactors

Rotating Biological Contactor (RBC)	Moving Bed Biofilm Reactor (MBBR)	Aerobic Granular Sludge in a Sequencing Batch Reactor (AGS in SBR)
Strengths		
• Mechanically simple and reliable process. • Large scale applications used successfully worldwide. • Inherent aeration by nature of shaft rotation. • Recycle loops are not required due to continued microbial growth and thus water treatment. • Does not require very skilled operators. • Able to handle lower substrate concentrations. • Creates a microbial niche – organisms present in the wastewater naturally adhere to the disks. • Rotating disks agitated the mixed liquor keeping sloughed biomass in suspension and well mixed at each stage of treatment. • Not affected by shock variations in hydraulic and organic loading.	• Versatile technology allowing creative solutions. • Active biomass is retained in the reactor continuously. • The suspended carriers promote the formation of active biomass, resulting in higher efficiencies and process stability. • Continuous flow process. • Multiple stages can be achieved through arrangement in series, without the need to pumping (similar to AS process). • Density of carriers close to water, thus minimizing mixing energy required to keep them in suspension. • Does not require skilled operators. • Simple aeration grid designed on base of reactor eliminates the need for diffuser replacements and maintenance.	• Hydraulic Load variations are readily handled by for AGS systems • High biomass retention • SBRs can be used as a continuous process • Does no require additional clarifiers downstream • Obtains high treatment efficiencies at low oxygen saturation concentrations (De Kreuk, et al., 2005; Verster, et al., 2013) • Granules have a fast settling rate • Recycles and mixers are not required saving on energy costs and maintenance • Land footprint of AGS is significantly decreased compared to other technologies •
Weaknesses		
• Requires primary clarification and pre-treatment as it does not handle particulate matter well. • If insufficient wetting of the biomass occurs, it leaves the disks vulnerable to nuisance organisms such as algae and worms. • Development of uneven biofilm growth.	• Good screening and grit removal is required to prevent build-up of inert material • Foaming is known to occasionally form at start up. Antifoam added into the process can cause decreased diffusion to the biofilm.	• AGS that is formed by slow growing bacteria is more stable than when fast growing bacteria are present • Competency of operators running a relatively new technology at a large scale • Requires pre-treatment to remove solids
Opportunities		
• Modifications to the reactor design could be incorporated to continuously remove surface biomass for product harvesting.	• Great potential for modifications to the design to facilitate easy liquid-solid separations. • Versatility allows makes MBBRs suitable for retrofit installation into existing tanks. In South Africa, the predominant technology is Activated Sludge. MBBRs could be fitted into these existing tanks.	• Successful large-scale application of AGS in a SBR in Gansbaai and Wemmershoek have been implemented and showed excellent treatment efficiencies. This shows great promise for this technology on a large scale •

Rotating Biological Contactor (RBC)	Moving Bed Biofilm Reactor (MBBR)	Aerobic Granular Sludge in a Sequencing Batch Reactor (AGS in SBR)
Threats		
• Temperatures below 12 °C in colder seasons will result in decreased efficiency. • Enclosures are often needed around the RBCs to minimize effects of sunlight, nuisance organisms and temperature fluctuations. These enclosures require odour control and often heat control to avoid condensation and corrosion of the units. • Process failures due to inadequate designs of the shaft system	• Too much sloughing in the reactor could cause the biomass to flow out with the media, and prevent water treatment and product formation.	• Process is unstable as washout can easily occur • Technology is not well understood on a large scale application • Effects of a biopolymer forming microorganism on a granular formation are not well understood
References		
(Antonie, 1976; Adav, et al., 2008; Grady, et al., 2011; Stensel & Burton F, 2003; WEF, 2010)	(WEF, 2010; Grady, et al., 2011; Borghei & Hosseini, 2002; Henze, et al., 2008)	(De Kreuk, et al., 2005; Gademan, et al., 2010; Verster, et al., 2013; Henze, et al., 2002)

In order to further assess the viability of these technologies, and to compare the actual performance of the three, experimental studies must be conducted. To this end a laboratory scale MBBR and AGS-SBR have been built and commissioned for production of the polymer PGA which was selected in Section 5.4.2 as a suitable candidate product for the biological reactor. This is an ongoing project and results to date are reported in Appendix E. This study should ultimately contribute to the ability to select the most suitable bacterial bioreactor for different WWBR systems.

6.5 Bioreactor Selection for the Integrated WWBR

The design of bioreactors suitable for use with a wastewater feedstock poses specific challenges, as does the placement of the bioreactor within the greater whole of the biorefinery. The factors involved have been given consideration (Section 6.1) with respect to the bacterial reactor. The approach taken in this study is applicable to the selection of the other bioreactors within the WWBR and can be used as the starting point for initial choices. Once the options have been reviewed and a shortlist created the process developed in this chapter can be applied, using the key criteria and SWOT analysis to make a final selection. As here, however, the process may yield two or three potential candidate bioreactor configurations which should then be assessed experimentally.

Incorporation of the system developed here into the conceptualisation of WWBRs in South Africa, or its use for a particular WWBR design, is explored further in Chapter 9.

7 GENERIC FLOWSHEETS AND MASS BALANCES FOR WASTEWATER BIOREFINERY DESIGN

This study has recognised the need for multiple unit operations to be included in the WWBR flowsheet to allow multiple specifications to be met i.e. the harvesting and beneficiation of different components of the wastewater as well as meeting the requisite water quality. This requires the maximising of conversion to product and maximising of quality of product water to be separated. In the preceding project run in the Centre for Bioprocess Engineering Research (Verster, et al., 2014), this approach was recognised through the compiling of a generalised flow sheet, given in Chapter 2, Figure 2-1.

Wastewater treatment generally consists of settling, primary treatment, secondary treatment and possibly polishing steps. It is expected that a wastewater biorefinery will include similar stages in order to produce water compliant to the specified quality as one of the products of defined quality. The optimisation of each unit operation is required with respect to its yield and efficiency as well as its product quality. Furthermore, the optimisation of the integrated process is required to maximise the overall product outputs and to ensure compliance with respect to water quality. In this section, key features of the wastewater biorefinery flowsheet mass balances are considered.

7.1 Approach to Flow Sheet Development for Biorefineries

Each biorefinery case study results in a unique process flowsheet; however, these encompass common building blocks including unit operations focussed on solids removal, on conversion of the soluble organic carbon component, on utilisation of N and P, on removal of trace contaminants and on delivering required water quality. Some unit operations may serve more than one purpose. The flowsheet development is guided by heuristic assumptions that make a first order feasibility analysis possible and contribute to understanding the potential of the biorefinery. These are discussed in more detail in the validation study in Chapter 8.

7.1.1 An overview flowsheet for WWBRs

An overview flowsheet for a generalised wastewater biorefinery is presented in Figure 7-1, with its accompanying lists of unit operations and process streams presented in Table 7-1 and Table 7-2. The mass balance equations for this flowsheet are given in Section 7.1.2.

The generic WWBR uses one or more wastewater streams (A1-4) as feedstock for the production of products, including compliant water. More than one wastewater inflow may be used, either simply because these are the streams that need remediation, or because the streams complement each other in terms of nutrients available for product formation. The combined feedstock is separated into a solids stream (U1) and a raw wastewater stream (B1). The latter is treated in a series of bioreactors, making use of the diversity of functions offered by varying the focus in each reactor. The bacterial reactor (1), algal reactor (2) and macrophyte reactor (3) each improve the quality of water, with the separated effluent of the prior reactor becoming the influent (D1 & F1) of the next, and the solution separated from the final effluent completely compliant water-as-product (Z). Each reactor also produces one or more value-added products (V, W & X) which are separated out for further processing, as well as a solids slurry (U2, U3, U4&5) which is combined with the feedstock solids. This combined slurry forms the influent to the solids reactor (4), which is likely to be a fungal reactor. The solids reactor produces products (Y), including the final "catch-all" compost. Each of the four bioreactors may need one or more supplement streams (B2-4; D3-5; F2-4 & U6-8) for optimal functioning. Each reactor also has carbon dioxide (photosynthesis and respiration) and water (precipitation and evaporation) flows, each either forming a net inflow or a net outflow. The generic flow diagram allows provision for a biomass recycle (C4) in the bacterial reactor and a feedstock bypass (D2) to the algal reactor which may be used to achieve optimal performance.

A more detailed version of the flow diagram for the generalised WWBR is split into flowsheets for each reactor train. Each of these flowsheets is presented in Sections 7.4 and 7.5 together with the equations for the relevant mass balance.

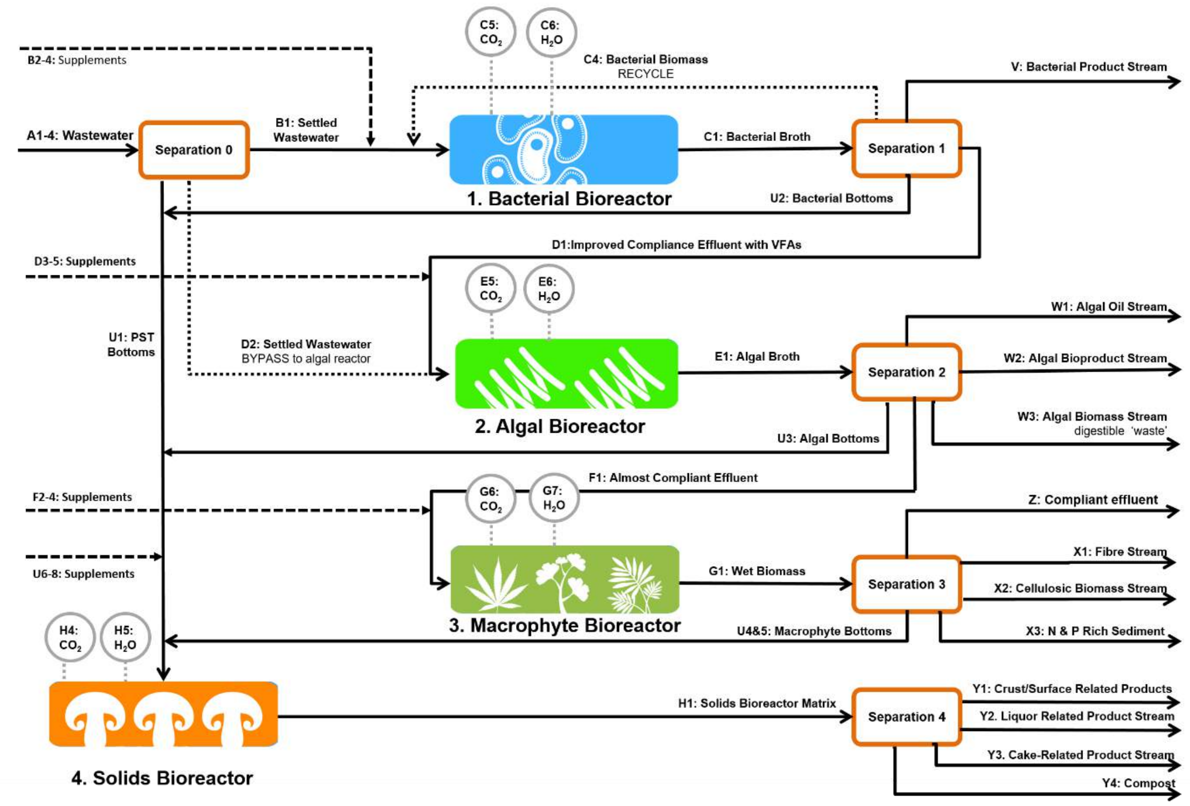


Figure 7-1: Generic wastewater biorefinery overview flowsheet (see Table 7-1 and Table 7-2)

Table 7-1: Overview of operations in unit groups for a generic wastewater biorefinery (see Figure 7-1)

Unit Group Numbers	Type	Unit Group Description
0.1-0.2	Separation 0	Separation of raw influent streams, with primary settling and splitting
1.1	Bioreactor	Bacterial bioreactor, preceded by a holding/mixing tank
1.2-1.4	Separation 1	Separation of bacterial product, bacterial biomass and improved effluent (to algal reactor)
2.1	Bioreactor	Algal bioreactor, preceded by a holding/mixing tank
2.2-2.5	Separation 2	Separation of algal products, algal biomass and almost compliant effluent (to macrophyte reactor)
3.1	Bioreactor	Macrophyte bioreactor, preceded by a holding/mixing tank
3.2-3.6	Separation 3	Separation of fibre, cellulosic biomass, sediment and compliant effluent (leaving system) some processes seasonal
4.1	Bioreactor	Solids reactor, preceded by a holding/mixing tank
4.2-4.4	Separation 4	Separation of solids reactor product, separated into crust-associated products, liquor-associated products and cake-associated products, the remainder being compost.

Table 7-2: Overview of streams for a generic wastewater biorefinery (see Figure 7-1)

Stream number	Stream description	Relation to process units	Relation to other streams (equations refer to mass balance, not volume)
A1-A4	Raw Wastewater	Into Separation 0 (Units 0.1-0.2-3)	Mixed incoming stream
B1	Settled Raw Wastewater	From Separation 0 Into Unit 1: Bacterial Bioreactor	B1 = A1-4 - U1 - D2 Composition same as D2
B2-4	Supplementary Feed	Into Unit 1: Bacterial Bioreactor	Determined by process needs
C1	Bacterial Broth	From Unit 1: Bacterial Bioreactor Into Separation 1	C1 = B1 + B2-4 + C4 + C5 + C6 Composition changed from B1 including increased VFA content
C4	Bacterial Biomass Recycle	From Separation 1 Into Unit 1: Bacterial bioreactor	C4 = C1 - U2 - D1 - V1 Composition changed from C1 Low liquid content
C5	CO_2	From Unit 1: Bacterial Bioreactor To atmosphere	CO_2 only
C6	H_2O	Between Unit 1: Bacterial Bioreactor and atmosphere	H_2O only, rainfall and/or evaporation
D1	Improved Compliance Effluent with VFA content	From Separation 1 Into Unit 2: Algal Bioreactor	D1 = C1 - C4 - U2 - V1 Composition similar to dissolved composition C1
D2	Settled Raw Wastewater, bypass stream	From Separation 1 Into Unit 2: Algal Bioreactor	D2 = A1-4 - B1 - U1 Composition same as B1.
D3-5	Supplementary Feed	Into Unit 2: Algal Bioreactor	Determined by process needs
E1	Algal Broth	From Unit 2: Algal Bioreactor Into Separation 2	E1 = D1 +D2 + D3-5 + E5 + E6 Composition changed from D
E5	CO_2	From atmosphere Into Unit 2: Algal Bioreactor	CO_2 only
E6	H_2O	Between Unit 2: Algal Bioreactor and atmosphere	H_2O only, rainfall and/or evaporation
F1	Almost Compliant Effluent	From Separation 2 Into Unit 3: Macrophyte Bioreactor	F1 = E1 - W1 - W2 - W3 - U3 Composition same as dissolved composition E1
F2-4	Supplementary Feed	Into Unit 3: Macrophyte Bioreactor	Determined by process needs
G1	Wet Macrophyte Biomass	From Unit 3: Macrophyte Bioreactor Into Separation 3	G1 = F1 + F2-4 + G6 + G7 Composition changed from F1 Combination of liquid, fibre and sediment
G6	CO_2	From atmosphere Into Unit 3: Macrophyte Bioreactor	CO_2 only
G7	H_2O	Between Unit 3: Macrophyte Bioreactor and Atmosphere	H_2O only, Precipitation/Evaporation
H1	Solids Matrix	From Unit 4: Solids Reactor Into Separation 4	H1 = U1 + U2 + U3 + U4&5 + U6-8 – H4 + H5 Composition complex.
H4	CO_2	From Unit 4: Solids Reactor To atmosphere	CO_2 only
H5	H_2O	Between Unit 4: Solids Bioreactor and Atmosphere	H_2O only, Precipitation/Evaporation
U1	Primary Settling Tank Bottoms	From Separation 0 Into Unit 4: Solids Reactor	Volume and composition dependent on incoming streams. U1 = A1-4 - B1 - D2 Dependent on PST efficiency
U2	Bacterial Bottoms	From Separation 1 Into Unit 4: Solids Reactor	U2 = C1 - (D1 + I + C4) Composition based on bacterial biomass
U3	Algal Biomass not to product streams	From Separation 2 Into Unit 4: Solids Reactor	Total algal biomass = U3 + L U3 = E1 – (F1 + W1 + W2 + W3) Composition based on algal biomass

Stream number	Stream description	Relation to process units	Relation to other streams (equations refer to mass balance, not volume)
U4&U5	Cellulosic Biomass & N & P Rich Sediment	From Separation 3 Into Unit 4: Solids Reactor	U4+U5 = G1 - (Z + X1 +X2 + X3) U4: Composition based on macrophyte (above ground) biomass, U5: Composition based on sediment accumulation (not directly related to input streams), composition the same as X3
U6-8	Supplementary Feed	Into Unit 4: Solids Reactor	Determined by process needs
V1	Bacterial Product Stream	From Separation 1 Exit system	V1 = (B1+ B2-4) * Bacterial bioproduct yield coefficient Stream needs further processing for pure product.
W1	Algal Oil Stream	From Separation 2 Exit system	W1 = (D1 + D2 + D3-5 + E5) * Algal oil yield coefficient Stream needs further processing for pure product.
W2	Algal Bioproduct Stream	From Separation 2 Exit system	W2 = (D1 + D2 + D3-5 + E5) * Algal bioproduct yield coefficient Stream needs further processing for pure product.
W3	Algal Biomass (digestible 'waste')	From Separation 2 Exit system	W3 = (D1 + D2 + D3-5 + E5) - (W1 + W2 + F1) Note U3 can be 0 Composition same as U3
X1	Fibre Stream	From Separation 3 Exit system	X1 = G1 * (1- moisture content fraction) * Fibre compositional fraction
X2	Cellulosic Biomass Stream	From Separation 3 Into further processing and/or leave system	X2 = G1 * (1- moisture content fraction) * Cellulosic compositional fraction
X3	N & P Rich Sediment	From Separation 3 Exit system	Composition based on sediment accumulation (not directly related to input streams)
Y1	Crust/Surface Product Stream	From Separation 4 Exit system	Y1 = (U1 + U2 + U3 + U4&5 + U6-8) * Crust product yield coefficient
Y2	Liquor Associated Product Stream	Separation 4 Exit system	Y2 = (U1 + U2 + U3 + U4&5 + U6-8) * Liquor associated product yield coefficient
Y3	Cake-Related Product Stream	Separation 4 Exit system	Y3 = (U1 + U2 + U3 + U4&5 + U6-8) * Cake-related product yield
Y4	Compost	Separation 5 Exit system	Y4 = H1 - (Y1 + Y2 + Y3)
Z	Compliant Effluent	From Separation 4 Exit system	Composition must comply with discharge standards (either for discharge into natural water body or for irrigation or for re-use)

7.1.2 Mass balance equations for overview flowsheet

The generalised flow diagram gives a simplified view of the WWBR and allows for an overall mass balance to be constructed. (The approach to mass balances for the detailed flowsheets for the four bioreactor trains is given in Section 7.3). In the mass balance the following apply:

It is considered as a continuous system, with an assumption of no accumulation over the time interval of analysis. For some sections of the process, this means that the mass balance must be calculated over a relatively long time period and averaged to the per day basis. In this model a year was used. In particular, aspects of the macrophyte bioreactor train will operate on an annual cycle. Thus the overall mass balance is considered to have zero accumulation over a full year.

The symbol for each stream represents the combined value of concentration (C) multiplied by flow rate (Q).

For each process portion (separation or reactor), components with overall negative signs are net outflows and positive components are net inflows. The CO_2 uptake or respiration rates, streams C5, E5, G6 and H4, and rain or evaporation streams, streams C6, E6, G7 and H5 are assigned a positive sign by default, because their net value could be an in- or outflow depending on site specific factors, including the wastewater concentration and the geographic location. Should these streams actually be outflows, their stream flowrate is quantified as less than zero. The yield coefficients then determine the final sign, for example positive (inflow) for photosynthetic carbon uptake, negative (outflow) for respiration, positive for rainfall and negative for evaporation.

Table 7-3: Mass balance equations for the overview flowsheet

Type	Overall Mass Balance
Separation 0	(A1-4) – (B1 + D2 + U1) = 0
1. Bacterial Bioreactor	(B1 + [B2-4] + C4 + C5 + C6) – (C1) = 0
Separation 1	(C1) – (C4 + D1 + V1 + U2) = 0
2. Algal Bioreactor	(D1+ D2 + [D3-5] + E5 + E6) – (E1) = 0
Separation 2	(E1) – (F1 + W1 + W2 + W3 + U3) = 0
3. Macrophyte Bioreactor	(F1 + [F2-4] + G6 + G7) – (G1) = 0
Separation 3	(G1) – (Z + X1 + X2 + X3 + [U4&5]) = 0
4. Solids Bioreactor	(U1 + U2 + U3 + [U4&5] + [U6-8] + H4 + H5) – (H1) =0
Separation 4	(H1) – (Y1 + Y2 + Y3 + Y4) = 0

7.2 A Note on the Energy Balance for a Wastewater Biorefinery

Most existing biorefineries are primarily aimed at producing energy (Ghatak, 2011) or biomass for energy production, whereas the third generation biorefinery focuses on higher value products and only considers energy as a final use of the remaining chemical potential, once maximum value has been extracted for other uses (Sections 2.2 and 2.3). This generic WWBR does not specifically include an energy production unit, although potential does exist to focus on biofuel or bioenergy production in each of the three reactors or to add an additional bioenergy unit. The focus on energy as a primary product is an area of significant distinction between conventional biorefinery thinking and the third generation biorefinery in general, and the WWBR in particular.

The exclusion of an energy production unit is also a response to the fact that there are a number of different scenarios regarding the placement of an energy recovery unit. One of these is to use the algal biomass product stream for anaerobic digestion on site (Inglesby, et al., 2015; Olguín, 2012). Alternatively, anaerobic digestion can be used as pre-treatment for the solids reactor, and a potential compliance step before the final composting (Ferry & Giljova, 2015). In either case, the fuel can be used to heat the bacterial bioreactor to increase the reaction rates, to heat the anaerobic digester itself, to produce electrical power for other energy needs or a combination of these. Moreover, there is the possibility of creating a microbial fuel cell using one of the streams in the WWBR (Cerrillo, et al., 2016). Further, most energy savings are involved in plant design and layout, with smart co-location of units and their connecting pipes, using pinch technology to cascade (Isafiade, et al., 2015). For these reasons, the scope of this model has been limited to material flows.

Several factors are important to note in advance of the analysis of WWBRs. Firstly, WWBRs work with waste streams that are not sterilised, therefore the energy cost associated with sterilisation can be omitted, or reduced to a maintenance cleaning role (Mooij, et al., 2015; Verster, et al., 2014). Since wastewater streams are usually dilute in comparison with other feedstocks, energy requirements per mass of nutrient for pumping may be higher (Ekama, et al., 2011). The required energy density of the units should be assessed, to determine the feasibility of using renewable energy sources where appropriate. In particular, the potential for energy production from "residual" streams within the WWBR should be included (Ghatak, 2011).

7.3 Approach to Mass Balances for Detailed Flowsheets of Bioreactor Trains

The first step in analysing a process flowsheet is to construct material and energy balances. This can inform techno-economic feasibility as well as environmental performance. To close the material and energy balances, the likely conversions, yields and efficiencies of the unit processes must be estimated. This is a work in progress focussed on material balances only to describe material flows. Estimates used in a study substantiating the mass balances for a bacterial bioreactor are explained in Section 8.2 followed by a demonstration of mass balances for an integrated WWBR (Section 8.3). These are explored further in the possible scenarios presented in Sections 8.3, 8.4.2 and 8.4.3.

7.3.1 The approach to the mass balances

For each biorefinery case, a lead commercial product is selected to suit the wastewater and the surrounding market. In addition to the lead product being well suited to manufacture from the particular wastewater, a market analysis establishing the local needs and demand for products contributes to the choice of lead product. Further to this, production of water quality compliant with specifications is a prerequisite. All other products produced from the wastewater are secondary. If, for example, the main product is an algal product, the entire bacterial reactor can be considered a pre-treatment to produce VFAs or liberate N and P to supplement the algal process. In this way the unit processes are optimised for one commercial product and water while, as secondary priority, the robustness of the system is considered. A selection of case studies illustrating this approach are presented in Chapter 8.

In this chapter, a preliminary set of material balances is presented for the bacterial bioreactor (Section 7.4). Similar material balances have been constructed for each of the other bioreactors, and are included in Appendix F, with only the bacterial reactor unit presented in this chapter.

7.3.2 General symbol conventions

C-inflow: The amount of carbon in the inflow streams, excluding CO_2 uptake (see Section 7.3.3), available to be converted into biomass, product or CO_2, used as basis for calculations. Where CO_2 is utilised it is recorded as a separate entity and added to C-inflow for the mass balance.

C-product: The amount of carbon in the product, used as basis for calculations.

Q_{STREAM} = Volumetric flowrate of the specified stream (m^3/day)

$C_{S(STREAM)}$ = Concentration of element in the specified stream (C = Concentration, $_{S} = _{C,N,P}$)

$C_{C(STREAM)}$ = soluble carbon (kg/m^3) in stream

$C_{N(STREAM)}$ = soluble nitrogen (kg/m^3) in stream

$C_{P(STREAM)}$ = soluble phosphorous (kg/m^3) in stream

$N_{S(STREAM)}$ = Total constituent in specified stream (kg/day) (N = Total amount in kg/day, $_{S} = _{C,N,P,W}$)

$N_{C(STREAM)}$ = Total carbon in specified stream (kg/day)

$N_{N(STREAM)}$ = Total nitrogen in specified stream (kg/day)

$N_{P(STREAM)}$ = Total phosphorous in specified stream (kg/day)

$N_{W(STREAM)}$ = Total water in specified stream (kg/day)

In any given stream, N = IN + X + P i.e. the stream flow rate is the sum of the residual unconverted component from the inflow, biomass component and the product component(s).

$X_{React,S(STREAM)}$ = Biomass fraction from specified reactor in specified stream (kg/day) (X = biomass, React = Reactor, S = C,N,P)

$X_{React,C(STREAM)}$ = Carbon in Biomass fraction of specified stream (kg/day)

$X_{React,N(STREAM)}$ = Nitrogen in Biomass fraction of specified stream (kg/day)

$X_{React,P(STREAM)}$ = Phosphorous in Biomass fraction of specified stream (kg/day)

$P_{i,S(STREAM)}$ = Product i fraction of specified stream (kg/day) ('i' is specified in terms of exiting product stream e.g. X1, Y2, W3…, S = C,N,P)

$P_{i,C(STREAM)}$ = Carbon in Product i fraction of specified stream (kg/day)

$P_{i,N(STREAM)}$ = Nitrogen in Product i fraction of specified stream (kg/day)

$P_{i,P(STREAM)}$ = Phosphorus in Product i fraction of specified stream (kg/day)

$IN_{S(STREAM)}$ = Unconverted inflow component, in specified stream (kg/day) (IN = inflow component, S = C,N,P,W).

Inflow component may consist of unconverted substrate, biomass or product, entering the specified reactor unit, and available to biological conversion.

$IN_{C(STREAM)}$ = Carbon in unconverted inflow component fraction of specified stream (kg/day)

$IN_{N(STREAM)}$ = Nitrogen in unconverted inflow component fraction of specified stream (kg/day)

$IN_{P(STREAM)}$ = Phosphorous in unconverted inflow component fraction of specified stream (kg/day)

$F_{N/C,component}$ = ratio of Nitrogen to Carbon in the specified component.

For example, the $F_{N/C,XBact}$ is the ratio of nitrogen to carbon in the bacterial biomass (wt% N)/(wt% C) which is 0.049/0.487 or 0.101 g-N/g-C using default model values provided by Wu (2015). The set of values, $F_{N/C,Xreact}$, $F_{N/C,INreact}$, $F_{N/C,PXi}$, link the carbon and nitrogen balances.

$F_{P/C,component}$ = ratio of Phosphorous to Carbon in the specified component.

For example, the $F_{P/C,XBact}$ is the ratio of phosphorus to carbon in the bacterial biomass (wt% P)/(wt% C) which is 0.025/0.487 or 0.051 g-P/g-C using default model values from Wu (2015). This set of values ($F_{P/C,Xreact}$, $F_{P/C,INreact}$, $F_{P/C,PXi}$) link the Carbon and Phosphorous balances.

SC = fraction of solids in suspension = (mass of solids) / (mass of total sludge)

7.3.3 Reactor conversion value conventions for carbon mass balance and associated assumptions

The reactor conversion values used to describe the bacterial reactor (Bioreactor 1) are set out in Table 7-4. In this study, these have been defined on an elemental basis and are presented in terms of carbon here. The yields commonly found in literature are calculated on the full mass of product (full composition, including e.g. C,H,O,N,P) per mass of substrate used (full composition, including e.g. C,H,O,N,P), and are therefore converted to the C-specific values to comply with a carbon mass balance used here. A similar approach can be taken for the N and P balances.

The yield for carbon dioxide is only relevant for the carbon mass balance, and not relevant for the nitrogen or phosphorous mass balances.

Literature values may refer to CO_2 yield per biomass concentration. In this project the biomass yield per C-inflow has been combined with the CO_2 yield per biomass concentration, to give a stoichiometric CO_2 yield per C-inflow.

Table 7-4: Carbon mass balance yield factors

Conversion description	Unit	Symbol of factor
Mass of carbon reporting to biomass as a fraction of that present in influent stream to reactor	kgC(reactor biomass)/kg C(inflow to reactor)	$Y_{C,X/IN}$
Mass of carbon reporting to product as a fraction of that present in influent stream to reactor	kgC(product)/kg C(inflow to reactor)	$Y_{C,Pi/IN}$
Mass of carbon entering or leaving as CO_2 as a fraction of that present in influent stream to reactor	kgC(CO_2)/kg C(inflow to reactor)	$Y_{C,CO2/IN}$
Mass of carbon remaining unconverted as a fraction of that present in influent stream to reactor	kgC (unconverted)/kgC(inflow to reactor)	$Y_{C,IN,unconverted/IN}$ = 1 – ($Y_{C,X/IN}$ + $Y_{C,Xi/IN}$ + $Y_{C,CO2/IN}$)

7.3.4 Nitrogen and phosphorus mass balances

The material balance for each reactor train is set up based on a mass balance on the element carbon. For each reactor, the yield based on carbon is determined for the conversion from the inflow organic components to biomass and products. The nitrogen and phosphorus material balances are estimated from the carbon balance, using factors of relative mass fractions normalised to carbon for each component.

The factors defining the relative mass fractions of the element of interest to carbon are given as follows: $F(J_k)_{i/C}$ where J_k refers to the component of interest i.e. biomass or product stream and i refers to the element of interest i.e. N or P. For example, the relative mass fraction for N normalised to C for bacterial biomass, $F(X_{Bact})_{N/C}$ is given by the mass % N per mass % C. The relative weight fractions of nitrogen and phosphorus normalised to carbon for various stream components are shown in Appendix Section **Error! Reference source not found.**

7.3.5 Assumptions for mass balances in separation steps

In the integrated generic flowsheet for WWBRs (Figure 7-1), each separation is represented as a lumped operation i.e. as a single step. In the detailed generic flowsheets for each reactor train (Figure 7-2, Figure 7-3, Figure 7-4 and Figure 7-5) the individual units involved are enumerated. Each separation step involves one or more separation unit with outflow streams of different compositions, and one or more splitter units with outflow streams having identical composition. In each bioreactor train, the outflow streams include a solids stream that is separated out as a concentrated bottoms and/or product slurry.

Solids content of slurry

Solids content (SC) is defined as the mass of solids (dry mass) in slurry, divided by the total mass of the slurry.

Solids Content Fraction (SC) = (mass of solids) / (mass of total slurry)

Liquid Content Fraction (LC) = (mass of liquid) / (mass of total slurry)

SC + LC = 1

Determination of the liquid content when the SC and the mass of solids are known:

mass of total slurry = mass of solids / SC

Similarly, mass of total slurry = mass of liquid / LC

thus mass of solids / SC = mass of liquid / LC

and LC = 1 – SC

thus mass of solids / SC = mass of liquid / (1 – SC)

rearranging: mass of liquid = ((1-SC)/SC) * mass of solids

The solids dry mass is calculated by dividing the total carbon in that stream by the carbon composition of the main component. For example, in Separator 1.2:

$$N_{W(C2)} = (N_{C(C2)}/C_{comp,bact}) * ((1-SC_{C2})/SC_{C2})$$

Table 7-5: Overview of separation steps for removal of solids in a generic wastewater biorefinery

Unit number	Separation description	Relevant parameters	Solids Content symbol
0	Primary Settling	Slurry solids content in “Solids to Bottoms” U1	$SC_{A1-4,U1}$
1	Bacterial Bioreactor Separation Train	Slurry solids content in “Solids (biomass) to Bottoms” U2	$SC_{C1, U2}$
2	Algal Bioreactor Separation Train	Slurry solids content in “Solids (biomass) to Bottoms” U3 & Product W3	$SC_{E1,U3}$ $SC_{E1,W3}$
3	Macrophyte Bioreactor Separation Train	Slurry solids content in “Solids to Bottoms” U4-5 and Products X1, X2 & X3	$SC_{G1, U4}$ $SC_{G1, X1}$ $SC_{G1, X2}$ $SC_{G1, X3}$
4	Solids Bioreactor Separation Train	Solids content in “Solids to Products” streams H2, H3, & Y4	SC_{H2} SC_{H3} SC_{Y4}

Factors used for separator units

In the detailed generic flowsheets, the type of separation which must take place is specified, but not the form of each separator. For each unit it is assumed that product recovery will be optimised for the main product, so that residual biomass, secondary products and unconverted inflow goes to the bottoms with high recovery. The bottoms for each unit are assumed to behave as an entity, so that there is one recovery value for the entire secondary stream even though it may contain several separable constituents. The secondary stream may then undergo further separation.

eff_{STREAM} = separator unit efficiency with respect to the specified stream

Factors used for splitter units

Each splitter divides an entry stream into two exit streams of identical composition. One exit stream is regarded as primary, and the splitter ratio (r_{STREAM}) is assigned the subscript of that stream. In the model this stream has been chosen to be the product containing stream. Thus the splitter streams which are bypass streams or which are directed to the solids reactor are always the secondary streams. The ratio for both streams sums up to 1.

$$IN_{(primary\ exit\ STREAM)} = IN_{(entry\ STREAM)} * r_{primary\ exit\ STREAM}$$

$$IN_{(secondary\ exit\ STREAM)} = IN_{(entry\ STREAM)} * (1 - r_{primary\ exit\ STREAM})$$

7.4 Flowsheet and Mass Balance for the Bacterial Bioreactor Train

In the generalised WWBR flowsheet, the bacterial bioreactor is placed as the first treatment and production step in the WWBR. This was selected because the bacterial bioreactions are generally the most intensively operated, resulting in the greatest productivity per land area. It is also the best understood biological conversion system available, well developed to produce bioproducts with an established market. In addition, bacterial reactions usually demand a well-balanced nutrient supply and often produce VFAs as a byproduct. These are retained in the improved compliance effluent and later removed by forming an important substrate supplement for the algal reactions.

The flowsheet for the primary handling of the feedstock followed by the bacterial reactor train is presented in Figure 7-2, with the accompanying unit descriptions and equations for the overall mass balance in Table 7-6 and the stream descriptions in Table 7-7. The symbols used for bacterial bioreactor yields (Table 7-8) and separator and splitter factors (Table 7-9) are presented. The equations for the mass balances for each unit are spelled out in the order in which they appear in the bacterial bioreactor train in Table 7-10 to Table 7-16.

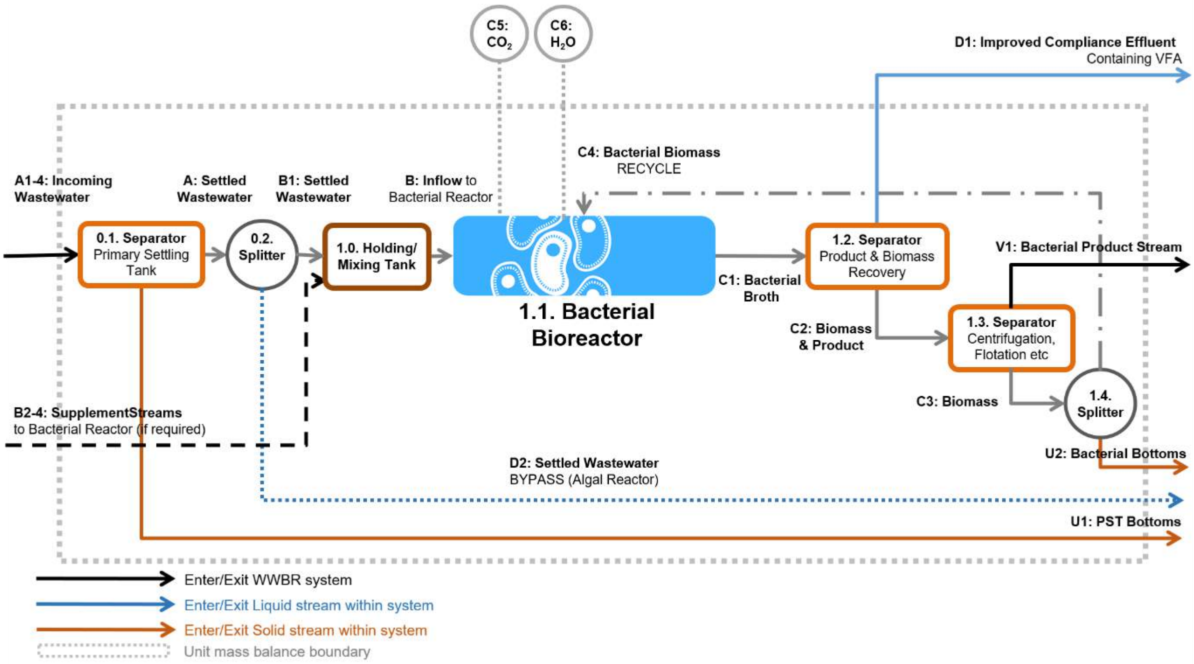


Figure 7-2: Bacterial bioreactor train detailed flowsheet

Table 7-6: Overall mass balance for bacterial bioreactor train

Unit number	Type	Unit description	Overall mass balance
0.1	Solid/Liquid Separator	Primary Settling Tank (PST) settling raw wastewater, removing the bulk of the solids	(A1 + A2 + A3 + A4) – (A + U1) = 0
0.2	Splitter	Settled, raw wastewater to bacterial and algal reactors	(A) – (B1 + D2) = 0
1.0	Mixing tank	Mixing supplementary substrate streams and providing buffer capacity to average flows and compositions	(B1 + B2 + B3 + B4) – (B) = 0
1.1	Reactor	Bacterial bioreactor	(B + C4 + C5 + C6) – (C1) = 0
1.2	Product & Biomass recovery	Separates product & bacterial biomass from improved effluent (to algal reactor): this may occur within reactor	(C1) – (C2 + D1) = 0
1.3	Downstream processing	Downstream processing for separation of bacterial product from biomass or residual biomass: for example centrifugation, flotation	(C2) – (C3 + V1) = 0
1.4	Splitter	Bacterial biomass to recycle and to Solids bioreactor	(C3) – (C4 + U2) = 0

It is noted that Tank 1.0 may be used as a holding tank if required. Under these conditions, intermittent accumulation occurs and the material balance given will not apply on an instantaneous basis, but on a cyclical basis. Further, depending on product purity required and nature of product, DSP unit 1.3 may consist of multiple units.

Table 7-7: Streams in bacterial bioreactor train

Stream number	Stream description	Relation to process units	Relation to other streams Equations refer to mass balance (kg/day)
A1	Raw Wastewater A1	Into Unit 0.1: Primary Setting Tank, Separator	Incoming stream, volume and composition chosen by user.
A2	Raw Wastewater A2	Into Unit 0.1: Primary Setting Tank, Separator	Incoming stream, volume and composition chosen by user. (Optional stream)
A3	Raw Wastewater A3	Into Unit 0.1: Primary Setting Tank, Separator	Incoming stream, volume and composition chosen by user. (Optional stream)
A4	Raw Wastewater A4	Into Unit 0.1: Primary Setting Tank, Separator	Incoming stream, volume and composition chosen by user. (Optional stream)
A	Settled Raw Wastewater	Into Unit 0.2: Splitter	Mixed incoming stream, volume and composition a function of A1-A4, with solids removed. A = A1-4 – U1
B1	Settled Raw Wastewater	From Unit 0.2: Splitter Into Unit 1.0: Holding tank	B1 = A - D2 Composition same as A, D2.
B2	Supplementary Feed	Into Unit 1.0: Holding tank	Incoming stream, volume and composition set by user. (Optional stream)
B3	Supplementary Feed	Into Unit 1.0: Holding tank	Incoming stream, volume and composition set by user. (Optional stream)
B4	Supplementary Feed	Into Unit 1.0: Holding tank	Incoming stream, volume and composition set by user. (Optional stream)
B	Mixed Inflow Stream	From Unit 1.0: Holding tank Into Unit 1.1: Bacterial Bioreactor	B = B1 + B2 + B3 + B4 Composition composite
C1	Bacterial Broth	From Unit 1.1: Bacterial Bioreactor Into Unit 1.2: Separator	C1 = B + C4 + C5 + C6 Composition changed from B1
C2	Bacterial Biomass & Product	Main Solids Component from Unit 1.2 Into Separator Unit 1.3	Solids composition similar to Solids in C1. Volume low, wet biomass.
C3	Biomass	From Unit 1.3: Separator Into Unit 1.4: Splitter	Composition changed from C2, Volume also less.
C4	Bacterial Biomass Recycle	From Unit 1.4: Splitter Into Unit 1.1: Bacterial Bioreactor	C4 = C3 - U2 Composition same as C3.
C5	CO_2	From Unit 1.1: Bacterial Bioreactor To Atmosphere	CO_2 only
C6	H_2O	Between Unit 1.1: Bacterial Bioreactor and Atmosphere	H_2O only
D1	Improved Compliance Effluent	From Unit 1.2: Separator Into Unit 2.1: Algal Bioreactor	D = C1 - C2 Composition same as dissolved composition C1
D2	Settled Raw Wastewater	From Unit 0.2: Splitter Into Unit 2.0: Holding Tank for Algal Bioreactor	D2 = A - B1 Composition same as A, B1.
U2	Bacterial Biomass	From Unit 1.4: Splitter Into Unit 4.1: Solids Bioreactor	U2 = C3 - C4 Composition based on bacterial biomass
V1	Bacterial Product Stream	From Unit 1.3: Separator Exit system	V1 = B * Bacterial bioproduct yield coefficient * Separation efficiencies Composition as specified by user

Table 7-8: Bacterial bioreactor yields

Conversion description	Unit	Symbol of factor
Mass of carbon reporting to bacterial biomass as a fraction of that present in influent stream to bacterial reactor (B)	kgC(Bacterial Biomass)/kg C(inflow Bacterial Bioreactor)	$Y_{C,XBact/IN}$
Mass of carbon reporting to product V1 as a fraction of that present in influent stream to bacterial reactor (B)	kgC(Product V1)/kg C(Inflow Bacterial Bioreactor)	$Y_{C,V1/IN}$
Mass of carbon reporting to interim product VFA as a fraction of that present in influent stream to bacterial reactor (B)	kgC(VFA)/kg C(Inflow Bacterial Bioreactor)	$Y_{C,VFA/IN}$
Mass of carbon leaving as CO_2 as a fraction of that present in influent stream to reactor (B)	kgC(CO_2 Bacterial Respiration)/kg C(Inflow Bacterial Bioreactor)	$Y_{C,CO2Bact/IN}$
Mass of carbon remaining unconverted as a fraction of that present in influent stream to reactor (B)	kgC (Unconverted)/kgC(Inflow Bacterial Bioreactor)	$Y_{C,INBact,unconverted/IN} = 1 - (Y_{C,XBact/IN} + Y_{C,V1/IN} + Y_{C,VFA/IN} + Y_{C,CO2Bact/IN})$

Table 7-9: Factors for separator and splitter units in bacterial bioreactor train

Unit number	Separator description	Relevant parameters	Factor symbol
0.1	Primary Settling	Slurry solids content Solids to Bottoms U1	SC_{U1} eff_{U1}
1.2	Product & Biomass Recovery	Slurry solids content Solids to Bottoms C2	SC_{C2} eff_{C2}
1.3	Bacterial Product Recovery	Slurry solids content Bacterial Product Recovery efficiency Solids (Biomass) to Bottoms C3	SC_{C3} eff_{V1} eff_{C3}
Unit number	**Splitter Description**	**Streams split**	**Split ratio symbol**
0.2	Raw Settled Wastewater	Fraction to Bacterial Bioreactor B1 Fraction to Algal Bioreactor D2	r_{B1} $1 - r_{B1}$
1.4	Bacterial Biomass Recycle	Fraction to Bacterial Bioreactor C4 Fraction to Solids Bioreactor U2	r_{C4} $1 - r_{C4}$

7.4.1 Mass balances for primary handling of feedstock

Before the bacterial bioreactor train per se, the wastewater feedstock streams must be mixed (if there are multiple streams) and separated to remove solids and potentially to allow a bypass. The primary settling tank (0.1; Table 7-10) receives the feedstock and the liquid component of settled wastewater (A) flows to the splitter (0.2; Table 7-11) where the main stream (B1) goes into the bacterial bioreactor train and a secondary stream (D2) is sent in a bypass directly to the algal bioreactor train (Section 7.4.1). This is an optional stream which may be needed if the effluent from the bacterial bioreactor stream contains insufficient total nutrients for the operation of the algal bioreactor. The solids slurry (U1) is taken as bottoms direct to the solids bioreactor train (Section 7.4.1).

Table 7-10: Mass balance for Unit 0.1 Separator: Primary Settling Tank

Carbon Mass Balance: Unit 0.1: Separator: Primary Settling Tank			
Carbon Fraction	**A1,A2,A3,A4: Incoming Wastewater**	**A: Settled Wastewater**	**U1: PST Bottoms to Solids Bioreactor**
Unconverted Carbon $IN_{C,liq}$ Liquid fraction	$IN_{C(A1-A4)liq} = Q_{(A1)liq} * C_{C(A1)liq} + Q_{(A2)liq} * C_{C(A2)liq} + Q_{(A3)liq} * C_{C(A3)liq} + Q_{(A4)liq} * C_{C(A4)liq}$	$IN_{C(A)liq} = IN_{C(A1-A4)liq} * (N_{W(A)}/N_{W(A1-A4)})$	$IN_{C(U1)liq} = IN_{C(A1-A4)liq} * (N_{W(U1)}/N_{W(A1-A4)})$
Unconverted Carbon $IN_{C,sol}$ Solid fraction	$IN_{C(A1-A4)sol} = Q_{(A1)sol} * C_{C(A1)sol} + Q_{(A2)sol} * C_{C(A2)sol} + Q_{(A3)sol} * C_{C(A3)sol} + Q_{(A4)sol} * C_{C(A4)sol}$	$IN_{C(A)sol} = IN_{C(A1-A4)sol} * (1-eff_{U1})$	$IN_{C(U1)sol} = IN_{C(A1-A4)sol} * eff_{U1}$
Totals	$N_{C(A1-A4)} = IN_{C(A1-A4)liq} + IN_{C(A1-A4)sol}$	$N_{C(A)} = IN_{C(A)liq} + IN_{C(A)sol}$	$N_{C(U1)} = IN_{C(U1)liq} + IN_{C(U1)sol}$
Checks: Total stream amounts: $(N_{C(A1-A4)}) - (N_{C(A)} + N_{C(U1)}) = 0$ After the PST, it is assumed that any solids still in the stream is hydrolysed and incorporated into the dissolved component. The dissolved component in the solids fraction is assumed to be easily biodegradable and follows the biocatalysis in the solids reactor like the solids.			
Nitrogen Mass Balance: Unit 0.1: Separator: Primary Settling Tank			
Nitrogen Fraction	**A1,A2,A3,A4: Incoming Wastewater**	A: Settled Wastewater	**U1: PST Bottoms to Solids Bioreactor**
Nitrogen Liquid Fraction	$IN_{N(A1-A4)liq} = Q_{(A1)liq} * C_{N(A1)liq} + Q_{(A2)liq} * C_{N(A2)liq} + Q_{(A3)liq} * C_{N(A3)liq} + Q_{(A4)liq} * C_{N(A4)liq}$	$IN_{N(A)liq} = IN_{N(A1-A4)liq} * (N_{W(A)}/N_{W(A1-A4)})$	$IN_{N(U1)liq} = IN_{N(A1-A4)liq} * (N_{W(U1)}/N_{W(A1-A4)})$
Unconverted Nitrogen Solid Fraction	$IN_{C(A1-A4)sol} = Q_{(A1)sol} * C_{C(A1)sol} + Q_{(A2)sol} * C_{C(A2)sol} + Q_{(A3)sol} * C_{C(A3)sol} + Q_{(A4)sol} * C_{C(A4)sol}$	$IN_{N(A)sol} = IN_{N(A1-A4)sol} * (1-eff_{U1})$	$IN_{N(U1)sol} = IN_{N(A1-A4)sol} * eff_{U1}$
Totals	$N_{N(A1-A4)} = IN_{N(A1-A4)liq} + IN_{N(A1-A4)sol}$	$N_{N(A)} = IN_{N(A)liq} + IN_{N(A)sol}$	$N_{N(U1)} = IN_{N(U1)liq} + IN_{N(U1)sol}$
Checks: Total stream amounts: $(N_{N(A1-A4)}) - (N_{N(A)} + N_{N(U1)}) = 0$ After the PST, it is assumed that any solids still in the stream is hydrolysed and incorporated into the dissolved component. The dissolved component in the solids fraction is assumed to be easily biodegradable and follows the biocatalysis in the solids reactor like the solids.			
Phosphorous Mass Balance: Unit 0.1: Separator: Primary Settling Tank			
Phosphorous Fraction	**A1,A2,A3,A4: Incoming Wastewater**	**A: Settled Wastewater**	**U1: PST Bottoms to Solids Bioreactor**
Unconverted Phosphorous Liquid Fraction	$IN_{P(A1-A4)liq} = Q_{(A1)liq} * C_{P(A1)liq} + Q_{(A2)liq} * C_{P(A2)liq} + Q_{(A3)liq} * C_{P(A3)liq} + Q_{(A4)liq} * C_{P(A4)liq}$	$IN_{P(A)liq} = IN_{P(A1-A4)liq} * (N_{W(A)}/N_{W(A1-A4)})$	$IN_{P(U1)liq} = IN_{P(A1-A4)liq} * (N_{W(U1)}/N_{W(A1-A4)})$
Unconverted Phosphorous Solid Fraction	$IN_{P(A1-A4)sol} = Q_{(A1)sol} * C_{P(A1)sol} + Q_{(A2)sol} * C_{P(A2)sol} + Q_{(A3)sol} * C_{P(A3)sol} + Q_{(A4)sol} * C_{P(A4)sol}$	$IN_{P(A)sol} = IN_{P(A1-A4)sol} * (1-eff_{U1})$	$IN_{P(U1)sol} = IN_{P(A1-A4)sol} * eff_{U1}$
Totals	$N_{P(A1-A4)} = IN_{P(A1-A4)liq} + IN_{P(A1-A4)sol}$	$N_{P(A)} = IN_{P(A)liq} + IN_{P(A)sol}$	$N_{P(U1)} = IN_{P(U1)liq} + IN_{P(U1)sol}$
Checks: Total stream amounts: $(N_{P(A1-A4)}) - (N_{P(A)} + N_{P(U1)}) = 0$ After the PST, it is assumed that any solids still in the stream is hydrolysed and incorporated into the dissolved component. The dissolved component in the solids fraction is assumed to be easily biodegradable and follows the biocatalysis in the solids reactor like the solids.			

Water Mass Balance: Unit 0.1: Separator: Primary Settling Tank			
Water Fraction	**A1,A2,A3,A4: Incoming Wastewater**	**A: Settled Wastewater**	**U1: PST Bottoms to Solids Bioreactor**
Total Water	$N_{W(A1-A4)} = N_{W(A1)liq} + N_{W(A2)liq} + N_{W(A3)liq} + N_{W(A4)liq}$	$N_{W(A)} = N_{W(A1-A4)} - N_{W(U1)}$	$N_{W(U1)} = N_{TOTAL(A1-4)sol} * ((1-SC_{U1})/SC_{U1})$
Checks: Total stream amounts: $N_{W(A1-A4)} – N_{W(A)} - N_{W(U1)} = 0$ This only considers the water in the liquid fraction. While the solids component has H and O, (C + N + P < 1), this is associated with e.g. carbohydrates. While there may be interstitial water associated between solids particles, these are not considered for this mass balance. The value of the total solids content of stream U1 is set by the solids content of the incoming streams. The water in the stream is determined by the Solids Content (SC) in the slurry after settling.			

Table 7-11: Mass balance for Unit 0.2 Splitter: settled wastewater to bacterial bioreactor and bypass

Carbon, Nitrogen, Phosphorous and Water Mass Balance: Unit 0.2: Splitter			
Fraction	**A: Settled Wastewater**	**B1: Settled Wastewater**	**D2: Settled Wastewater BYPASS (Algal Reactor)**
Total Carbon	$N_{C(A)}$	$N_{C(B1)} = N_{C(A)} * r_{B1}$	$N_{C(D2)} = N_{C(A)} * (1 - r_{B1})$
Total Nitrogen	$N_{N(A)}$	$N_{N(B1)} = N_{N(A)} * r_{B1}$	$N_{N(D2)} = N_{N(A)} * (1 - r_{B1})$
Total Phosphorous	$N_{P(A)}$	$N_{P(B1)} = N_{P(A)} * r_{B1}$	$N_{P(D2)} = N_{P(A)} * (1 - r_{B1})$
Total Water	$N_{W(A)}$	$N_{W(B1)} = N_{W(A)} * r_{B1}$	$N_{W(D2)} = N_{W(A)} * (1 - r_{B1})$
Checks: Total stream amounts: $(N_{C(A)}) – (N_{C(B1)} + N_{C(D2)}) = 0$ $(N_{N(A)}) – (N_{N(B1)} + N_{N(D2)})) = 0$ $(N_{P(A)}) – (N_{P(B1)} + N_{P(D2)}) = 0$ $(N_{W(A)}) – (N_{W(B1)} + N_{W(D2)}) = 0$			

7.4.2 Mass balances of mixing tank and bacterial bioreactor

The Bacterial Bioreactor Train begins with a mixing tank (1.0; Table 7-12) which receives the settled wastewater from the primary handling (B1) as influent together with any supplementary nutrient streams (B2-4). This unit may perform a holding function if the bacterial bioreactor is operated in semi-batch mode or if the incoming wastewater feedstock streams have an inconstant flowrate; however, this mass balance ignores temporary accumulation in these situations with the assumption that this is adequate for early-stage feasibility assessment. The combined emerging stream (B) forms the inflow to the bacterial reactor (1.1; Table 7-13). Many bacterial reactors will need a mechanism for increasing the biomass residence time, and an optional biomass recycle stream (C4) is included. The bacterial respiration will release carbon dioxide to atmosphere (C5) and, depending on the reactor type and configuration, water may enter or leave the system (C6) through precipitation or evaporation.

Table 7-12: Mass balance for Unit 1.0 Mixing Tank: bacterial bioreactor inflow

Carbon, Nitrogen, Phosphorous and Water Mass Balance: Unit 1.0: Mixing tank			
Fraction	**B1: Settled Wastewater**	**B2-4 Supplement Streams**	**B: Inflow to Bacterial Bioreactor**
Total Carbon	$N_{C(B1)} = N_{C(A)} * r_{B1}$	$N_{C(B2\text{-}4)} = Q_{(B2)} * C_{C(B2)} + Q_{(B3)} * C_{C(B4)} + Q_{(B4)} * C_{C(B4)}$	$N_{C(B)} = N_{C(B1)} + N_{C(B2\text{-}4)}$
Total Nitrogen	$N_{N(B1)} = N_{N(A)} * r_{B1}$	$N_{N(B2\text{-}4)} = Q_{(B2)} * C_{N(B2)} + Q_{(B3)} * C_{N(B3)} + Q_{(B4)} * C_{N(B4)}$	$N_{N(B)} = N_{N(B1)} + N_{N(B2\text{-}4)}$
Total Phosphorous	$N_{P(B1)} = N_{P(A)} * r_{B1}$	$N_{P(B2\text{-}4)} = Q_{(B2)} * C_{P(B2)} + Q_{(B3)} * C_{P(B3)} + Q_{(B4)} * C_{P(B4)}$	$N_{P(B)} = N_{P(B1)} + N_{P(B2\text{-}4)}$
Total Water	$N_{W(B1)} = N_{W(A)} * r_{B1}$	$N_{W(B2\text{-}4)} = N_{W(B2)} + N_{W(B3)} + N_{W(B4)}$	$N_{W(B)} = N_{W(B1)} + N_{W(B2\text{-}4)}$
Checks: Total stream amounts: $(N_{C(B1)} + N_{C(B2\text{-}4)}) - (N_{C(B)}) = 0$ $(N_{N(B1)} + N_{N(B2\text{-}4)}) - (N_{N(B)}) = 0$ $(N_{P(B1)} + N_{P(B2\text{-}4)}) - (N_{P(B)}) = 0$ $(N_{W(B1)} + N_{W(B2\text{-}4)}) - (N_{W(B)}) = 0$ The Substrate Streams B2, B3 and B4 are assumed to have negligible solids component.			

Table 7-13: Mass balance for Unit 1.1 Bacterial Bioreactor

Carbon Mass Balance: Unit 1.1: Bacterial Bioreactor					
Carbon Fraction	**B: Inflow to Bacterial Bioreactor**	**C1: Bacterial Broth**	**C4: Bacterial Biomass RECYCLE**	**C5: CO_2 Release = Outflow**	**C6: H_2O**
Biomass $X_{Bacterial}$		$X_{C(C1)} = N_{C(B)} * Y_{XBacterial/C} + X_{C(C4)}$	$X_{C(C4)} = X_{C(C3)} * r_{C4}$		
Product P_{V1}		$P_{V1,C(C1)} = N_{C(B)} * Y_{P,V1/C} + P_{V1,C(C4)}$	$P_{V1,C(C4)} = P_{V1,C(C3)} * r_{C4}$		
Product P_{VFA}		$P_{VFA,C(C1)} = N_{C(B)} * Y_{P,VFA/C} + P_{VFA,C(C4)}$	$P_{VFA,C(C4)} = P_{VFA,C(C3)} * r_{C4}$		
Carbon Dioxide $CO_{2Bacterial}$				$CO_{2C,Bacterial(C5)} = N_{C(B)} * Y_{CO2Bacterial/C}$	
Unconverted Carbon	$IN_{C(B)} = N_{C(B)} = N_{C(B1)} + N_{C(B2\text{-}4)}$	$IN_{C(C1)} = N_{C(B)} * (1- (Y_{XBacterial/C} + Y_{P,V1/C} + Y_{P,VFA/C} + Y_{CO2Bacterial/C}))$	$IN_{C(C4)} = IN_{C(C3)} * r_{C4}$		
Totals	$N_{C(B)} = IN_{C(B)}$	$N_{C(C1)} = X_{C(C1)} + P_{V1,C(C1)} + P_{VFA,C(C1)} + IN_{C(C1)}$	$N_{C(C4)} = X_{C(C4)} + P_{V1,C(C4)} + P_{VFA,C(C4)} + IN_{C(C4)}$	$N_{C(C5)} = CO_{2Bacterial(C5)}$	
Checks: Total stream amounts: $(N_{C(B)} + N_{C(C4)} + N_{C(C5)}) - (N_{C(C1)}) = 0$					

Nitrogen Mass Balance: Unit 1.1: Bacterial Bioreactor					
Nitrogen Fraction	**B: Inflow to Bacterial Bioreactor**	**C1: Bacterial Broth**	**C4: Bacterial Biomass RECYCLE**	**C5: CO_2 Release = Outflow**	**C6: H_2O**
Biomass $X_{Bacterial}$		$X_{N(C1)} = X_{C(C1)} * f(X_{bact})_{N/C}$	$X_{N(C4)} = X_{C(C4)} * f(X_{bact})_{N/C}$		
Product P_{V1}		$P_{V1,N(C1)} = P_{V1,C(C1)} * f(V1)_{N/C}$	$P_{V1,N(C4)} = P_{V1,C(C4)} * f(V1)_{N/C}$		
Unconverted Nitrogen	$IN_{N(B)} = N_{N(B)} = N_{N(B1)} + N_{(B2\text{-}4)}$	$IN_{N(C1)} = IN_{N(B)} - X_{N(C1)} - P_{V1,N(C1)}$	$IN_{N(C4)} = IN_{N(C3)} * r_{C4}$		
Totals	$N_{N(B)} = IN_{N(B)}$	$N_{N(C1)} = X_{N(C1)} + P_{V1,N(C1)} + IN_{N(C1)}$	$N_{N(C4)} = X_{N(C4)} + P_{V1,N(C4)} + IN_{N(C4)}$		
Checks: Total stream amounts: $(N_{N(B)} + N_{N(C4)}) - (N_{N(C1)}) = 0$					

Phosphorous Mass Balance: Unit 1.1: Bacterial Bioreactor					
Phosphorous Fraction	B: Inflow To Bacterial Bioreactor	C1: Bacterial Broth	C4: Bacterial Biomass RECYCLE	C5: CO2 Release = outflow	C6: H2O
Biomass XBacterial		$X_{P(C1)} = X_{C(C1)} * f_{(XBact)P/C}$	$X_{N(C4)} = X_{C(C4)} * f_{(XBact)P/C}$		
Product PV1		$P_{V1,P(C1)} = P_{V1,C(C1)} * f_{(V1)P/C}$	$P_{V1,N(C4)} = P_{V1,C(C4)} * f_{(V1)P/C}$		
Unconverted Phosphorous	$IN_{P(B)} = N_{P(B)} = N_{P(B1)} + N_{P(B2\text{-}4)}$	$IN_{P(C1)} = IN_{P(B)} - X_{P(C1)} - P_{V1,P(C1)}$	$IN_{P(C4)} = IN_{P(C3)} * r_{C4}$		
Totals	$N_{P(B)} = IN_{P(B)}$	$N_{P(C1)} = X_{P(C1)} + P_{V1,P(C1)} + IN_{N(C1)}$	$N_{P(C4)} = X_{P(C4)} + P_{V1,P(C4)} + IN_{P(C4)}$		
Checks: Total stream amounts: $(N_{P(B)} + N_{P(C4)}) - (N_{P(C1)}) = 0$					

Water Mass Balance: Unit 1.1: Bacterial Bioreactor					
	B: Inflow to Bacterial Bioreactor	**C1: Bacterial Broth**	**C4: Bacterial Biomass RECYCLE**	**C5: CO_2 Release = Outflow**	**C6: H_2O**
Total Water	$N_{W(B)}$	$N_{W(C1)} = N_{W(B)} + N_{W(C4)} + N_{W(C6)}$	$N_{W(C4)}$		$N_{W(C6)} = (N_{W(B)} + N_{W(C4)})*(F_{rain} - F_{evap})$
$(N_{W(B)} + N_{W(C4)} + N_{W(C6)}) - (N_{W(C1)}) = 0$					

7.4.3 Mass balance for first separation step for bacterial bioreactor outflow

The bacterial broth (C1) emerging from the reactor includes product, biomass and the changed composition liquid; this stream enters a series of separator and splitter units in order to recover the necessary streams. The first separator (1.2; Table 7-14) is operated to remove all biomass and product, sending the changed-composition water stream (D1) to the algal reactor train as the main influent (Section 7.5.1). This stream has both improved compliance towards ultimate reuse, through the removal of nutrients and increased suitability as an inflow feed for the algal reactor through the VFAs produced and N and P components liberated as interim products in the bacterial reactor and potential nutrients for the algal bioreactor.

Table 7-14: Mass balance for Unit 1.2 Separator: bacterial biomass & bacterial product V1 from improved compliance effluent

Carbon Mass Balance: Unit 1.2: Separator			
Carbon Fraction	**C1: Bacterial Broth outflow**	**C2: Biomass & Product**	**D1: Improved Compliance Effluent**
Biomass $X_{Bacterial}$	$X_{C(C1)} = ((N_{C(B2)} + N_{C(B4\text{-}6)}) * Y_{XBacterial/C}) + X_{C(C4)}$	$X_{C(C2)} = X_{C(C1)} * eff_{C2}$	$X_{C(D1)} = X_{C(C1)} * (1 - eff_{C2})$
Product P_{V1}	$P_{V1,C(C1)} = N_{C(B)} * Y_{P,V1/C} + P_{V1,C(C4)}$	$P_{V1,C(C2)} = P_{V1,C(C1)} * eff_{C2}$	$P_{V1,C(D1)} = P_{V1,C(C1)} * (1 - eff_{C2})$
Product P_{VFA}	$P_{VFA,C(C1)} = N_{C(B)} * Y_{P,VFA/C} + P_{VFA,C(C4)}$	$P_{VFA,C(C2)} = P_{VFA,C(C1)} * (N_{W(C2)}/N_{W(C1)})$	$P_{VFA,C(D1)} = P_{VFA,C(C1)} * (N_{W(D1)}/N_{W(C1)})$
Unconverted Carbon	$IN_{C(C1)} = N_{C(B)} * (1- (Y_{XBacterial/C} + Y_{P,V1/C} + Y_{P,VFA/C} + Y_{CO2Bacterial/C}))$	$IN_{C(C2)} = IN_{C(C1)} * (N_{W(C2)}/N_{W(C1)})$	$IN_{C(D1)} = IN_{C(C1)} * (N_{W(D1)}/N_{W(C1)})$
Totals	$N_{C(C1)} = X_{C(C1)} + P_{V1,C(C1)} + P_{VFA,C(C1)} + IN_{C(C1)}$	$N_{C(C2)} = X_{C(C2)} + P_{V1,C(C2)} + P_{VFA,C(C2)} + IN_{C(C2)}$	$N_{C(D1)} = X_{C(D1)} + P_{V1,C(D1)} + P_{VFA,C(D1)} + IN_{C(D1)}$
Checks: Total stream amounts: $(N_{C(C1)}) – (N_{C(D1)} + N_{C(C2)}) = 0$ The fraction dissolved components (e.g. unconverted Carbon, VFA) depend on the water split, which depends on the solids content (SC) of the bottoms stream.			

Nitrogen Mass Balance: Unit 1.2: Separator			
Nitrogen Fraction	**C1: Bacterial Broth outflow**	**C2: Biomass & Product**	**D1: Improved Compliance Effluent**
Biomass $X_{Bacterial}$	$X_{N(C1)} = X_{C(C1)} * f(X_{Bact})_{N/C}$	$X_{N(C2)} = X_{N(C1)} * eff_{C2}$	$X_{N(D1)} = X_{N(C1)} * (1 - eff_{C2})$
Product P_{V1}	$P_{V1,N(C1)} = P_{V1,C(C1)} * f(V1)_{N/C}$	$P_{V1,N(C2)} = P_{V1,N(C1)} * eff_{C2}$	$P_{V1,N(D1)} = P_{V1,N(C1)} * (1 - eff_{C2})$
Product P_{VFA}	$P_{VFA,N(C1)} = P_{VFA,C(C1)} * f(VFA)_{N/C}$	$P_{VFA,N(C2)} = P_{VFA,N(C1)} * (N_{W(C2)}/N_{W(C1)})$	$P_{VFA,N(D1)} = P_{VFA,N(C1)} * (N_{W(D1)}/N_{W(C1)})$
Unconverted Nitrogen	$IN_{N(C1)} = (N_{N(B)}) – (X_{N(C1)} + P_{V1,N(C1)} + P_{VFA,N(C1)})$	$IN_{N(C2)} = IN_{N(C1)} * (N_{W(C2)}/N_{W(C1)})$	$IN_{N(D1)} = IN_{N(C1)} * (N_{W(D1)}/N_{W(C1)})$
Totals	$N_{N(C1)} = X_{N(C1)} + P_{V1,N(C1)} + P_{VFA,N(C1)} + IN_{N(C1)}$	$N_{N(C2)} = X_{N(C2)} + P_{V1,N(C2)} + P_{VFA,N(C2)} + IN_{N(C2)}$	$N_{N(D1)} = X_{N(D1)} + P_{V1,N(D1)} + P_{VFA,N(D1)} + IN_{N(D1)}$
Checks: Total stream amounts: (NN(C1)) – (NN(D1) + NN(C2)) = 0			

Phosphorous Mass Balance: Unit 1.2: Separator			
Phosphorous Fraction	**C1: Bacterial Broth outflow**	**C2: Biomass & Product**	**D1: Improved Compliance Effluent**
Biomass $X_{Bacterial}$	$X_{P(C1)} = X_{C(C1)} * f(X_{Bact})_{P/C}$	$X_{P(C2)} = X_{P(C1)} * eff_{C2}$	$X_{P(D1)} = X_{P(C1)} * (1 - eff_{C2})$
Product P_{V1}	$P_{V1,P(C1)} = P_{V1,C(C1)} * f(V1)_{P/C}$	$P_{I,P(C2)} = P_{I,P(C1)} * eff_{C2}$	$P_{I,P(D1)} = P_{I,P(C1)} * (1 - eff_{C2})$
Product P_{VFA}	$P_{VFA,P(C1)} = P_{VFA,C(C1)} * f(VFA)_{P/C}$	$P_{VFA,P(C2)} = P_{VFA,P(C1)} * (N_{W(C2)}/N_{W(C1)})$	$P_{VFA,P(D1)} = P_{VFA,P(C1)} * (N_{W(D1)}/N_{W(C1)})$
Unconverted Phosphorous	$IN_{P(C1)} = (N_{P(B)}) – (X_{P(C1)} + P_{V1,P(C1)} + P_{VFA,P(C1)})$	$IN_{P(C2)} = IN_{P(C1)} * (N_{W(C2)}/N_{W(C1)})$	$IN_{P(D1)} = IN_{P(C1)} * (N_{W(D1)}/N_{W(C1)})$
Totals	$N_{P(C1)} = X_{P(C1)} + P_{V1,P(C1)} + P_{VFA,P(C1)} + IN_{P(C1)}$	$N_{P(C2)} = X_{P(C2)} + P_{V1,P(C2)} + P_{VFA,P(C2)} + IN_{P(C2)}$	$N_{P(D1)} = X_{P(D1)} + P_{V1,P(D1)} + P_{VFA,P(D1)} + IN_{P(D1)}$
Checks: Total stream amounts: $(N_{P(C1)}) – (N_{P(D1)} + N_{P(C2)}) = 0$			

Water Mass Balance: Unit 1.2: Separator			
	C1: Bacterial Broth outflow	**C2: Biomass & Product**	**D1: Improved Compliance Effluent**
Total Water	$N_{W(C1)} = N_{W(B2)} + N_{W(B4\text{-}6)} + N_{W(C4)} - N_{W(C5)}$	$N_{W(C2)} = (N_{C(C2)}/C_{comp,bact}) * ((1\text{-}SC_{C2})/SC_{C2})$	$N_{W(D1)} = N_{W(C1)} - N_{W(C2)}$
Checks: Total stream amounts: $(N_{W(C1)}) – (N_{W(D1)} + N_{W(C2)}) = 0$ The value of the total solids content of stream C2 is estimated by dividing the kg Carbon in stream C2 ($N_{C(C2)}$) by the Carbon composition of bacterial biomass. This is an overestimation but is simplified from using the compositions of the product stream and residual VFA and unconverted Carbon substrate.			

7.4.4 Mass balances for subsequent separation steps for bacterial bioreactor outflow

The biomass-and-product stream (C2) flows to a second, and probably more complex, separator or set of separators (1.3; Table 7-15) which is operated to select for a very pure product stream (V1) and sends the biomass slurry (C3) to a splitter (1.4; Table 7-16). Here a biomass recycle stream (C4) is returned to the bacterial reactor, with the balance of the slurry sent as bottoms (U2) to combine with the primary feedstock slurry (U1) in the solids bioreactor train (Section 7.5.3).

Table 7-15: Mass balance for Unit 1.3 Separator: bacterial biomass from bacterial product V1

Carbon Mass Balance: Unit 1.3: Separator			
Carbon Fraction	**C2: Biomass & Product**	**C3: Biomass**	**V1: Bacterial Product Stream**
Biomass $X_{Bacterial}$	$X_{C(C2)} = X_{C(C1)} * eff_{C2}$	$X_{C(C3)} = X_{C(C2)} * eff_{C3}$	$X_{C(V1)} = X_{C(C2)} * (1 - eff_{C3})$
Product P_{V1}	$P_{V1,C(C2)} = P_{V1,C(C1)} * eff_{C2}$	$P_{V1,C(C3)} = P_{V1,C(C2)} * (1 - eff_{V1})$	$P_{V1,C(I)} = P_{V1,C(C2)} * eff_{V1}$
Product P_{VFA}	$P_{VFA,C(D1)} = P_{VFA,C(C1)} * (N_{W(D1)}/N_{W(C1)})$	$P_{VFA,C(C3)} = P_{VFA,C(C2)} * (N_{W(C3)}/N_{W(C2)})$	$P_{VFA,C(V1)} = P_{VFA,C(C2)} * (N_{W(V1)}/N_{W(C2)})$
Unconverted Carbon	$IN_{C(C2)} = IN_{C(C1)} * (N_{W(D1)}/N_{W(C1)})$	$IN_{C(C3)} = IN_{C(C2)} * (N_{W(C3)}/N_{W(C2)})$	$IN_{C(V1)} = IN_{C(C2)} * (N_{W(V1)}/N_{W(C2)})$
Totals	$N_{C(C2)} = X_{C(C2)} + P_{V1,C(C2)} + P_{VFA,C(C2)} + IN_{C(C2)}$	$N_{C(C3)} = X_{C(C3)} + P_{V1,C(C3)} + P_{VFA,C(C3)} + IN_{C(C3)}$	$N_{C(V1)} = X_{C(V1)} + P_{V1,C(V1)} + P_{VFA,C(V1)} + IN_{C(V1)}$
Checks: Total stream amounts: $(N_{C(C2)}) – (N_{C(V1)} + N_{C(C3)}) = 0$ The emphasis here is on recovery of Product V1, and it is assumed that the processes involved here bring about a concentration change of Product V1 as well, so that the Carbon (and the other nutrients) mass balance of Product V1 cannot simply be linked to the water split. Product stream V1 is not pure product V1, and there is some water still associated with the product stream. If this is processed further, this water stream, $N_{W(V1)}$ is lost to downstream processing.			
Nitrogen Mass Balance: Unit 1.3: Separator			
Nitrogen Fraction	**C2: Biomass & Product**	**C3: Biomass**	**V1: Bacterial Product Stream**
Biomass $X_{Bacterial}$	$X_{N(C2)} = X_{N(C1)} * eff_{C2}$	$X_{N(C3)} = X_{N(C2)} * eff_{C3}$	$X_{N(V1)} = X_{N(C2)} * (1 - eff_{C3})$
Product P_{V1}	$P_{V1,N(C2)} = P_{V1,N(C1)} * eff_{C2}$	$P_{V1,N(C3)} = P_{V1,N(C2)} * (1 - eff_{V1})$	$P_{V1,N(V1)} = P_{V1,N(C2)} * eff_{V1}$
Product P_{VFA}	$P_{VFA,N(C2)} = P_{VFA,N(C1)} * (N_{W(C2)}/N_{W(C1)})$	$P_{VFA,N(C3)} = P_{VFA,N(C2)} * (N_{W(C3)}/N_{W(C2)})$	$P_{VFA,N(V1)} = P_{VFA,N(C2)} * (N_{W(V1)}/N_{W(C2)})$
Unconverted Nitrogen	$IN_{N(C2)} = IN_{N(C1)} * (N_{W(C2)}/N_{W(C1)})$	$IN_{N(C3)} = IN_{N(C2)} * (N_{W(C3)}/N_{W(C2)})$	$IN_{N(V1)} = IN_{N(C2)} * (N_{W(V1)}/N_{W(C2)})$
Totals	$N_{N(C2)} = X_{N(C2)} + P_{V1,N(C2)} + P_{VFA,N(C2)} + IN_{N(C2)}$	$N_{N(C3)} = X_{N(C3)} + P_{V1,N(C3)} + P_{VFA,N(C3)} + IN_{N(C3)}$	$N_{N(V1)} = X_{N(V1)} + P_{V1,N(V1)} + P_{VFA,N(V1)} + IN_{N(V1)}$
Checks: Total stream amounts: $(N_{N(C2)}) – (N_{N(V1)} + N_{N(C3)}) = 0$			

Phosphorous Mass Balance: Unit 1.3: Separator			
Phosphorous Fraction	**C2: Biomass & Product**	**C3: Biomass**	**V1: Bacterial Product Stream**
Biomass $X_{Bacterial}$	$X_{P(C2)} = X_{P(C1)} * eff_{C2}$	$X_{P(C3)} = X_{P(C2)} * eff_{C3}$	$X_{P(V1)} = X_{P(C2)} * (1 - eff_{C3})$
Product P_{V1}	$P_{V1,P(C2)} = P_{V1,P(C1)} * eff_{C2}$	$P_{V1,P(C3)} = P_{V1,P(C2)} * (1 - eff_{V1})$	$P_{V1,P(V1)} = P_{V1,P(C2)} * eff_{V1}$
Product P_{VFA}	$P_{VFA,P(C2)} = P_{VFA,P(C1)} * (1 - eff_{D1})$	$P_{VFA,P(C3)} = P_{VFA,P(C2)} * (N_{W(C3)}/N_{W(C2)})$	$P_{VFA,P(V1)} = P_{VFA,P(C2)} * (N_{W(V1)}/N_{W(C2)})$
Unconverted Phosphorous	$IN_{P(C2)} = IN_{P(C1)} * (1 - eff_{D1})$	$IN_{P(C3)} = IN_{P(C2)} * (N_{W(C3)}/N_{W(C2)})$	$IN_{P(V1)} = IN_{P(C2)} * (N_{W(V1)}/N_{W(C2)})$
Totals	$N_{P(C2)} = X_{P(C2)} + P_{V1,P(C2)} + P_{VFA,P(C2)} + IN_{P(C2)}$	$N_{P(C3)} = X_{P(C3)} + P_{V1,P(C3)} + P_{VFA,P(C3)} + IN_{P(C3)}$	$N_{P(V1)} = X_{P(V1)} + P_{V1,P(V1)} + P_{VFA,P(V1)} + IN_{P(V1)}$
Checks: Total stream amounts: $(N_{P(C2)}) – (N_{P(V1)} + N_{P(C3)}) = 0$			

Water Mass Balance: Unit 1.3: Separator			
	C2: Biomass & Product	**C3: Biomass**	**V1: Bacterial Product Stream**
Total Water	$N_{W(C2)} = (N_{C(C2)}/C_{comp, bact})*((1-SC_{C2})/SC_{C2})$	$N_{W(C3)} = (N_{C(C3)}/C_{comp, bact})*((1-SC_{C3})/SC_{C3})$	$N_{W(V1)} = N_{W(C2)} - N_{W(C3)}$
Checks: Total stream amounts: $(N_{W(C2)}) – (N_{W(V1)} + N_{W(C3)}) = 0$ The value of the total solids content of stream C3 is estimated by dividing the kg Carbon in stream C3 ($N_{C(C3)}$) by the Carbon composition of bacterial biomass .			

Table 7-16: Mass balance for Unit 1.4 Splitter: bacterial biomass to recycle and bottoms

Carbon, Nitrogen, Phosphorous and Water Mass Balance: Unit 1.4: Splitter			
Fraction	**C3: Biomass**	**C4: Bacterial Biomass RECYCLE**	**U2: Bacterial Bottoms**
Total Carbon	$N_{C(C3)}$	$N_{C(C4)} = N_{C(C3)} * r_{C4}$	$N_{C(U2)} = N_{C(C3)} * (1 - r_{C4})$
Total Nitrogen	$N_{N(C3)}$	$N_{N(C4)} = N_{N(C3)} * r_{C4}$	$N_{N(U2)} = N_{N(C3)} * (1 - r_{C4})$
Total Phosphorous	$N_{P(C3)}$	$N_{P(C4)} = N_{P(C3)} * r_{C4}$	$N_{P(U2)} = N_{P(C3)} * (1 - r_{C4})$
Total Water	$N_{W(C3)}$	$N_{W(C4)} = N_{W(C3)} * r_{C4}$	$N_{W(U2)} = N_{W(C3)} * (1 - r_{C4})$
Checks: Total stream amounts: $(N_{C(C3)}) – (N_{C(C4)} + N_{C(U2)}) = 0$ $(N_{N(C3)}) – (N_{N(C4)} + N_{N(U2)}) = 0$ $(N_{P(C3)}) – (N_{P(C4)} + N_{P(U2)}) = 0$ $(N_{W(C3)}) – (N_{W(C4)} + N_{W(U2)}) = 0$			

7.5 Flowsheets and Mass Balances for Other Bioreactor Units

Having presented the overview flowsheet for the generic WWBR (Figure 7-1), the detailed generic flowsheet and complete tables of mass balance equations were reported for the primary feedstock handling and bacterial bioreactor train (Figure 7-2, Section 7.3). The correspondingly detailed flowsheets for the algal (Figure 7-3, Section 7.5.1), macrophyte (Figure 7-4, Section 7.5.2) and solids (Figure 7-5, Section 7.5.3) bioreactor trains are now presented together with overview mass balance equations. All the detailed mass balance equations for these three reactor trains in the generic WWBR can be found in Appendix F.

7.5.1 Flowsheet and mass balance for the algal bioreactor

In the generalised WWBR flowsheet presented as an example in this study, the algal bioreactor follows the bacterial bioreactor. The purpose in terms of the wastewater remediation aspect of the biorefinery is that the algal processes are expected remove a high proportion of the nitrogen and phosphorus entering in the feedstock streams. In addition, the placement after the bacterial bioreactor allows for VFAs produced in the bacterial processes to become part of the inflow substrate for the algal bioreactor, enhancing its performance.

Figure 7-3 displays the algal bioreactor train, with descriptions of units and related overall mass balance equations presented in Table 7-17 and the streams enumerated in Table 7-18. The symbols used for algal bioreactor yields (Table 7-19) and separator and splitter factors (Table 7-20) are then given. Detailed equations for mass balances are presented in Appendix Section F.2.

The algal bioreactor train begins with a mixing tank (2.0) which receives the inflow streams. The inflow comprises primarily the improved compliance effluent (D1) from the bacterial bioreactor separator (1.2) which also contains VFAs produced in the bacterial process. Secondary inflow includes a possible stream of settled wastewater (D2) direct from the primary handling splitter (0.2) which bypasses the bacterial reactor; this option would be used only in the case where the main inflow from the bacterial bioreactor is too carbon-poor to adequately serve the algal bioreactor. Additional minor inflow streams (D3-5) allow for supplementary nutrients. The mixed stream (D) exiting the mixing tank forms the inflow to the algal bioreactor (2.1). Most algal reactions include photosynthesis, with a net absorption of carbon dioxide from atmosphere (E5) to supplement carbon available in the inflow stream and most algal bioreactor designs will have a net inflow or outflow of water (E6) through precipitation and evaporation.

The algal broth (E1) consists of the two algal products, biomass and changed composition liquid. It flows out into the first separation unit (2.2) following the algal bioreactor where the now almost compliant effluent (F1) is extracted, becoming the inflow for the macrophyte reactor train (Section 7.5.2). The bottoms from this separator is the biomass and product stream (E2) which is subjected to a more complex separation, possibly including cell breakage or other extraction methods. The algal products stream (E3) exiting this separator (2.3) undergoes a further (biphasic) separation (2.4), resulting in the algal bioproduct stream (W1), which is probably low-volume high-value, and the algal oil product stream (W2), both leaving the biorefinery system. Finally, the biomass stream (E4) may be split into a stream leaving the system (W3) as a biomass product and/or an algal bottoms stream (U2) which is sent to the solids bioreactor train (Section 7.5.3).

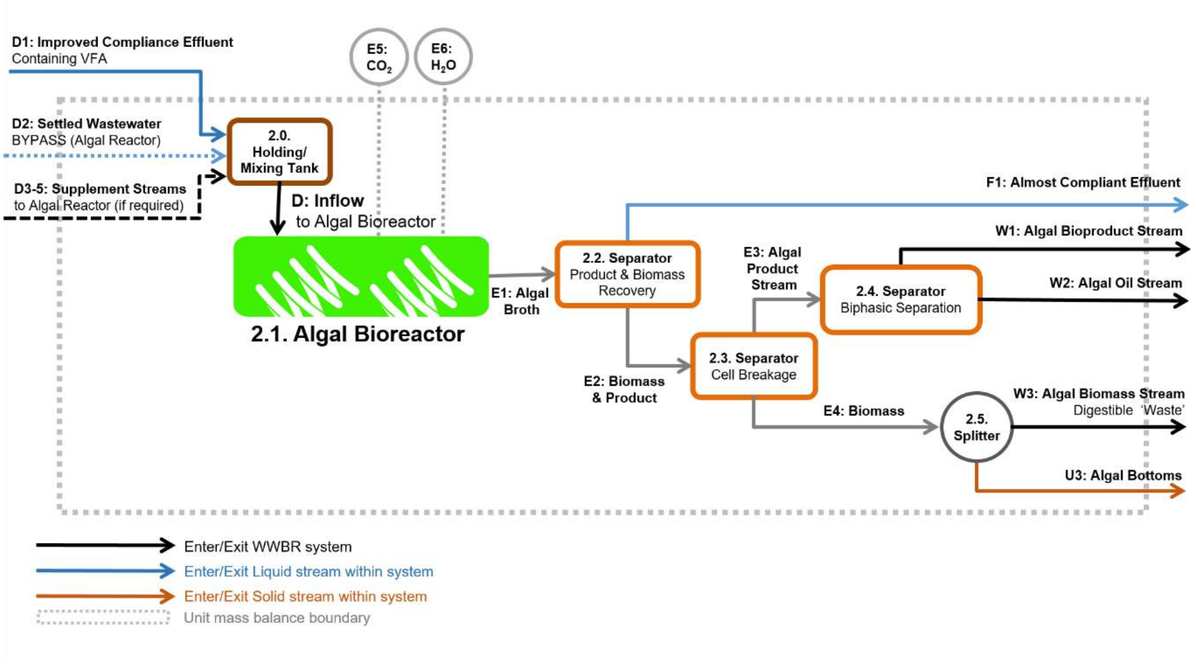


Figure 7-3: Algal bioreactor train detailed flowsheet

Table 7-17: Overall mass balance for algal bioreactor train

Unit number	Type	Unit description	Overall mass balance (In) – (Out) = 0
2.0	Holding tank	Mixing supplementary substrate streams and providing buffer capacity to average flows and compositions	(D1 + D2 + D3 + D4 + D5) – (D) = 0
2.1	Algal Bioreactor	Algal bioreactor	(D + E5 + E6) – (E1) = 0
2.2	Product & Biomass Recovery	Separates product + algal biomass from improved effluent (to macrophyte bioreactor)	(E1) – (E2 + F1) = 0
2.3	Separator	Downstream processing: cell breakage	(E2) – (E3 + E4) = 0
2.4	Separator	Downstream processing: separates lipids and water-based products	(E3) – (W1 + W2) = 0
2.5	Splitter	Algal biomass to product stream (digestible algal biomass) and solids bioreactor	(E4) – (W3 + U3) = 0

Table 7-18: Streams in algal bioreactor train

Stream number	Stream description	Relation to process units	Relation to other streams Equations refer to mass balance (kg/day)
D1	Improved Compliance Effluent	From Unit 1.2: Separator Into Unit 2.0: Holding tank for Algal Bioreactor	D1 = C1 - C2 Composition same as dissolved composition C1
D2	Settled Raw Wastewater	From Unit 0.2: Splitter Into Unit 2.0: Holding tank for Algal Bioreactor	D2 = A - B1 Composition same as A, B1.
D3	Supplementary Feed	Into Unit 2.0: Holding tank for Algal Bioreactor	Incoming stream, volume and composition set by user. (Optional stream)
D4	Supplementary Feed	Into Unit 2.0: Holding tank for Algal Bioreactor	Incoming stream, volume and composition set by user. (Optional stream)
D5	Supplementary Feed	Into Unit 2.0: Holding tank for Algal Bioreactor	Incoming stream, volume and composition set by user. (Optional stream)
D	Mixed Inflow Stream	From Unit 2.0: Holding tank for Algal Bioreactor Into Unit 2.1: Algal Bioreactor	D = D1 + D2 + D3 + D4 + D5
E1	Algal Broth	From Unit 2.1: Algal Bioreactor Into Unit 2.2: Separator	E1 = D + E5 + E6 Composition changed from D
E2	Biomass & Product	From Unit 2.2: Product & Biomass recovery Into Unit 2.3: Downstream Processing	E2 = E1 – F1 Composition similar to solids component of E1
E3	Algal Product Stream	From Unit 2.3: Product & Biomass recovery Into Unit 2.4: Downstream Processing	E3 = E2 – E4 Composition changed from E2
E4	Biomass	From Unit 2.3: Product & Biomass recovery Into Unit 2.5: Splitter	E4 = E2 – E3 Composition changed from E2
E5	CO_2	From atmosphere Into Unit 2.1: Algal Bioreactor	CO_2 only
E6	H_2O	Between Unit 2.1: Algal Bioreactor and atmosphere	H_2O only
F1	Almost Compliant Effluent	From Unit 2.2: Separator Into Unit 3.0: Holding tank for Macrophyte Bioreactor	F1 = E1 - E2 Composition same as dissolved composition E1
U3	Algal Biomass Not To Product Streams	From Unit 2.5: Splitter Into Unit 4.0: Holding tank for Solids Bioreactor	Total algal biomass = U3 + W3 U3 = E1 – (F1 + W1 + W2 + W3) Composition same as W3
W1	Algal Bioproduct Stream	From Unit 2.4: Separator Exit system	W1 = D * Algal bioproduct yield coefficient * Separation efficiencies Composition as specified by user
W2	Algal Oil Stream	From Unit 2.4: Separator Exit system	W2 = D * Algal oil yield coefficient * * Separation efficiencies Composition as specified by user
W3	Algal Biomass (digestible 'waste')	From Unit 2.5: Splitter Exit system	W3 = D – (F1 + W1 + W2 + U3) Note U3 can be 0 Composition same as U3

Table 7-19: Algal bioreactor yields

Conversion description	Unit	Symbol of factor
Mass of carbon reporting to algal biomass as a fraction of that present in influent stream to reactor (D)	kgC(Algal Biomass)/kg C(Inflow Algal Bioreactor)	$Y_{C,XAlgal/IN}$
Mass of carbon reporting to algal product W1 as a fraction of that present in influent stream to reactor (D)	kgC(Product W1)/kg C(Inflow Algal Bioreactor)	$Y_{C,W1/IN}$
Mass of carbon reporting to algal product W2 as a fraction of that present in influent stream to reactor (D)	kgC(Product W2)/kg C(Inflow Algal Bioreactor)	$Y_{C,W2/IN} + Y_{CO2Algal/IN}$
Mass of carbon entering or leaving as CO_2 as a fraction of that present in influent stream to reactor (D)	kgC(CO_2 Algal Uptake)/kg C(Inflow Algal Bioreactor)	$Y_{C,CO2Algal/IN}$
Mass of carbon remaining unconverted as a fraction of that present in influent stream to reactor (D)	kgC (Unconverted)/kgC(Inflow Algal Bioreactor)	$Y_{C,INAlgal,unconverted/IN} = 1 - (Y_{C,XAlgal/IN} + Y_{C,W1/IN} + Y_{C,W2/IN})$

Table 7-20: Factors for separator and splitter units in algal bioreactor train

Unit number	Separator description	Relevant parameters	Efficiency symbol
2.2	Product & Biomass Recovery	Slurry solids content Solid to Bottoms E2	SC_{E2} eff_{E2}
2.3	Algal Product Recovery	Algal Bioproduct recovery efficiency Solids (Biomass) to Bottoms E4 Solids to Bottoms E4	eff_{E3} eff_{E4} SC_{E4}
2.4	Algal Product Separation	Algal High-Value Bioproduct recovery efficiency Algal Oil recovery efficiency Solids content in oil recovery Solids content in algal bioproduct	eff_{W1} eff_{W2} SC_{W2} SC_{W1}
Unit number	**Splitter description**	**Streams split**	**Ratio symbol**
2.5	Algal Biomass	Fraction to Algal Product W3 Stream Fraction to Solids Bioreactor U3	r_{W3} $1 - r_{W3}$

7.5.2 Flowsheet and mass balance for the macrophyte bioreactor

The generalised WWBR flowsheet places the macrophyte bioreactor immediately before release of the (now compliant) water stream into the environment, or to reuse. The macrophyte bioreactor functions as a long residence time, slow acting reactor with multiple simultaneous mechanisms removing the last of the nutrients from the wastewater.

The macrophyte bioreactor train is diagrammed in Figure 7-4 and the units with the corresponding overall mass balance equations (Table 7-21) and stream descriptions (Table 7-22) follow. Macrophyte bioreactor yield symbols are presented in Table 7-23, with the symbols for separator and splitter factors given in Table 7-24. The detailed mass balance equations for the macrophyte bioreactor train can be found in Appendix Section F.3.

The macrophyte bioreactor train may begin with a mixing tank (3.0) should supplementary nutrient streams (F2-4) be deemed necessary. The main influent component is the almost compliant effluent stream (F1) from the algal bioreactor train (Section 7.5.1); once combined with possible supplementary nutrients this forms the inflow (F) to the macrophyte bioreactor (3.1). Macrophytes always have a net inflow of carbon dioxide from atmosphere (G4) through photosynthesis which is considerably greater than respiration, and macrophyte bioreactors are usually exposed to the elements. Depending on the local climate, they have a net inflow or outflow of water (G7) from precipitation and evaporation.

The wet biomass (G1) harvested from the macrophyte bioreactor, usually on a batch basis, goes through a number of separation processes. The first separator (3.2) removes the compliant effluent (Z), the key product of the biorefinery which is either discharged into the environment or reused. The solids element from this separation is sent to a following separator (3.3) producing a fibre and biomass stream (G3) and a nitrogen and phosphorus rich sediment stream (G4). The fibre and biomass is separated in a further separation unit (3.4), with a fibre product stream (X1) and a cellulosic biomass stream emerging. The cellulosic biomass may be split into a product stream (X2) which exits the system and/or a cellulosic biomass bottoms stream (U4) which is sent to the solids bioreactor train (Section 7.5.3). Likewise, the sediment may be split into a product stream (X3) and/or a sediment bottoms stream (U5) combining with the solids bioreactor train (Section 7.5.3) inflow.

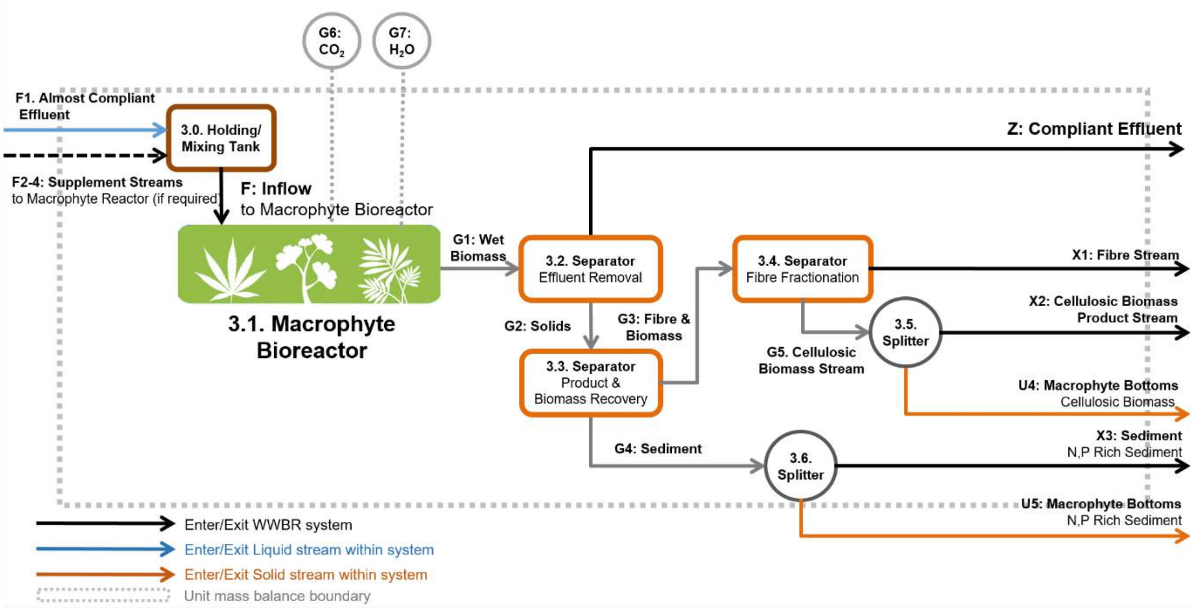


Figure 7-4: Macrophyte bioreactor train detailed flowsheet

Table 7-21: Overall mass balance for macrophyte bioreactor train

Unit number	Type	Unit description	Overall mass balance (In) – (Out) = 0
3.0	Holding / Mixing Tank	Mixing supplementary substrate streams and providing buffer capacity to average flows and compositions	(F1 + F2 + F3 + F4) – (F) = 0
3.1	Macrophyte Bioreactor	Macrophyte Bioreactor	(F + G6 + G7) – (G1) = 0
3.2	Solid/Liquid Separator	Separates Macrophyte Biomass from Compliant Effluent (leaving system)	(G1) – (G2 + Z) = 0
3.3	Solid/Solid Separator	Separates Biomass from Sediment. This may involve separate steps, e.g. manual harvesting (seasonal), and sediment de-sludging (annual)	(G2) – (G3 + G4) = 0
3.4	Size Fractioning Separator	Macrophyte Biomass harvested and fractionated into high quality Fibre and lower quality Cellulosic Biomass	(G3) – (G5 + X1) = 0
3.5	Splitter	Lower quality Cellulosic Biomass to Solids Bioreactor and to product stream (further processing)	(G5) – (X2 + U4) = 0
3.6	Splitter	N & P Rich Sediment to product stream and to Solids Bioreactor	(G4) – (X3 + U5) = 0

Table 7-22: Streams in macrophyte bioreactor train

Stream number	Stream description	Relation to process units	Relation to other streams Equations refer to mass balance (kg/day)
F1	Almost Compliant Effluent	From Unit 2.2: Separator Into Unit 3.0: Holding tank for Macrophyte Bioreactor	F = E1 - E2 Composition same as dissolved composition E1
F2	Supplementary Feed	Into Unit 3.0: Holding tank for Macrophyte Bioreactor	Incoming stream, volume and composition set by user. (Optional stream)
F3	Supplementary Feed	Into Unit 3.0: Holding tank for Macrophyte Bioreactor	Incoming stream, volume and composition set by user. (Optional stream)
F4	Supplementary Feed	Into Unit 3.0: Holding tank for Macrophyte Bioreactor	Incoming stream, volume and composition set by user. (Optional stream)
G1	Wet Macrophyte Biomass	From Unit 3.1: Macrophyte Bioreactor Into Unit 3.2: Separator	G1 = (F + G6 + G7) * Macrophyte yield coefficient * Separation efficiencies Composition changed from F1, a combination of liquid, fibre and sediment.
G2	Solids	From Unit 3.2: Separator (Effluent Removal) Into Unit 3.3: Separator (Product & Biomass Recovery)	G2 = G1 – Z Macrophyte biomass as well as any sediment
G3	Fibre & Biomass	From Unit 3.3: Separator (Product & Biomass Recovery) Into Unit 3.4: Separator	G3 = G2 – G4
G4	Sediment	From Unit 3.3: Separator (Product & Biomass Recovery) Into Unit 3.6: Splitter	Slow accumulating sediment consisting of algal(dead) biomass, rich in N and P.
G5	Cellulosic Biomass Stream	From Unit 3.4: Separator Into Unit 3.5: Splitter	Similar composition to G3 Volume G5 = G3 – X1
G6	CO_2	From Atmosphere Into Unit 3.1: Macrophyte Bioreactor	CO_2 only
G7	H_2O	Between atmosphere and Unit 3.1: Macrophyte Bioreactor	H_2O only
U4	Macrophyte Bottoms (Cellullosic Biomass)	From Unit 3.5: Splitter Into Unit 4.0: Holding tank for Solids Bioreactor	U4 = G5 – X2 Composition same as G5, X2
U5	Macrophyte Bottoms (N,P Rich Sediment)	From Unit 3.6: Splitter Into Unit 4.0: Holding tank for Solids Bioreactor	U5 = G4 – X3 Composition same as G4, X3
X1	Fibre Stream	From Unit 3.4: Separator Exit system	G*(1- moisture content fraction) * Fibre compositional fraction * Separation efficiencies
X2	Cellulosic Biomass Product Stream	From Unit 3.5: Splitter Exit system	X2 = G5 – U4
X3	N,P Rich Sediment	From Unit 3.6: Splitter Exit system	X3 = G4 – U5
Z	Compliant Effluent	From Unit 3.2: Separator Exit system	Composition must comply with discharge standards (either for discharge into natural water body or for irrigation)

Table 7-23: Macrophyte bioreactor yields

Conversion description	Unit	Symbol of factor
Mass of carbon reporting to macrophyte biomass as a fraction of that present in influent stream to reactor (F)	kgC(Macrophyte Biomass)/kgC(Inflow Macrophyte Reactor)	$Y_{C,X,Macr/IN}$ = $CO_{2C,Macrophyte(G6)}$
Mass of carbon entering as CO_2 as a fraction of that present in influent stream to reactor (F)	kgC(CO_2 Macrophyte Uptake)/kgC(Inflow Macrophyte Bioreactor)	$(Y_{C,macrophyte} * C_{macrophyte} * N_{W(F)}) / 365$
Mass of carbon reporting to bacterial biomass, as a sediment component, as a fraction of that present in influent stream to reactor (F)	kgC(Bacterial Biomass)/kgC(Inflow Macrophyte Reactor)	$Y_{C,X,Bact/IN}$
Mass of carbon remaining unconverted as a fraction of that present in influent stream to reactor (F)	kgC (Unconverted)/kgC(Inflow Macrophyte Bioreactor)	$1 - Y_{C,X,Bact/IN}$

Table 7-24: Factors for separator and splitter units in macrophyte bioreactor train

Unit number	Separator description	Relevant parameters	Efficiency symbol
3.2	Effluent removal	Solids to Bottoms G2 Slurry solids contents	eff_{G2} SC_{G2}
3.3	Product & Biomass recovery	Biomass to biomass stream efficiency Sediment to sediment stream efficiency	eff_{G3} eff_{G4}
3.4	Fibre fractionation	Macrophyte fibre recovery Cellulosic Biomass Stream	eff_{X1} eff_{G5}
Unit number	**Splitter description**	**Streams split**	**Ratio symbol**
3.5	Macrophyte Bottoms Cellulosic Biomass	Fraction to Cellulosic Product X2 stream Fraction to Solids Bioreactor U4	r_{X2} $1 - r_{X2}$
3.6	Macrophyte Bottoms N&P Rich Sediment	Fraction to Sediment Product X3 stream Fraction to Solids Bioreactor U5	r_{X3} $1 - r_{X3}$

7.5.3 Flowsheet and mass balance for the solids bioreactor

The solids bioreactor train is placed in the generalised WWBR to valorise and remediate the solids slurries from various parts of the WWBR. The detailed flowsheet for the solids bioreactor train is given in Figure 7-5, with a list of units and overall mass balance equations (Table 7-25) and a list of stream descriptions (Table 7-26) following. For the detailed mass balance equations see Appendix Section F.4.

The bottoms stream from the primary separation (0.1) of the combined influent wastewater streams (A1-4, Section 7.4) entering the WWBR, as well as the bottoms streams from each of the reactor trains are indicated. Thus the solids bioreactor train begins with a mixing tank (4.0) in which the primary separation bottoms (U1, Section 7.4.1), bacterial biomass (U2, Section 7.4.4), algal biomass (U3, Section 7.5.1), macrophyte biomass (U4, Section 7.5.2) and macrophyte bioreactor sediment (U5, Section 7.5.2) are combined with supplementary nutrient streams (U4-6) which may be added if necessary, giving the inflow (U) to the solids bioreactor. As with other bioreactors, the solids bioreactor is expected to be a heterotrophic process, potentially fungal, hence has an outflow of carbon dioxide (H4) to atmosphere from respiration. Similarly, depending on the configuration of the bioreactor, it may have in- or outflow of water (H5) from precipitation and evaporation.

The solids bioreactor (4.1) produces a solids matrix (H1) which is most likely harvested periodically. This matrix goes to the first separator (4.1) in the solids train which recovers the crust/surface related product (Y1) and sends the subsurface matrix (H2) to the second separator (4.3). Here a liquor related product stream (Y2) is retrieved, with the pressed cake (H3) going to the final separator (4.4) yielding cake related product (Y3) and compost (Y4). All these product streams exit the WWBR.

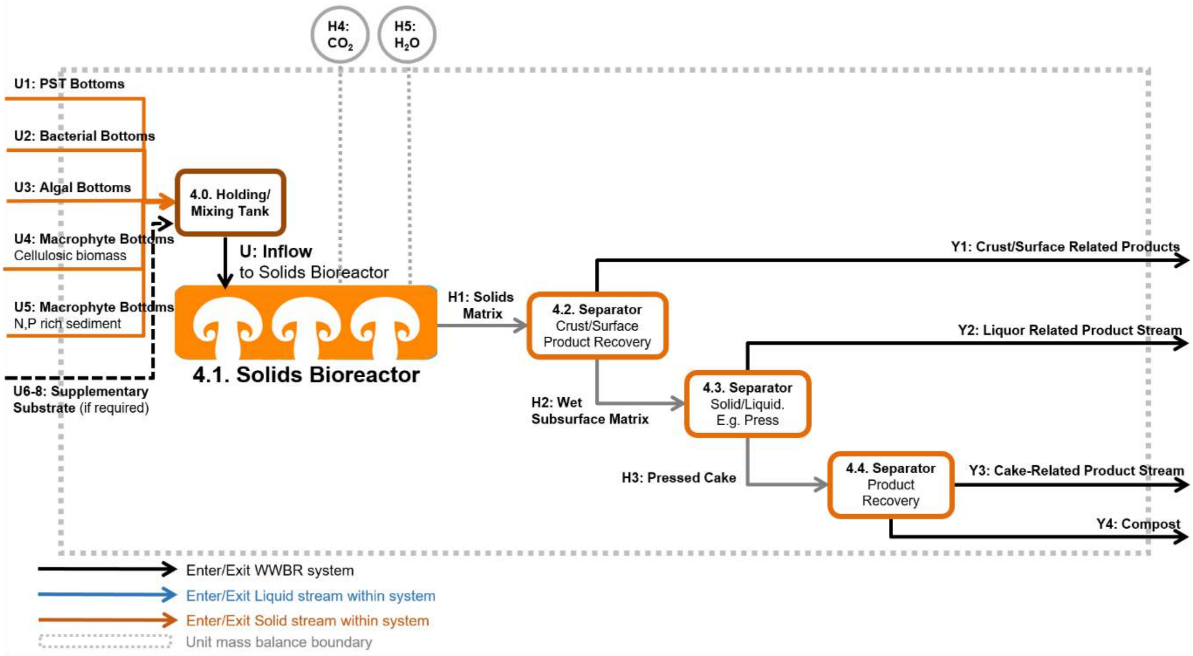


Figure 7-5: Solids bioreactor train detailed flowsheet

Table 7-25: Overall mass balance for solids bioreactor train

Unit number	Type	Unit description	Overall Mass Balance (In) – (Out) = 0
4.0	Holding Tank for Solids Bioreactor	Mixing Supplementary Feed and providing buffer capacity to average flows and compositions	(U1 + U2 + U3 + U4 + U5 + U6 + U7 + U8) - (U) = 0
4.1	Solids Bioreactor	Solids Bioreactor	(U + H4 + H5) – (H1) = 0
4.2	Separator	Separates crust-associated (surface) products from rest of growth matrix	(H1) – (H2 + Y1) = 0
4.3	Separator	Solid/Liquid separation, e.g. Press to separate liquid medium from support matrix	(H2) – (H3 + Y2) = 0
4.4	Separator	Cake-related product recovery from residual compost	((H3) – (Y3 + Y4) = 0

Table 7-26: Streams in solids bioreactor train

Stream number	Stream description	Relation to process units	Relation to other streams Equations refer to mass balance (kg/day)
H1	Solids Matrix	From Unit 4.1 Solids Bioreactor Into Unit 4.2: Separator	H1 = U +H4 + H5 Composition complex.
H2	Wet Subsurface Matrix	From Unit 4.2: Separator Into Unit 4.3: Separator	Composition different from H1,H3
H3	Pressed Cake	From Unit 4.3: Separator Into Unit 4.4: Separator	H3 = H2 – Y2 Low volume, less wet. Composition: Similar to solids fraction of H2
H4	CO_2	From Unit 4.1: Solids Bioreactor To Atmosphere	CO_2 only
H5	H_2O	Between atmosphere and Unit 4.1: Solids Bioreactor	H_2O only
U1	Biosolids (Main Fraction)	From Unit 0.1: Separator Into Unit 4.0: Holding Tank for Solids Bioreactor	Volume and composition set by user. Dependent on PST efficiency set by user.
U2	Bacterial biomass	From Unit 1.4: Splitter Into Unit 4.0: Holding Tank for Solids Bioreactor	U2 = C1 - (D + V1 + C4) Composition based on bacterial biomass as set by user
U3	Algal biomass not to product streams	From Unit 2.5: Splitter Into Unit 4.0: Holding Tank for Solids Bioreactor	Total algal biomass = U3 + W3 U3 = E1 – (F1 + W1 + W2 + W3) Composition same as L
U4	Macrophyte Bottoms: Cellulosic biomass	From Unit 3.5: Splitter Into Unit 4.0: Holding tank for Solids Bioreactor	U4 = G5 – X2 Composition same as X2
U5	Macrophyte Bottoms: N,P rich sediment	From Unit 3.6: Splitter Into Unit 4.0: Holding tank for Solids Bioreactor	U5 = G4 – X3 Composition same as X3
U6	Supplementary Feed	Into Unit 4.0: Holding tank for Solids Bioreactor	Incoming stream, volume and composition set by user. (Optional stream)
U7	Supplementary Feed	Into Unit 4.0: Holding tank for Solids Bioreactor	Incoming stream, volume and composition set by user. (Optional stream)
U8	Supplementary Feed	Into Unit 4.0: Holding tank for Solids Bioreactor	Incoming stream, volume and composition set by user. (Optional stream)
Y1	Crust-Surface Related Product Stream	From Unit 4.2: Separator Exit system	H1 * Crust related product yield * Separation efficiencies
Y2	Liquor -Related Product Stream	From Unit 4.3: Separator Exit system	Y2 = H1 – H2 Y2 = H1 * (e.g.) Organic acid yield coefficient * Separation efficiencies Composition: Similar to dissolved fraction of H2
Y3	Cake-Related Product Stream	From Unit 4.4: Separator Exit stream	Y3 = H1 * Cake-related Product Yield * Separation efficiencies
Y4	Compost	From Unit 4.4: Separator Exit stream	Y4 = H3 – Y3

Table 7-27: Solids bioreactor yields

Conversion description	Unit	Symbol of factor
Mass of carbon reporting to biomass as a fraction of that present in influent stream to reactor (U)	kgC(Biomass)/kgC(Inflow Solids Bioreactor)	$Y_{C,XSolids/IN} = Y_{C,Y1/IN} + Y_{C,Y3/IN}$
Mass of carbon reporting to product Y1 (organic content in surface-crust) as a fraction of that present in influent stream to reactor (U)	kgC(Product Y1)/kgC(Inflow Solids Bioreactor)	$Y_{C,Y1/IN}$
Mass of carbon reporting to product Y2 (liquor-related product stream) as a fraction of that present in influent stream to reactor (U)	kgC(Product Y2)/kgC(Inflow Solids Bioreactor)	$Y_{C,Y2/IN}$
Mass of carbon reporting to product Y3 (cake-related product stream) as a fraction of that present in influent stream to reactor (U)	kgC(Product Y3)/kgC(Inflow Solids Bioreactor)	$Y_{C,Y3/IN}$
Mass of carbon leaving as CO_2 as a fraction of that present in influent stream to reactor (U)	kgC(CO_2 Respiration)/kgC(Inflow Solids Bioreactor)	$Y_{C,CO2,Solids/IN}$
Mass of carbon reporting to product Y4 (compost) as a fraction of that present in influent stream to reactor (U)	kgC(Compost)/kgC(Inflow Solids Bioreactor)	$Y_{C,Y4/IN} = 1 - (Y_{C,Y1/IN} + Y_{C,Y2/IN} + Y_{C,Y3/IN} + Y_{C,CO2Solids/IN})$

Table 7-28: Factors for separator and splitter units in solids bioreactor train

Unit number	Separator description	Relevant parameters	Efficiency symbol
4.2	Crust/Surface Product recovery	Solids to Bottoms H2 Slurry solids contents	eff_{H2} SC_{H2}
4.3	Solid/Liquid separator	Product Y2 Pressed cake solids contents	eff_{Y2} SC_{H3}
4.4	Product recovery	Product Y3 Solids contents: Product Y4	eff_{Y3} SC_{Y4}

7.6 Using the Generic WWBR Flowsheet and Mass Balances

The flowsheets and mass balances presented in this chapter are a springboard for exploring the relevance of the WWBR concept. The generalised WWBR flowsheet allows, in its concise form (Figure 7-1), the development of an appreciation for the WWBR concept and opens the space for exploring its application into varied situations within the South African context. The detailed generic flowsheet, presented in four sections (Figure 7-2, Figure 7-3, Figure 7-4 & Figure 7-5), then enables the in-depth consideration of specific options in particular conditions. The factors enumerated in the accompanying tables for each flowsheet reveal the various types of information required. These are sought, first from the literature and subsequently through empirical demonstration, for locations in which a WWBR installation is intended. Further, the detailed mass balance equations enable first order estimations of the efficacy of envisaged scenarios. This is followed through by means of a simulation tool developed as part of a PhD project and presented in Chapter 8. The insights provided by the generalised flowsheets and mass balances perform an important function in assessing the establishment of the WWBR as a new and desirable option and in positioning the concept for future application. This feeds into Chapter 9, South African Wastewater Biorefineries: Conceptual Approach Emerging from this Study.

8 SIMULATION FOR PRELIMINARY EXPLORATION OF POTENTIAL WASTEWATER BIOREFINERY DESIGN

To pursue the potential for WWBRs in the South Africa industrial and municipal wastewater context, a tool is needed to allow a first order evaluation of specific opportunities in order to stimulate future-thinking and assess potential benefit. Thus a mass balancing tool centred around a generic WWBR flowsheet was developed as part of this project and the PhD of Bernelle Verster. It is intended to serve both as an early stage feasibility assessment and as a communication and facilitation tool between potential industry partners, and not a (proprietary) modelling tool.

The flowsheets and mass balances for this approach are presented in Chapter 7 and Appendix F, listing the required factors. In Section 8.1 a range of values is determined from literature for each factor. These values are estimates and can be changed in different model runs depending on the scenario being investigated.

Using data from a wide literature search, this simulation model, is applied with mid-range selected values to provide a set of preliminary mass balances for a particular group of feedstocks, providing estimated final outflow values. In Section 8.2 the model is demonstrated across a single bioreactor with values from an experimental study reported in the literature. Section 8.3 reports an integrated WWBR mass balance using municipal wastewater as feedstock.

The model also gives a visualisation of the flows into the WWBR, between the units and out of the WWBR. This numerical and visual presentation of the simulation allows an early stage evaluation of potential opportunities to resource recovery using the limited information available.

The utility of the model lies in the facility to take the initial simulation and rework the estimations through a number of differing scenarios to explore the consequences of changing the various factors and configurations used. The results of several changes of scenario are reported in Section 8.4 and are used to establish the value of this tool as an initial consideration of application of the WWBR concept to any local setting.

8.1 Selection of Factors for Unit Mass Balances

Considering the generic flowsheets and mass balances presented in Sections 7.1.1 and 7.1.2, it is apparent that any preliminary overview of the options for a WWBR in a particular location requires a set of inflows, bioreactor yields, separation efficiencies and splitter ratios which are reasonable for the conceptualised scenario. The potential inflows and combinations of inflow are determined by the anticipated setting, but estimated ranges for the other factors can be determined from literature for the initial analysis. Although these values are clearly dependent on specific requirements, bioreactor configurations and local conditions, an order of magnitude estimation is useful for preliminary analysis of alternatives.

Where a range of literature values are available, or a single value that is highly optimised for the specific system, a conservative value is applied to take into account the likely lower yields available from wastewater, as well lower yield values that may be more appropriate for an integrated system as opposed to a maximised value for a single unit system. Where possible, concerns regarding the highest attainable yield values are discussed.

8.1.1 Bacterial bioreactor factors for mass balances

Bacterial growth rates and specific product formation rates vary widely, depending on physiological conditions, dominant metabolism and bacterial grouping (Harding, 2009). For example, the energy efficiency of aerobic and anaerobic growth results in differing growth yields and rates. Similarly the metabolic load of photosynthesis affects yields and rates. Further, metabolite production can be growth associated or produced during the stationary phase (Doran, 1995). All these factors affect the stoichiometry and rate of production, however a theoretical mass balance is possible.

The critical factors affecting the bacterial bioreactor were highlighted in Chapter 7 through the establishment of the material balances around this reactor system. The need for biomass retention in the WWBR bioreactor was presented in Chapter 6 and is recognised to affect the model presented here. Recycling or retaining biomass enables higher apparent biomass concentration, and thereby rates of metabolism for removal of contaminants and production of products. In some cases, biomass retention or recycling may not be feasible, depending in particular on the location of product. For example, where an intracellular bioproduct is produced, the cells need to be broken to recover the product which renders them unviable.

Bacterial biomass factors

Typical biomass yields for aerobic bacterial processes lie in the range 0.38 to 0.5 g-biomass per g-organic-carbon-source where this carbon source is a carbohydrate (Bailey & Ollis, 1986). Higher yields are expected from less oxidised materials such as long chain fatty acids and oils, owing to their lower oxygen content. Harding (2009) provides biomass yields across a range of families of carbon source. The bacterial biomass yield produced during PHA production from confectionary wastewater was reported as 0.34 g-biomass/g-substrate-COD (Fernández-Dacosta, et al., 2015; Tamis, et al., 2014), which translates to 0.427 g-biomass-C/g-substrate-C, where g-substrate-C is equal to TOC (see Section 8.2), while the bacterial biomass concentrations obtained during PGA production, reported in Appendix section G.1.1, is 4.98 g/ℓ at 37 °C and 4.40 g/ℓ at 30 °C, which translates to 0.185 and 0.164 g-biomass-C/g-substrate-C, respectively. The conservative value of 0.164 g-biomass-C/g-substrate-C is used in Section 8.3. These values compare reasonably well with the review of PGA production of Madonsela (2013), reporting a biomass concentration in the range of 2 - 5 g/ℓ. The articles used in the review mainly reported biomass concentrations and not yield, thus it is not possible to calculate exact yield values.

The bacterial biomass composition used in this model is for aerobic growth, $CH_{1.8}O_{0.5}N_{0.2}P_{0.01}$ (Roels, 1983) and the calculations to obtain the mass percent contributions are illustrated in Table 8-1.

Table 8-1: Conversion of composition to mass percent for bacterial biomass

Element	**Composition: Normalised to C** (Wu, 2015) (mol element per mol C in molecule)	**Molar mass of element** (g/mol element)	**Mass** (g element/mol molecule)	**Biomass Composition** (wt fraction: g / g total dry biomass) **values used in model**
C	1	12	12	0.48 (TOC bacterial biomass)
N	0.2	14	2.8	0.11
P	0.01	31	0.31	0.012
H	1.8	1	1.8	0.072
O	0.5	16	8	0.32
Total	N/A	N/A	24.6	1.00

Bacterial bioproducts factors

The production of bacterial bioproducts is usually reported in terms of volumetric concentrations in the form g-product/ℓ-broth. These may be converted to a yield, given in terms of g-product/g-substrate, and more specifically, a carbon-based yield given as g-product-C/g-substrate-C fraction for use in the mass balance. Bacterial bioproducts can be intracellular (reside inside the cell) or extracellular (exported to outside of the cell). The location of the product affects the potential of the biomass to be recycled as well as the downstream processing required. These may have implications on the optimum yields possible, especially in the integrated WWBR.

The reported datasets on bioproduction are mostly generated in shake flask and laboratory experiments, and are thus are not entirely suitable for calculations modelling commercial scale bioreactors. However, the first experimental values for any specific situation will be from this level of experiment, and the resultant values for yields are assumed to be acceptable for a first level approximation.

Intracellular bacterial bioproduct V1

As an example of the literature data required for the modelling of the bacterial bioreactor, production of PHA from confectionary wastewater, containing (7.8 soluble + 0.8 solid) g-COD/ℓ is described. Tamis et al. (2014) performed the experiment, while a techno-economic study was performed using the data. Polyhydroxyalkanoates (PHA) are a group of bio-based biodegradable polymers with wide application, as discussed in Section 5.4.1. PHA production from wastewater is well investigated, especially through using an aerobic granular sludge reactor (De Bruin, et al., 2004). One of the most studied of the PHAs is polyhydroxybutyrate (PHB) with the molecular formula $C_4H_6O_2$ and a carbon fraction of 0.56. A case study producing PHB from confectionary wastewater is used as the intracellular bacterial bioproduct for a demonstration of the model simulation for a simple bacterial bioreactor train presented in Section 8.2. The carbon-based yields, given as g-product-C/g-substrate-C, are calculated as shown here.

The incoming COD was fermented to volatile fatty acids. No significant COD loss was observed in the anaerobic fermentation steps. While the VFA profile consisted of various acids, the greatest fraction was acetic acid (32%) (Tamis, et al., 2014), hence in this model it is assumed that all incoming COD is in the form of acetic acid, for simplicity of calculation. Thus

COD value of 1.07 g-COD/g-acetic acid

(7.8 soluble + 0.8 solid) g-COD/1.07 = 8.04 g-acetic acid/ℓ

C-fraction of acetic acid = 0.4

8.04 g-acetic acid/ℓ * 0.4 = 3.21 g-C/ℓ-incoming substrate (= TOC = TC)

The overall process yields were 0.34 g-biomass/g-COD, 0.11 g-PHA/g-COD, and 0.55 g-CO_2/g-COD. For the WWBR model used in this project, the TOC yield values of the PHB and biomass is required, thus these values need to be converted, first to g/ℓ components using the g-COD/g-component values, and then to g-C/ℓ using the carbon composition values.

Table 8-2 lists the outgoing concentration of the biomass, PHA and CO_2 in g-C/ℓ, based on the specified incoming feed stream, as well as the carbon-based yields which are independent of volumetric flowrate and concentration of the feed stream. These are used in the model. PHA for the purposes of this model is assumed to be composed of polyhydroxybutyrate (PHB) only.

Table 8-2: Carbon yields for PHA, biomass and CO_2 produced from acetic acid

Component	**COD-Yield** (g-component/ g-COD-substrate)	g-COD-component /ℓ	g-COD/g-component	g- component /ℓ	**Fraction C** (g-C/g-component)	**Total concentration C-in-**component (g-C-component /ℓ)	**Yield** (g-C-component / g-C-substrate)
Substrate-content							
Incoming substrate as acetic acid		7.8 + 0.8 = 8.6	1.07	8.04	0.4	3.21	
Product-content							
PHB produced	0.11			0.946	0.558	0.528	0.165
Bacterial Biomass	0.34			2.924	0.48	1.404	0.437
CO_2 produced	0.55			4.73	0.27	1.277	0.398
Sum		8.6		9.88		3.21	1
Rationale							
Calculation			funda-mental value	= g-COD/ℓ / g-COD/g-component (CO_2 back-calculated)	funda-mental value	=C-composition * g-component/ℓ (CO_2-C remainder)	g-C-component /g-C-substrate (CO_2-C remainder)
Balance				no need to balance (H,O,N,P not accounted for)		carbon	yield

Extracellular bacterial bioproduct V1

Polyglutamic acid (PGA) is another biopolymer with a variety of applications (Section 5.4.2) and is used here as an example of an extracellular product. A similar approach would be taken for other extracellular products. The production of PGA from wastewater is also the focus of a previous WRC report, (Verster, et al., 2014). Subsequent analysis of this extracted and purified γ-PGA showed a γ-PGA suitable for wastewater applications, but not for areas which require a specific composition of high molecular weight stereoisomers. The molecular formula of a PGA monomeric unit is $C_5H_7O_3N$, which translates to $CH_{1.4}O_{0.6}N_{0.2}$, and an elemental composition in terms of a mass % C: 0.465, N: 0.109, P: 0.000. It is used in Section 8.3 as the extracellular bioproduct in the demonstration of the model using an integrated system.

Reported concentrations for PGA production vary widely, from less than 1 g/ℓ-broth to more than 100 g/ℓ-broth (Madonsela, 2014). Typical substrate compositions reported to date follow a 'Medium E' recipe (Birrer et al. (1994)). A modified version of this medium was used in this report (Appendix Section D.3.2), as shown in Table 8-3, with a maximum PGA concentration of 3.4 g/ℓ obtained at 37 °C compared to 6 g/ℓ at 30° (Appendix Section D.4), which translates to 0.123 and 0.216 g-C-product/g-C-substrate as shown in Table 8.3

The conservative value of 0.123 g-C-product/g-C-substrate is used in Section 8.3.

Table 8-3: Carbon yields for PGA and biomass produced from Modified Medium E

Component	**g-component/ℓ**	**Molecular Formula**	**Fraction C** (g-C/g-component)	**Total C** (g/ℓ)	**Yield** (g-C-component/ g-C-substrate)
Substrate-content					
Glucose	20	$C_6H_{12}O_6$	0.4	8.0	
Glycerol	1	$C_3H_8O_3$	0.39	0.4	
Citric acid	12	$C_6H_8O_7$	0.375	4.5	
Total g/ℓ	**33**			**12.9**	
Product-content					
Biomass produced	5.0, 4.4	$CH_{1.8}O_{0.5}N_{0.2}P_{0.025}$	0.48	2.4, 2.1	0.185, 0.164
PGA produced	3.4, 6	$C_5H_7O_3N$	0.465	1.6, 2.8	0.123, 0.216

Bacterial interim product VFAs

Volatile fatty acids (VFAs) are generally a mixture of acetic acid (C fraction 0.4), propionic acid (C fraction 0.486) and butyric acid (C fraction 0.545), and are produced as an interim product in the production of PHAs, biogas and hydrogen. VFA production through fermentation is a common way to convert organic material to a more biologically available form for use in, for example, PHB production or algal bioreactors. Fernández-Dacosta et al. (2015) used a yield of 0.91 g-product-COD/g-substrate-COD (translating to 0.97 g-product-C/g-substrate-C). Wijekoon et al. (2011) reported VFA yields at different organic loading rates, translating to g-product-C/g-substrate-C in the range of 0.7 to 0.95.

There is no significant COD loss in the conversion of incoming (complex) COD to VFA. In conventional single unit bioreactor systems, as illustrated in Section 8.2, this VFA is then used to produce biomass and PHA, for example, and the value of exiting VFA is much lower. In this model, the VFA yield is determined by subtracting the product and biomass yield from the VFA yield.

Bacterial respiration factors

Bacterial respiration depends on the solid residence time. For activated sludge wastewater treatment, a higher endogenous respiration rate translates to less sludge production, but also to higher aeration costs. In the WWBR, endogenous respiration should be minimised to allow a greater product yield, but as the only available design value relevant to wastewater, the endogenous respiration rate used in Henze et al. (2008) of 0.24/day is selected for use in the runs of the model reported here.

Summary of yield factors used for Bacterial Bioreactor

The values which will be used in Section 8.3 for the demonstration of simulated mass balance for the integrated system, using the extracellular product PGA as hypothetical example are presented in Table 8-4.

Table 8-4: Carbon-based yield factors for bacterial bioreactor (Section 7.3.3)

Conversion description	Symbol of factor	Estimated range of factor values in literature (g C/g C substrate)	Selected factor value for start-point (g C/g C substrate)
Mass of carbon reporting to biomass as a fraction of that present in influent stream to reactor (B)	$Y_{C,XBact/IN}$	0.164 – 0.185	0.164
Mass of carbon reporting to extracellular product V1 as a fraction of that present in influent stream to reactor (B)	$Y_{C,V1/IN}$	0.123 – 0.216	0.123
Mass of carbon reporting to interim product VFA as a fraction of that present in influent stream to reactor (B)	$Y_{C,VFA/IN}$	0.7 to 0.95	0.7- $Y_{V1/IN}$ - $Y_{C,XBact/IN}$
Mass of carbon leaving as CO2 as a fraction of that present in influent stream to reactor (B)	$Y_{C,CO2Bact/IN}$	up to 0.24	0.24
Mass of carbon remaining unconverted as a fraction of that present in influent stream to reactor (B)	$Y_{C,INBact,unconverted/IN} = 1 - (Y_{C,XBact/IN} + Y_{C,V1/IN} + Y_{C,VFA/IN} + Y_{C,CO2Bact/IN})$	remainder	remainder

8.1.2 Algal bioreactor factors for mass balances

While this model does not specify the type of algal reactor used, it is noted that most literature on algal production from wastewater has focused on high rate algal ponds (HRAPs) or adaptations of it (Section 3.4.2). The algal reactor is likely to be the main reactor when waste streams are used that are high in N and P but low in COD. Algal reactors are not likely to be the main reactor where there are spatial constraints, due to the large land requirement. The reactor should be designed to create a selective environment to favour the desired algal growth (Mooij, et al., 2015).

While total COD removal is a factor of residence time and thus of the size of the algal reactor, the total COD removal is reported to be in the order of 31 – 53% in HRAPs combined with Advanced Settling Ponds (ASP) (Rose, et al., 2007).

Algal biomass factors

This model makes no assumptions about the specific species present in the bacterial or algal bioreactors. The reactor environment represents a dynamic ecosystem, and it is possible to design a selective environment to favour a specific product, rather than an algal species (Mooij, et al., 2015). Algal biomass is not recycled, as higher biomass or nutrient concentration in the almost compliant effluent is not needed, apart from which the algal cells are generally broken during product recovery and are thus no longer viable.

The biomass concentration in the algal bioreactor is generally not as high as found in conventional algal biorefinery conditions: Typical nitrogen concentrations of 15–20 mg/ℓ in domestic municipal wastewater effluent would stoichiometrically support a microalgae growth density of approximately 0.2 g/ℓ, far lower than the densities achievable in ideal, nutrient-replete conditions, which range from 2 to 10 g/ℓ (Peccia, et al., 2013). The model values for algal biomass ($C_{106}H_{181}O_{46}N_{16}P$) are based on Park et al (2011), with a C fraction of 0.520. In a review of heterotrophic algal cultivation (Bumbak, et al., 2011), biomass yields on different substrates, but most commonly acetate and glucose (with a C fraction of 0.407 and 0.4, respectively) ranged from 0.41 to 0.81 g-CDW/g-substrate. Converting into a g-C-biomass/g-C-substrate yields gives a range of 0.524 – 1.053. The biomass concentration ranged from 30 to 117 g/ℓ, which, using data in Bumbak et al. ((2011), Appendix G.1.2) translates to a

C-biomass/C-substrate yield of 0.3 – 1.1. The biomass content of high lipid producers tended to be lower, and using one example reported in the review, the biomass producing docosahexanoic acid from ethanol was 83 g/ℓ from 217 g/ℓ, translating to a g-biomass-C/g-substrate-C yield of 0.381.

Algal bioproducts factors

Algal high-value bioproducts W1

In a WWBR approach, the nitrogen should be directed to product, or biomass, rather than lost to the atmosphere through denitrification. Potential products include phycocyanin and antioxidants like astaxanthin. The yield values for these products are expected to be very low, but their production may still be justified through the high price obtainable, as well as the potential for co-production with commodity products like algal lipids.

For example, high value pigment yields from algae are reported in the range of 0.03 – 2.9 g/ℓ, with around 0.3 g/ℓ a conservative estimate produced in heterotrophic cultivation using 50 g/ℓ glucose (Bumbak, et al., 2011). Using phycocyanin (C fraction 0.68), this translates to a g-product-C/g-substrate-C yield of 0.010.

Algal lipids W2

Griffiths and Harrison (2009) compared algal lipid productivity in photo-autotrophic cultivations from literature. They found a wide range of reported values ranging from 13 to 31% dry weight for green algae (most being freshwater species), averaging 23%, and an average lipid content of 41% under nitrogen deprivation. Other taxa had a wider range, but with a similar average. Olguín (2012) reports similar values, and a range of 20-50% oil content for heterotrophic cultures, which is more appropriate to wastewater. Bumbak et al. (2011) compared fed-batch heterotrophic cultivations. The example for docosahexanoic acid was used with a concentration of 11.7 g/ℓ, in a fed-batch culture containing (accumulated) 217 g/ℓ ethanol, complete conversion is assumed. The g-product-C/g-substrate-C yield is then 0.083.

Algal photosynthesis and respiration factors

Addition of carbon dioxide has been shown to enhance algal productivity by about 30% as well as reducing nitrogen loss through ammonia volatilization (Park, et al., 2011). At a WWBR facility the CO_2 from the bacterial bioreactors could be reused at the algal bioreactor, with a low increase in cost. Algal hetero- or mixotrophic growth, meaning growth on dissolved carbon instead of or in addition to CO_2, respectively, gives 3 to 10 times greater biomass concentrations than autotrophic growth (Dhull, et al., 2014). According to Chojnacka and Marquez-Rocha (2004) biomass is produced from the organic carbon, while chemical energy, for example in the form of lipids, is converted from light energy. Autotrophic growth results in CO_2 uptake, heterotrophic growth results in CO_2 production, and mixotrophic growth showed no appreciable change in CO_2 in work by (Kim, et al., 2013). The model assumes a ratio of CO_2 uptake proportional to the incoming C-substrate, which can be altered by the user. The default value is 0.1, or 10% of the incoming g-substrate-C.

Summary of yield factors used for Algal Bioreactor

The values which will be used in Section 8.3 for the simulated mass balance for the algal bioreactor are presented in Table 8-5.

Table 8-5: Carbon-based yield factors for algal bioreactor (Sections 7.3.3; 3.4.3)

Conversion description	**Symbol of factor**	**Range of factor values in literature**	**Selected factor value for start-point**
Mass of carbon reporting to algal biomass as a fraction of that present in influent stream to reactor (D)	$Y_{C,XAlgal/IN}$	0.3 – 1.1	0.345
Mass of carbon reporting to product W1 as a fraction of that present in influent stream to reactor (D)	$Y_{C,W1/IN}$	0.01 – 0.098	0.01
Mass of carbon reporting to product W2 (algal lipids) as a fraction of that present in influent stream to reactor (D)	$Y_{C,W2/IN}$ + $Y_{C,CO2Algal/IN}$	0.042 – 0.210	0.083
Mass of carbon entering or leaving as CO2 as a fraction of that present in influent stream to reactor (D)	$Y_{C,CO2Algal/IN}$	negative to positive	-0.1
Mass of carbon remaining unconverted as a fraction of that present in influent stream to reactor (D)	$Y_{C,INAlgal,unconverted/IN} = 1 - (Y_{C,XAlgal/IN} + Y_{C,W1/IN} + Y_{C,W2/IN})$	remainder	remainder

8.1.3 Macrophyte bioreactor factors for mass balances

A macrophyte bioreactor is similar to a constructed wetland from an engineering design perspective, but different from an economic and operating perspective. Because a macrophyte reactor is also aimed at producing a valuable product (in addition to water) the installation and operating costs considered feasible are higher than for a treatment wetland, and the harvesting yields have qualitatively higher required efficiencies. Quantitative values are less well characterised for this reactor in the WWBR, with very little research to date on potential products and their recovery, working in parallel with the remediation function. Section 3.4.3 is a review of the factors affecting the macrophyte bioreactor. Here, more than with any of the other reactors, the need for environmental sensitivity in the choice of biological species runs closely with the selection of appropriate product. Using indigenous species is best from a biodiversity point of view, however the basic research on suitability, productivity, technical performance and market need for products from indigenous species is still needed.

Macrophyte biomass and bioproduct factors

There are high value bioproducts from macrophytes, such as colourants (Bechtold & Mussak, 2010), as well as potential agricultural-type products that do not use the entire plant, such as fruits and seeds. It may be more suitable to select products that use as high a proportion of biomass as possible, to allow a high ratio of removal of plant matter; thus fibre for (geo)textiles, composites and the construction industry is considered. While growing food on wetlands is possible (Kakuru, et al., 2013), wetlands used for wastewater treatment may be exposed to contaminants that are hazardous to (human) health and food products are not considered in this project.

For the demonstration of the model, flax (*Linum usitatissimum*) has been chosen as it is well known, and well characterised. The stem of the plant is used for textile production, and increasingly in building and structural applications, with about 25 – 30% yields of straw dry mass possible in the final product (Mussig, 2010). For fibre production, the final plant density is about 2000 plants per square meter, and they are harvested before seed production. The main shortcoming of flax production is various environmental issues associated with retting, a step in DSP (Mussig, 2010). Flax grown on treatment wetlands may be suitable, but the manner of harvesting may have to be adapted to avoid destabilisation of the rootzone. Floating wetlands do provide access to root harvesting, but this may reduce the filtering capacity and microbial activity associated with the root network, which is the principal mechanism of nutrient removal in floating wetlands. Evidence suggests that removal of shoots does not negatively affect the roots (Dodkins & Mendzil, 2014b).

Plant fibre compositions are generally reported only in terms of structural polymers - the polysaccharides, cellulose and hemicelluloses, and the aromatic polymer lignin – with little concern for the N and P content (Marques, et al., 2010). Flax contains 64% cellulose, 17% hemicellulose and 2% lignin (Bledzki & Gassan, 1999).

Flax contains between 0.56% and 0.91% N with the green ripe stage showing the highest N content (Ahmad, et al., 1982). The average value of 0.735% (0.00735) was chosen, and the model assumes that the N content is equal to the N uptake from the water (no N fixing from the atmosphere). For the P composition the average value for grasses of 0.23% P (0.0023) (Harper, et al., 1933), is used, while noting that grasses are higher in N than flax (2.53%).

For simplicity of calculation, the carbonaceous composition is assumed to be the remainder dry mass (1 – 0.00735 – 0.0023), and composed only of cellulose, with a C fraction of 0.444 to give a C composition of flax of 0.715.

In general, the largest nutrient removals can be achieved by perennial grasses and legumes that are cut frequently at early stages of growth, but the market value of grass-related products are less well established. Another aspect to consider is that annuals like flax only use part of the growing season for growth and active uptake (WEF FD-16, 2010), with two harvests possible. The model uses an average daily rate.

Macrophyte photosynthesis and respiration factors

Emergent (not completely submerged) macrophytes are considered photoautotrophic, meaning they obtain their CO_2 exclusively from the atmosphere. To estimate the contribution of CO_2-C to the C-balance, a few assumptions need to be made that cannot be determined at this early stage. These values, which need to be checked again by the user, are summarised in Table 8-6 and explained below.

Table 8-6: Estimation of CO_2 uptake of macrophyte bioreactor

Parameter description	Units	Range of factor values in literature / Calculation	Selected factor value for start-point
1. Estimated kg-C uptake, per harvest (kg-C/m^2.harvest):			
Macrophyte biomass per harvest, per m^2	kg macrophyte biomass / m^2 planted area	129.7 – 2883	0.92
C fraction of macrophyte	kg-C / kg macrophyte biomass	0.715	0.715
C-uptake	**Ymacrophyte = kg-C/m2 planted area.harvest**	**0.92 * 0.715**	**0.658**
2. Conversion of inflow fluid to planted area-dependent parameter (final unit: m^2 macrophyte area):			
Incoming liquid to macrophyte reactor (stream F_W in water mass balance)	kg Water (and mass balance is set over 1 day)	design specific, dependent on mass balance	F_W
conversion of kg to m^3 in model water mass balance (assuming density of 1 000kg/m^3)	m^3/kg Water	1/1000 (assumption)	1/1000
Estimated area of macrophyte bioreactor per m^3 incoming fluid, using a depth of 1.2m (See Section 8.1.7)	m^2 Water / m^3 Water	design specific	0.833
Planted area as fraction of total area	m^2 planted area / m^2 total area of macrophyte bioreactor	0.2 – 1.0	0.2
Planted area parameter	**F_W * m^2 planted area**	**F_W * 1/1000 * 0.833 * 0.2**	**F_W * 0.000 167**
3. Conversion of harvest values to daily value (final unit: /day):			
Harvests per year	/year	0 - 3	2
daily average	year/day	1/365 (assumption)	1/365
Harvest average per day	**harvest/day**	**2 * 1/365**	**0.0055**
4. Complete value (kg-C/day)			
$X_{C(G1)}$	**kg-C/m^2 planted area.harvest * F_W * m^2 macrophyte area * harvest/day = kg-C/day**	**0.658 * 0.000 167 * F_W * 0.0055**	**0.000 000 601 * F_W**

The carbon uptake, $Y_{macrophyte}$, is dependent on the planted biomass per m^2 of planted surface area in the macrophyte reactor, and the carbon composition of the macrophytes. In this model the planted surface area is calculated as a function of the water entering the macrophyte reactor, $N_{W(F)}$, in kg/day, and the residence time. The default value of $C_{macrophyte}$ is 0.715 g-C/g-macrophyte-biomass (Section: Macrophyte biomass and bioproduct factors, above).

The above-mat biomass ranges between 0.072 and 2.350 kg/m^2, and root biomass ranges from 0.043 to 0.533 kg/m^2, per growing season (or harvest), with a total mass ranging from 0.130 – 2.88 kg/m^2 (Dodkins & Mendzil, 2014b). For the model an average total biomass value of 0.920 kg/m^2 was used, calculated from the data in Dodkins and Mendzil (2014b), giving a $Y_{macrophyte}$ value of 0.658 kg-C/m^2.harvest.

Determining the surface area in the model requires an assumption of the depth and the hydraulic residence time of the pond. While this is highly system specific, a default value used for planted wetlands of 1.2 m deep and residence time of 1 day has been assumed, as used in the water balance (Section 8.1.7). These values can be changed by the user.

The recommended planted area of floating wetlands is 20% of the surface area of the pond, with higher planted area causing anoxic conditions in the pond. Ponds aimed at N removal through tightly controlled conditions (either high treatment rate aeration or nitrate removal anaerobic basin) should have 100%

cover (Dodkins & Mendzil, 2014b). Using a value of 20% cover, gives a heuristic value of F_W * 0.000 167 kg.m^2 planted area per day.

Lastly, the carbon uptake over the year needs to be averaged to a daily value to align with the day-basis of the mass balance. Two harvests per year are assumed, and the total kg plant mass obtained annually is then divided by 365 to give the daily contribution. Using these values the value of $X_{C(G1)}$ is 0.000 000 601 * F_W kg-C/day.

Sedimentation in the macrophyte bioreactor

Nutrient uptake through plants accounts for only about 6% of N and P removal. Nutrient removal, particularly P is mainly through settling into the sediment, and accounts for about 40 -60% of P (45 – 75g/m^2/year (Dodkins & Mendzil, 2014b)). Total N removal through floating wetlands includes denitrification processes as well and amounts to about 75%.

The model does not make explicit allowance for sedimentation phenomena, but assumes some sedimentation occurs through bacterial (microbial) growth from the remaining unconverted nutrients in the almost compliant effluent. This is an underestimation, as it does not account for non-biological means of P deposition, for example. It is an important factor to include in the model, however, because dredging (at a recommended rate of around once every 10 years) is ideal for sustained P removal (Dodkins & Mendzil, 2014b).

Summary of Carbon-based yield factors used for Macrophyte Bioreactor

The values which will be used in Section 8.3 for the simulated mass balance for the macrophyte bioreactor are presented in Table 8-7.

Table 8-7: Carbon-based yield factors for macrophyte bioreactor (Section 7.3.3)

Conversion description	Symbol of factor	Range of factor values in literature	Selected factor value for start-point
Mass of carbon reporting to macrophyte biomass as a fraction of that present in influent stream to reactor (F)	$Y_{C,macrophyte}$	range * 0.81	0.745
Separation efficiency between Fibres and cellulosic biomass product streams	eff_{X1}	0.8 – 1	0.8
Mass of carbon reporting to bacterial biomass committed to sediment, as a fraction of that present in influent stream to reactor (F)	$Y_{C,X,S,Bact/C}$	0.164 – 0.185	0.164
Mass of carbon entering as CO_2 as a fraction of that present in influent stream to reactor (F)	$CO_{2C,Macrophyte(G6)}$	See Table 8-6	0.000 000 68 * F_W
Mass of carbon remaining unconverted as a fraction of that present in influent stream to reactor (U)	1 – $Y_{C,X,S,Bact/C}$	remainder	1 – 0.164

8.1.4 Solids bioreactor factors for mass balances

The solids bioreactor aims to generate value from the bottoms components generated in the WWBR. Products produced using solid substrate type reactors are commonly reported on a g/kg-dry-substrate basis. While not the sole microbial component, solid substrate bioreactions can be dominated by fungi (Singhania, et al., 2009), and too high moisture levels lead to the unwanted dominance of bacteria. Data on valuable bioproduction using wastewater slurries is virtually non-existent (Susana forum on

SSF (Verster, 2016) on faecal sludge in particular), therefore much of this section is not yet corroborated in the context of the WWBR.

Solids Bioreactor biomass factors

Kalogeris et al. (2003) compared the impact of moisture and temperature changes on biomass production in solid substrate bioreactors using wheat straw as substrate. The biomass yields reported range between 28 and 52 g/kg-dry-substrate. The C fraction used for wheat straw is based on lignin, (using $C_9H_{10}O_2$, C fraction 0.72), and the same biomass composition as for bacteria was used (C fraction 0.47), giving a g-C-biomass/g-C-substrate yield range of 0.019 – 0.034. A mid-range value of 0.028 was used for the demonstration model.

Solids Bioreactor bioproducts factors

The products are separated into three broad categories; crust, liquor and cake related products, and the cake related products can be further split into compost and cake related product that is not compost.

Crust related bioproduct Y1

The crust related product category makes allowance for products produced at the air-matrix interface. This may be through fungal fruitbodies (commonly known as mushrooms) or a biofilm, and includes enzymes (Stamets, 1993), surfactants (Das & Mukherjee, 2007) and biopolymers (Wu, et al., 2004). Producing crust-related products would require that the matrix is not turned to improve mass transfer, which may be more suitable to the conventional sludge drying beds found in wastewater treatment.

PGA yield from solid substrate fermentation is in the range 36-99 mg-product/g-dry-substrate (Madonsela, 2013). Using dairy manure as substrate (C fraction 0.45, Patni and Jui (1987)), this translates to 0.037 – 0.1023 g-product-C/g-substrate-C.

Liquor related bioproduct Y2

The liquor related product stream contains products like organic acids. From a review of organic acid production using solid substrates, mainly bagasse, the yield of citric acid was in the range of 70 – 290 g-product/kg-substrate (Pandey, et al., 2010). The bagasse composition was assumed simplified to cellulose with a C fraction of 0.444 producing a g-product-C/g-substrate-C yield range of 0.030 – 0.136. Prado et al. (2005) report similar values ranging from 0.0.45 – 0.081 in different reactor configurations. A median value of 0.045 g-product-C/g-substrate-C was used in the demonstration model.

Cake-related bioproduct Y3

Cake-related product together with compost make up the remainder of the solids stream, with a separation coefficient / fractional split set by the user.

The cake related product stream makes allowance for bioproducts that may be used in applications where compost is not suitable, for example brick-making or packaging material (Arifin & Yusuf, 2013; Ecovative, 2016; Corpuscoli, 2016). The nutrient requirement is less important here, expected to be low, and dependent on the nutrients that remain after the entire WWBR process, but a bulk composition of non-digestible fibre (Pelletier, et al., 2013) may be more appropriate. This product category may include spent support matrix. The composition of product Y3 is based on fungal hyphae, with composition fraction value ranges of N 0.0042 - 0.202, P 0.0026 - 0.0044 and C 0.324 - 0.372 (Novaes-Ledieu, et al., 1967). The fraction values used for the first simulation are the averages N 0.0122, P 0.0035 and C 0.348.

Compost Y4

The compost produced does not have a user-set composition, but is dependent on the nutrients that remain after the entire WWBR process. The main fraction is organic matter, and most of the nitrogen and phosphate originates from the primary settlement tank. Compost is the remainder and last product of the WWBR process. Typical composition of compost nutrient values are in the range of 0.5 – 2% nitrogen, 0.3 – 1% phosphorous (as P_2O_5) and 84 – 89% organic matter (Lindsey & Hirt, 1999). Typical compost composition from mushroom waste is in the range of 1.8 – 3% nitrogen, 0.5 – 1.4% phosphorus and 33 - 37% carbon (William, et al., 2001).

Solids Bioreactor respiration factors

Sugama and Okazaki (1979) reported that the ratio of mg CO_2 evolved to mg dry mycelia formed by *Aspergillus oryzae* on rice ranged from 0.91 to 1.26 mg-CO_2 per mg-dry-mycelium. This translates to a CO_2 yield of 0.528 – 0.731 g-CO_2-C/g-biomass-C. Multiplying with the yield of biomass over substrate used in the model (0.028) gives a g-CO_2-C/g-substrate-C value in the range of 0.015 – 0.020. The value used in the model is the conservatively higher respiration value of 0.020.

Summary of yield factors used for Solids Bioreactor

A summary of yield values used as initial estimates for the demonstration of the model in an integrated system (Section 8.3) is shown in Table 8-8.

Table 8-8: Summary of Carbon-based yield values used for solids bioreactor

Conversion description	Symbol of factor	**Range of factor values in literature**	**Selected factor value for start-point**
Mass of carbon reporting to biomass as a fraction of that present in influent stream to reactor (U)	$Y_{C,XSolids/IN} = Y_{C,Y4/IN}$	0.019 – 0.034	0.028
Mass of carbon reporting to Product Y1 (Organic Content in Surface/Crust) as a fraction of that present in influent stream to reactor (U)	$Y_{C,Y1/IN}$	0.037 – 0.1023	0.037
Mass of carbon reporting to Product Y2 (Liquor-Related Product Stream) as a fraction of that present in influent stream to reactor (U)	$Y_{C,Y2/IN}$	0.030 – 0.136	0.045
Mass of carbon reporting to Product Y3 (Cake-Related Product Stream) as a fraction of that present in influent stream to reactor (U)	$Y_{C,Y3/IN}$	0.4	0.4
Mass of carbon lost as CO_2 as a fraction of that present in influent stream to reactor (U)	$Y_{C,CO2,Solids/IN}$	0.015 – 0.020	0.020
Mass of carbon remaining unconverted as a fraction of that present in influent stream to reactor (U)	$Y_{C,INSolids,unconverted/IN} = 1 - (Y_{C,XSolids/IN} + Y_{C,Y1/IN} + Y_{C,Y2/IN} + Y_{C,Y3/IN} + Y_{C,CO2Solids/IN})$	remainder	remainder

8.1.5 Separator efficiency factors

Separators and downstream processing units are generally well developed and well understood. Obtaining 100% separation between e.g. solids and liquids is possible in bioprocessing, but becomes a cost and time factor. A general compromise is a range of 80 – 95% separation of solids. General values for separator efficiencies used in bioprocessing are included in Table 8-9 (Harding, 2009). Where no specific information was available, a product recovery efficiency fraction value of 0.9 was used in the model.

Table 8-9: Product fractions recovered and waste fractions removed in bioprocessing concentration or purification units (Harding, 2009)

	Solid or product fraction removed*	Liquid or waste fraction removed*
Adsorption	0.99	0.95
Centrifugation	0.98	0.80
Chromatography	0.99	0.95
Evaporation	1.00	0.90
Filtration	0.95	0.95
Precipitation or crystallisation	1.00	0.00
Solvent extraction and decanting	0.99	0.95
OTHER	0.99	0.80

The majority of primary industrial wastewater-treatment solids-separation process units operate with clarifiers and flotation devices (Theobald, 2015). Many factors influence the settling characteristics of a given clarifier. Most common factors include temperature variation, short circuits, detention time, weir-overflow rate, surface-loading rate and solids loading, but a yield of 50% reduction in SS is an attainable design goal (range: 50 to 70%). BOD_5 can be reduced from 20 to 40% (Lopez, et al., 2015). Where no further specific information was available, a separation efficiency fraction value of 0.5 was used.

The other important factor in separations is the solids content of the resulting bottoms stream. A solids content of 1% is a common calculation value for primary settling without polymer addition, with values between 4 and 6% commonly required for solids handling, achieved with polymer addition. Typical values for solids contents of slurries found in wastewater treatment are shown in Table 8-10. A more comprehensive list of solids concentrations relevant to wastewater treatment can be found in "Metcalf and Eddy" *Wastewater Engineering. Treatment Disposal Reuse* (Tchobanoglous, et al., 2003).

Table 8-10: Representative solids contents of slurries found in wastewater treatment with relevance to WWBR

Type of slurry	Range of solids concentration (fraction dry solids)	Typical solids concentration (fraction dry solids)
Primary Settling Tank	0.05 – 0.09	0.06
Waste activated sludge with primary settling (similar to the bacterial biomass bottoms)	0.005 – 0.015	0.008
Waste activated sludge without primary settling (similar to the bacterial biomass bottoms, without Unit 0.1)	0.008 – 0.025	0.013
Rotating Biological Contactor waste sludge (similar to the bacterial biomass bottoms)	0.01 – 0.03	0.015
Gravity thickener of primary sludge	0.05 – 0.10	0.08
Aerobic digester of primary sludge	0.025 – 0.07	0.035
Aerobic digester of primary sludge and waste activated sludge	0.008 – 0.025	0.013

Specific considerations and factors relevant to the different reactor unit trains are discussed in the following subsections.

Bacterial Bioreactor Train separator efficiencies

The separator efficiencies for the bacterial bioreactor are based on the slurries found in wastewater treatment, as these are most closely related to bacterial processes. The values chosen are listed in Table 8-11.

Table 8-11: Bacterial bioreactor train separator efficiencies

Unit number	Relevant parameters	Efficiency symbol	Range of factor values in literature	Selected factor value for start-point
0.1	Slurry solids content Solids to Bottoms U1	SC_{U1} eff_{U1}	0.01 – 0.09 design specific	0.06 0.5
1.2	Slurry solids content Solids to Bottoms C2	SC_{C2} eff_{C2}	0.005 – 0.015 design specific	0.008 0.5
1.3	Slurry solids content Bacterial Product Recovery efficiency Solids (Biomass) to Bottoms C3	SC_{C3} eff_{V1} eff_{C3}	0.05 – 0.10 0.8 – 1.0 design specific	0.08 0.9 0.5

Algal Bioreactor Train separator efficiencies

The model does not specify specific downstream processing options, but does suggest likely recovery methods, in keeping with the design for downstream processing approach, discussed in the chapter on bacterial bioreactor design (Chapter 6). For primary dewatering, flocculation and sedimentation is suggested, while decanter or spiral plate centrifuges and rotary press are likely secondary dewatering steps. To recover algal lipids, a wet biomass processing route is strongly preferred (Louw, et al., 2016).

In terms of algal product recovery, there are some challenges to consider. Algal cells are larger than bacterial cells, but break fairly easily. In addition they are too small to filter well. Flotation, or skimming are therefore more suited to product recovery. Harvesting at a specific time of day may be advantageous as the algal metabolism changes during the night to include programmed cell death and respiration (Cowan, et al., 2016).

Downstream processing depends on, amongst other things, the resistance of the algal cells to disruption. The algal process will rely on ecological selection, which is likely to select for a product that fulfils an ecological role, but unlikely to select for easily-disrupted cells. While the method of cell disruption lies outside the scope of the model, a conservatively low disruption efficiency fraction value of 0.7 is assumed.

Inglesby et al. (2015) mention using an algal slurry of 20 g/ℓ into an anaerobic digester, which correlates with the representative solids contents of slurries found in wastewater treatment as listed in Table 8-12.

Table 8-12: Algal bioreactor train separation efficiencies

Unit number	Relevant parameters	Efficiency symbol	Range of factor values in literature	Selected factor value for start-point
2.2	Slurry solids content Solid to Bottoms E2	SC_{E2} eff_{E2}	0.008 – 0.08 design specific	0.02 0.5
2.3	Algal Bioproduct recovery efficiency Solids (Biomass) to Bottoms E4 Solids to Bottoms E4	eff_{E3} eff_{E4} SC_{E4}	0.8 – 1.0 design specific 0.008 – 0.08	0.9 0.5 0.08
2.4	Algal High-Value Bioproduct recovery efficiency Algal Oil recovery efficiency Water content in oil recovery	eff_{W1} eff_{W2} SC_{W2}	0.8 – 1.0 0.8 – 1.0 0 – 0.1	0.9 1 0.05

Macrophyte Bioreactor Train separator efficiencies

Macrophyte harvesting is likely to occur seasonally, which means the yield values are averaged for daily absorption rates. The almost compliant effluent moves through the wetland matrix and exits as compliant effluent (stream Z) containing very low levels of solid contaminants. The sediment and macrophytes that constitute the solid fraction (stream G2) remains quite wet, however.

The harvesting is likely to be done manually, or be manually assisted, as large machinery will disturb the wetland matrix, for example sink the floating wetlands. The bulk of the cellulosic biomass is the fibre in the main portion of the plants, and this is separated from the rootstock through cutting. The remainder rootstock is associated with the sediment (stream G4), and during (probably annual) desludging maintenance, this sediment together with the root mass underneath the floating islands is removed, and either sold as a nutrient rich soil additive (stream X3) or added to the solids bioreactor (stream U5). It is common practice to remove the rootstock with fibrous plants to achieve longer fibres, but this approach may need to be revised for the WWBR. If this approach is followed, the eff_{G3} value may be higher.

The bulk of the macrophyte is then processed to remove the main fibre sections. The cellulosic biomass product stream (stream X1) that leaves the WWBR system is not completely pure, but has most of the peripheral material, for example leaves, removed. These remnants become the cellulosic biomass, macrophyte bottoms stream (stream G5) that can either be sold as product (stream X2) or be used as support and carbon source in the solids bioreactor (stream U4).

For these reasons, the efficiencies of separation are expected to be quite low. Harvesting of the macrophytes is estimated at a fraction value of 0.8.

The moisture content of flax and hemp fibres are in the range of 10 – 30% (Kymäläinen & Pasila, 2000), translating to a solids content fraction of 0.7 – 0.9. The mid-range value of 0.8 was used in the model.

Table 8-13: Macrophyte bioreactor train separation efficiencies

Unit number	Relevant parameters	Efficiency symbol	Range of factor values in literature	Selected factor value for start-point
3.2	Solids to Bottoms G2 Slurry solids contents	eff_{G2} SC_{G2}	unknown 0.008 – 0.8	0.99 0.6
3.3	Biomass to biomass stream efficiency Sediment to sediment stream efficiency Slurry solids contents	eff_{G3} eff_{G4} SC_{G3}	0.8 – 1.0 0.8 – 1.0 unknown	0.9 0.9 0.6
3.4	Macrophyte fibre recovery Macrophyte fibre solids contents	eff_{X1} SC_{X1}	0.8 – 1.0 0.7 – 0.9	0.8 0.8

Solids Bioreactor Product Train separator efficiencies

The solids bioreactor involves two solid-solid separations (units 4.2 and 4.4) and one solid-liquid separation (unit 4.3), assumed to be a belt-press. While the belt-press as a choice for separation in this context has not been corroborated, values for the belt press in the treatment of biosolids have been used (WEF, 2005).

Separating the crust related products is likely to be a cutting, or skimming operation, with a high yield of crust recovery (eff_{Y1}), but with a fair amount of contaminants in the Y1 stream (1 - eff_{H2}). This separation is likely to be similar to an agricultural tilling or scooping operation.

Separating the cake related product stream (Y3) and the compost (Y4) is likely to be achieved through a (vibrating) sieving action. Efficiency values for this operation are unknown and likely highly specific to the process. Estimates of 60% recovery product Y3 have been used. Composting proceeds best at a moisture content of 40-60% by weight. At lower moisture levels, microbial activity is limited. At higher levels, the process is likely to become anaerobic and contaminated (Cornell Waste Management Institute, 1996). A mid-range value of 50% solids has been used.

Table 8-14: Solids bioreactor train separation efficiencies

Unit number	Relevant parameters	Efficiency symbol	Range of factor values in literature	Selected factor value for start-point
4.2	Solids to Bottoms H2 Crust to Top Slurry solids contents	eff_{H2} eff_{Y1} SC_{H2}	0.8 - 1.0 0.8 - 1.0 design specific	0.8 0.9 0.5
4.3	Solids to Bottoms H3 Pressed cake solids contents	eff_{H3} SC_{H3}	0.8 - 1.0 0.12 – 0.32	0.9 0.3
4.4	Product Y3 to product stream Product Y4 to product stream Solids contents: Product Y4	eff_{Y3} eff_{Y4} SC_{Y4}	unknown unknown 0.4 – 0.6	0.6 0.9 0.5

8.1.6 Splitter ratios

The splitters do not have a range of values typically found in literature, as their explicit function is to assist the integration of the respective bioreactor units. The impact of the splitters will be briefly illustrated in the contextualisation of an integrated WWBR in Section 8.4.

The splitter that directs settled wastewater to the algal bioreactor is informed by the amount of nutrients that is needed to supplement the algal bioreactor stream. It is optional and also dependent on what additional nutrient rich streams are available (streams D3 – D5).

The splitters that send a fraction of potential product, as substrate to the solids bioreactor (streams U2 – U5) is to provide nutrients or supportive substrate to the solids bioreactor from the WWBR as a source. The defining factor value would be evaluated from the needs of the solids reactor to optimise its productivity, and in the case of the cellulosic biomass, to effect efficient mass and heat transfer. This needs to be traded off with the economic value and market demands of the potential product, and the possibility of alternative substrates to replace the product. The purpose of this model is to assist in investigating these decisions.

The selected factor value for start-point is chosen to direct 90% of the flow to the main intended stream, which is indicated by the subscript of the ratio symbol, and summarised in Table 8-15.

Table 8-15: Splitter ratios for a generic WWBR

Unit number	Streams split	Ratio symbol	Range of factor values in literature	Selected factor value for start-point
0.2	Fraction to Bacterial Bioreactor B1 Fraction to Algal Bioreactor D2	r_{B1} $1 - r_{B1}$	0 – 1 1 - 0	0.9 0.1
1.4	Fraction to Bacterial Bioreactor C4 Fraction to Solids Bioreactor U2	r_{C4} $1 - r_{C4}$	0 – 1 1 - 0	0.9 0.1
2.5	Fraction to Algal Biomass Stream W3 Fraction to Solids Bioreactor U3	r_{W3} $1 – r_{W3}$	0 – 1 1 - 0	0.9 0.1
3.5	Fraction to Cellulosic Product X2 stream Fraction to Solids Bioreactor U4	r_{X2} $1 – r_{X2}$	0 – 1 1 - 0	0.9 0.1
3.6	Fraction to Sediment Product X3 stream Fraction to Solids Bioreactor U5	r_{X3} $1 – r_{X3}$	0 – 1 1 - 0	0.9 0.1

8.1.7 Water mass balance factors

Because the model is stoichiometric with limited consideration for volumes, an average depth was used to incorporate the surface evaporation per Mℓ water entering the system. Facultative and fermentative ponds, which are populated mainly by bacteria, are in the range of 3-6 m deep. A typical design parameter is 4 m depth, and this value was used for the bacterial reactor. High rate aeration algal ponds are about 30-45 cm deep (0.3 – 0.45 m), hence the algal reactor was estimated at 0.4 m. Wetlands are typically 1.2 m deep, as this depth is best for maintenance, and shallower ponds promote the growth of *Typha* and *Phragmites* which is considered a nuisance (Lynda Muller, personal communication 2015). Floating wetlands may be used in deeper ponds. Duck weed ponds and hyacinth ponds range from 1.5 – 4.5 m in depth, where non-aerated systems are shallower, and aerated systems deeper (WEF FD-16, 2010, pp. 211-258). The default depth for the macrophyte reactor used in the model is 1.2 m. The solid substrate bioreactor may be closed to aid in increasing temperature in composting, but likely will be open at least some of the time (or total area) to remove excess moisture. A default value of 1 m has been used as a conservative estimate.

The Area/Volume (m^2/m^3) heuristic was determined by considering a virtual 'block of water', of area dimension 1 x 1 m^2, which then gives a heuristic of area per m^3 unit volume liquid in the reactor, determined by the depth of the reactor, effectively = 1/depth.

Table 8-16: Bioreactor area sizing and evaporation

	Typical depth (m)	**Area factor** = volume/depth of liquid ($m^3/m = m^2$)	Average annual evaporation (mm)	Average daily evaporation (mm/day)	Volume (m^3) evaporation per m^3 liquid in reactor, per day	Water lost per kg liquid in reactor, per day (kg)
Bacterial Bioreactor	6.00	0.17	303	0.8301	0.0001	0.0001
Algal Bioreactor	0.50	2.00	303	0.8301	0.0017	0.0017
Macrophyte Bioreactor	1.20	0.83	303	0.8301	0.0007	0.0007
Solids Bioreactor	1.00	1.00	303	0.8301	0.0008	0.0008

Table 8-17: Bioreactor area sizing and precipitation

	Typical depth (m)	**Area** = volume/depth of liquid ($m^3/m = m^2$)	Average annual rainfall (mm)	Average daily rainfall (mm/day)	Volume (m^3) precipitation per m^3 liquid in reactor per day	Water gained per kg liquid in reactor (kg)
Bacterial Bioreactor	6.00	0.17	450	1.232	0.0002	0.0002
Algal Bioreactor	0.50	2.00	450	1.232	0.0025	0.0025
Macrophyte Bioreactor	1.20	0.83	450	1.232	0.0010	0.0010
Solids Bioreactor	1.00	1.00	450	1.232	0.0012	0.0012

The default value for annual evaporation used in the model is 303 mm/year (Jovanovic, et al., 2015), while the average annual precipitation used is 450 mm/year (Dedekind, et al., 2016). Note that these are very rough values averaged for the country, and meant more to alert the user to keep these aspects of the water balance in mind. Substituting more accurate values, and investigating scenarios based on seasonal variability may be worthwhile.

From these values, the volume of evaporation lost or precipitation gained can be correlated to the volume liquid in the reactor by multiplying the evaporation or precipitation (kg/kg water in reactor) with the kg water in the reactor. Note that the evaporation and precipitation data needs to be converted to a daily value, to fit with the basis of the model. The values are only applied to the bioreactor units, and not to other process units, which represents an underestimation.

8.2 Demonstration of Simulation for a Simple Bioreactor Train: PHA from Confectionary Waste

Although many types of wastewater can be used for the production of PHA, high concentrations of biologically available COD, relatively low nitrogen and solid concentrations and low toxicity promote process feasibility. From this perspective, food and paper industry effluents may be considered the most suitable substrates for waste-based PHA production (Tamis, et al., 2014).

The crux of enriching biomass with superior PHA-storing capacity in an open bioreactor system (an environment in which myriad species constantly invade the system for example by being present in the wastewater substrate) is the establishment of a selective environment. The cyclical presence and absence of volatile fatty acids (VFA) inherent in the sequencing batch aerobic granular sludge (AGS-SBR) process provides a competitive advantage for PHA storing species.

8.2.1 Input values for PHB from confectionary wastewater

Fernández-Dacosta, et al. (2015) performed a conceptual process design based on data from laboratory and pilot plant scale operations (Tamis, et al., 2014) using real industrial wastewater from the confectionary industry. They report a PHA yield of 70% dry cell weight, which translates to a g-C-product/g-C-substrate yield of 0.427 as covered in Section 8.1.1 Extracellular bacterial bioproduct V1. The PHA was polyhydroxybutyrate (PHB), and was produced in an aerobic conversion reaction using three sequential fermentation steps, with a microbial enrichment culture.

The wastewater from the Mars factory was pre-treated in a flotation-based fat separation unit before entering the influent tank of the pilot installation. No primary settlement of solids was employed.

The concentration of ammonium was maintained between 10 and 30 mg-N/ℓ at the end of the cycle, through dosing after measurement, if necessary. The resulting COD:N mass ratio in the feed stream was approximately 25:1. It was assumed that ammonium was the limiting growth nutrient with other elements required for microbial growth present in excess. In this set-up the bacterial reactor included a three step process (refer to Appendix section G.2). For the purposes of the model, the three steps are seen as a 'black box', with only the overall yield values used. The experiment was run as a fed-batch system To use the model an assumption of continuous operation was needed, with a reference value of 1 m^3 incoming, hence analysis over an averaged time period was considered.

The average soluble COD of the wastewater varied strongly over time (intrinsic to factory operation, e.g. semi-periodic cleaning of equipment) with an average concentration of 7.8 ± 4.1 g-COD/ℓ (average ± standard deviation over the dataset) and a concentration of 0.8 ± 0.5 g-COD/ℓ as solids not passing a 0.45 μm pore size filter. The soluble nitrogen concentration in the wastewater was negligible (<1 mg-N/ℓ).

These values are then incorporated into the model along with the separation and splitter values, as summarised in Table 8-19.

Table 8-18: Values for streams in PHA production (adapted from Fernández-Dacosta et al. (2015) and Tamis et al. (2014))

Stream	Value	Comments
B1: Mars candy bar factory	1 000 m^3 (unit volume chosen) 3.21 kg-C/m3.day total	7.8 ± 4.1 g-COD/ℓ soluble 0.8 ± 0.5 g-COD/ℓ solids Assume all COD is acetic acid, 1.07 g COD/g acetic acid
B2: Supplement nutrient stream (Urea + PO_4)	0.0041 m^3/m^3 B1 84 g-N/L 9.3 g-P/L 36 g-C/L	See Appendix section G.2 The target COD:N mass ratio was around 25:1. A nutrient solution containing 3 M nitrogen in the form of urea, 0.3 M phosphate, 0.3 M $MgSO_4$, 0.2 M K_2SO_4, and trace elements (64 mM $FeCl_3$, 3 mM $ZnSO_4$, 2.7 mM H_3BO_3 , 2.1 mM $NiCl_2$, 1.5 mM $CoSO_4$, 0.6 mM $CuSO_4$, 0.8 mM Na_2MoO_4) was provided to the bioreactor.

Table 8-19: Factors for units in PHA production (adapted from Fernández-Dacosta et al. (2015) and Tamis et al. (2014))

Process Unit	Conversion	Comments
0.1. Separator	SC_{U1} = 1 eff_{U1} = 0	A solids separator was not used. An initial fat separator was employed, but the data presented reflects the composition after this step, which makes the fat separator fall outside the system boundary.
0.2. Splitter	r_{B1} = 1	The entire volume is directed to the bacterial bioreactor.
1.1. Bacterial reactor: biomass	$Y_{C,XBact/IN}$ = 0.165	See Table 8-18.
1.1. Bacterial reactor: Product V1: PHA	$Y_{C,V1/IN}$ = 0.115	See Table 8-18.
1.1. Bacterial reactor: Product: VFA	0	All used up internally, converted to biomass, PHA or CO_2.
1.1. Bacterial reactor: Respiration CO_2	$Y_{C,CO2Bact/IN}$ = 0.437	See Table 8-18 .
1.1. Bacterial reactor: Unconverted	0.00	Remainder
1.2. Separator	eff_{C2} = 0.5 SC_{C2} = 0.008	Assume model default values. Fraction of wastewater to stream D1 "Impurities are about 9% of the solid phase"
1.3 Separator: Centrifugation	eff_{C3} = 0.9 eff_{V1} = 0.95 SC_{C3} = 0.08	Assume model default value for eff_{C3} and SC_{C3} Disruption efficiency 95% Final product purity 99.9%
1.4. Splitter	r_{C4} = 0	No biomass is recycled.
Global PHA recovery	0.735	Fraction of PHA in stream I / PHA in stream C1, bacterial broth.

8.2.2 The output values of model demonstration run

Figure 8-1 shows an example visualisation of the carbon mass balance as outlined in Section 8.1 in a Sankey diagram. It visually indicates a large fraction of carbon lost as CO_2, which may be due to a high endogenous respiration rate typical of wastewater treatment. According to the model 30% of carbon exits through D1, the improved compliance effluent, while the source data assumes that the water is treated well enough for discharge. This may be an artefact of imperfect separations used as default values in the model and requires further optimisation. It highlights the need for additional buffering unit processes, like the macrophyte bioreactor to improve resilience. Converting the exiting nutrient values into effluent concentration gives 0.0014 kg-C/m^3, 0.00022 kg-N/m^3 and 0.000027 kg-P/m^3, which translates into 1.36 mg-C/ℓ, 0.22mg-N/ℓ, and 0.027 mg-P/ℓ, values which are sufficiently low for discharge.

The Sankey diagrams are visual representations of the overall mass balance, showing the incoming and outgoing streams as they relate to each other, in kg/day. The white areas between the flows are not meaningful, but are merely space to separate the streams for legibility.

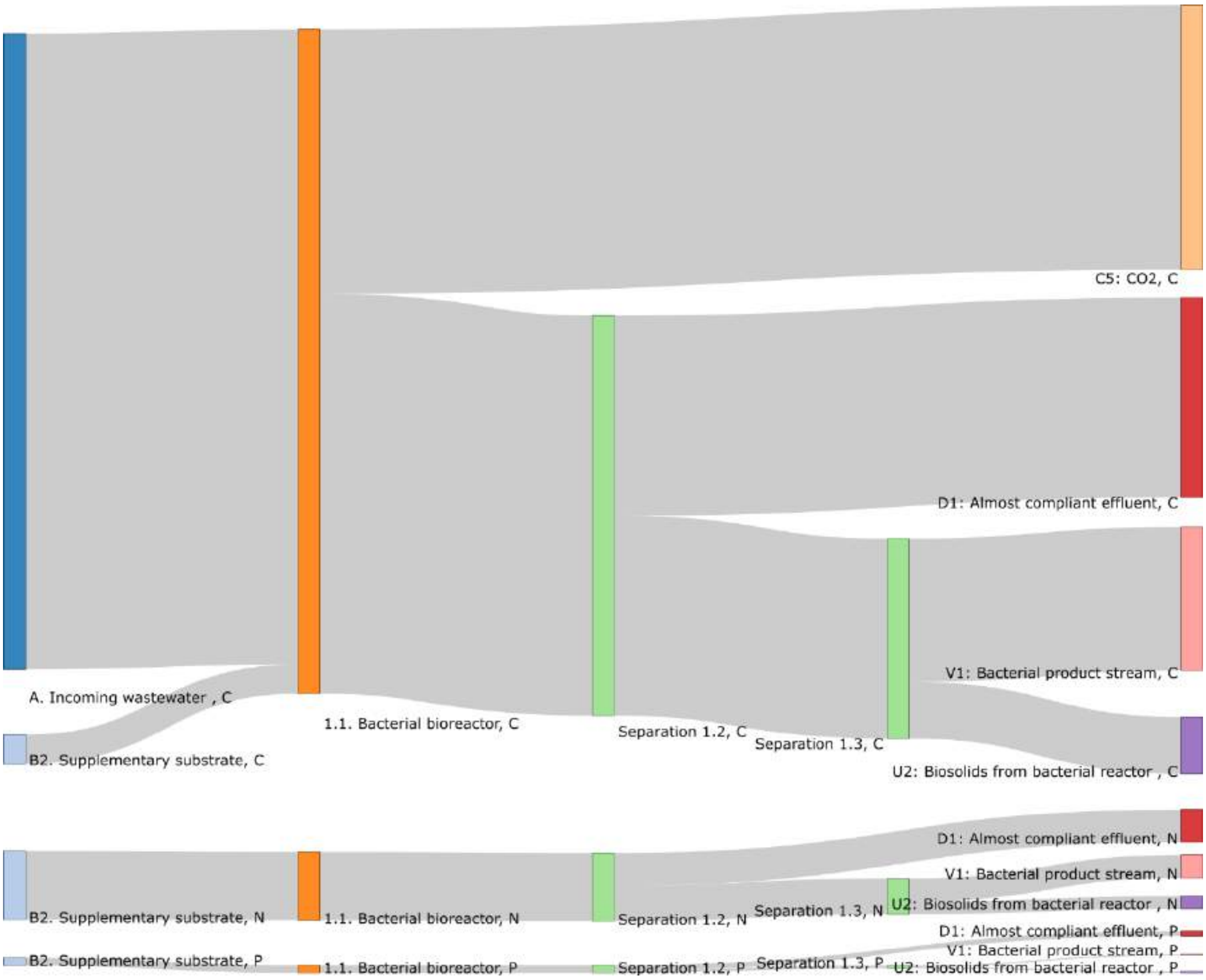


Figure 8-1: Sankey diagram of carbon, nitrogen and phosphorus mass balances for the simulation of PHB production in a bacterial bioreactor train using Mars confectionary factory wastewater

The corresponding values for the flows illustrated in Figure 8-1 are listed in Table 8-20.

Table 8-20: Inventory of carbon, nitrogen, phosphorus and water for bacterial bioreactor train using mars confectionary factory wastewater

Item	Stream Description	C kg/day	N kg/day	P kg/day	W kg/day
Raw, unsettled wastewater A1 to mixing tank	Mars confectionary factory wastewater	3 210	0	0	996 790
Urea supplement stream B2	3M Urea, 0.3M PO_4	148	344	38	3 570
Incoming (total)		**3 358**	**344**	**38**	**1 000 360**
CO_2 (out)		1 336	0	0	0
Precipitation/ Evaporation		0	0	0	907
Bacterial product V1 stream (not 100% pure)	PHA	725	117	7	250 575
D1: Improved compliance effluent		1 011	165	25	743 098
U2: Bacterial bottoms		286	62	6.61	6 753
Total outgoing		**3 358**	**344**	**38**	**100 0427**
Difference	*(should be 0)*	0.00	0.00	0.00	839.98
Difference (%)		0.00	0.00	0.00	0.08
Item		**% C of total**	**% N of total**	**% P of total**	**% Water of total**
Raw, unsettled wastewater A1 to mixing tank	Mars confectionary factory wastewater	95.60	0	0	99.64
Urea supplement stream B2	3M Urea, 0.3M PO4	4.40	100.00	100.00	0.36
Incoming (total)		100.00	100.00	100.00	100.00
CO_2 (total)		36.99	0.00	0.00	0.00
Precipitation/ Evaporation		0.00	0.00	0.00	0.09
Bacterial product V1	PHA	21.58	34.01	17.51	25.03
Improved compliance effluent D1		30.10	47.89	65.15	74.28
Bacterial bottoms U2		8.52	18.10	17.34	0.68
Difference	*(should be 0)*	0.00	0.00	0.00	0.00

8.2.3 Concluding remarks on simulating a single unit system

This section demonstrates the use of the model for resource recovery for a single-unit system. It also illustrates the format of data reporting in standard metric units of kg and m^3, reporting in terms of elements C, N and P rather than electrons in the form of COD. The use of elemental compositions is motivated on their direct usefulness in the mass balance; reporting in COD requires an additional assumption about the organic nature of the substrate, whereas TOC values are more useful for this mass balance. The model can be expanded to allow for an electron balance in future.

This section only considers a single unit, the Bacterial Bioreactor, using the literature study by Fernández-Dacosta et al. (2015) which is not designed for a multi-unit system. This limits the resilience of the system, and the system in its current configuration cannot absorb shock loads of high nutrient containing waters. It is a suitable system for a highly-defined, intensively managed waste stream like a food industry's wastewater, but less suitable for a complex wastewater. The model is demonstrated for an integrated WWBR in Section 8.3 and some examples of more complex wastewaters are evaluated in Section 8.4.

8.3 Demonstration of Simulation for an Integrated System

The single unit simulated in Section 8.2 is well suited to a stream that is low in nitrogen and phosphorus. For streams that have higher concentrations of nutrients, additional treatment is required. Further additional treatment steps can allow the concomitant meeting of multiple objectives e.g. compliant water, optimised productivity of the major carbon-based product and optimisation of N- and P-based products. In this section, a more dilute wastewater with higher concentrations of N and P is selected. PGA, an extracellular product, is now the chosen bacterial product. The yields are reduced by 20% to take into account a possibly non-optimal system, the potential impact of a higher dilution, and to allow interim product like VFA to continue through to the algal bioreactor in which algal lipids and a niche product are formed. This may also allow lower residence times within the bacterial bioreactor.

8.3.1 Municipal wastewater as feedstock for integrated WWBR simulation

A hypothetical municipal wastewater stream was used. For the purposes of comparison with the wastewaters used in Section 8.4, the incoming flow was standardised to 1 000 m^3 (1 Mℓ). The composition is based on the mid-range average values reported in Henze et al. (2008), and Tchobanoglous et al. (2003), with data relating to the sludge adapted from Strande et al. (2015). Section 4.2 looks at municipal wastewater as biorefinery feedstock, and Appendix section C.3 references background data.

The composition values used are shown in Table 8-21. For the demonstration of the integrated unit process, no supplementary streams were added to optimise the nutrient compositions.

Table 8-21: Summary of incoming wastewater values used to demonstrate an integrated multi-unit process

Incoming (Stream A1)	**Total flow** (m^3/day)	**C** (kg/m^3)	**N** (kg/m^3)	**P** (kg/m^3)
Liquid component	1 000	0.160 (as TOC)	0.050	0.008
	Solids (kg/m3)	**C** (kg C / kg solids)	**N** (kg C / kg solids)	**P** (kg C / kg solids)
Solids component	0.72	0.583	0.157	0.04

8.3.2 Values of factors for units in the integrated WWBR used in simulation

A summary of the factors used in this demonstration is listed in the following Tables: Table 8-22 the biomass composition and product compositions, Table 8-23 the yield factors and Table 8-24 the separator efficiencies.

Refer to flowsheets in Chapter 7: Figure 7-2: Bacterial bioreactor train detailed flowsheet; Figure 7-3: Algal bioreactor train detailed flowsheet; Figure 7-4: Macrophyte bioreactor train detailed flowsheet; and Figure 7-5: Solids bioreactor train detailed flowsheet.

Table 8-22: Summary of biomass and product composition values used to demonstrate an integrated multi-unit process

Biomass Composition (g / g total dry biomass)	**C**	**N**	**P**
Bacterial Bioreactor	0.48	0.11	0.013
Algal Bioreactor	0.52	0.092	0.013
Macrophyte Bioreactor	0.715	0.00735	0.0023
Solids Bioreactor	0.47	0.11	0.03
Product Composition (g / g total dry product)	**C**	**N**	**P**
Bacterial Bioproduct V1	0.465	0.109	0
Algal Bioproduct W1	0.68	0.096	0
Algal Bioproduct W2	0.805	0	0
Algal Bioproduct W3	0.52	0.092	0.013
Macrophyte Bioproduct X1	0.715	0.00735	0.0023
Macrophyte Bioproduct X2	0.715	0.00735	0.0023
Macrophyte Bioproduct X3	determined by process	determined by process	determined by process
Solids Bioproduct Y1	0.465	0.109	0
Solids Bioproduct Y2	0.375	0	0
Solids Bioproduct Y3	0.348	0.012	0.0013
Solids Bioproduct Y4	determined by process	determined by process	determined by process
Compliant Effluent Z	determined by process	determined by process	determined by process

Table 8-23: Summary of outgoing yield values used to demonstrate an integrated multi-unit process

Bioreactor Unit	**Conversion value (Y)**	
1.1. Bacterial bioreactor	Biomass: V1: Interim Product VFA: CO_2:	0.164 0.123 0.7 – 0.164 – 0.123 = 0.413 0.24
2.1. Algal bioreactor	Biomass: W1: W2: W3: CO_2:	0.345 0.01 0.083 (0.345) 0.1
3.1. Macrophyte Bioreactor	Biomass: X1: X2: X3: CO_2:	0.000 000 0601 0.000 000 0601 * eff_{X1} 0.000 000 0601 * (1 - eff_{X1}) dependent on process -0.000 000 0601
4.1. Solids Bioreactor	Biomass: Y1: Y2: Y3: Y4: CO_2:	0.028 0.037 0.045 0.4 remainder 0.020

Table 8-24: Summary of separator and splitter values used to demonstrate an integrated multi-unit process

Process Unit	Conversion value		Comments
0.1. Separator	SC_{U1} = eff_{U1} =	0.06 0.5	
0.2. Splitter	r_{B1} =	0.9	Assumption: 90% of the overall volume is directed to the bacterial bioreactor, with 10% bypass to the algal bioreactor.
1.2. Separator	SC_{C2} = eff_{C2} =	0.008 0.5	
1.3 Separator: Centrifugation	SC_{C3} = eff_{C3} = eff_{V1} =	0.08 0.9 0.5	
1.4. Splitter	r_{C4} =	0.1	Assumption: 10% of biomass is recycled.
2.2. Separator	SC_{E2} = eff_{E2} =	0.02 0.5	
2.3. Separator: Centrifugation	SC_{E4} = eff_{E3} = eff_{E4} =	0.9 0.5 0.08	
2.4. Separator	eff_{W1} = eff_{W2} = SC_{W2}	0.9 1 0.05	
2.5. Splitter	r_{W3} =	0.9	
3.2. Separator	SC_{G2} = eff_{G2} =	0.6 0.99	
3.3. Separator: Centrifugation	SC_{G3} = eff_{G3} = eff_{G4} =	0.6 0.9 0.9	
3.4. Separator	SC_{X1} = eff_{X1} =	0.8 0.8	
3.5. Splitter	r_{X2} =	0.9	
3.6. Splitter	r_{X3} =	0.9	
4.2. Separator	SC_{Y1} = eff_{Y1} = eff_{H2} =	0.8 0.9 0.5	
4.3. Separator: Centrifugation	SC_{H3} = eff_{H3} =	0.3 0.9	
4.4. Separator	SC_{Y4} = eff_{Y3} = eff_{Y4} =	0.5 0.6 0.9	

8.3.3 Results of applying the values simulating an integrated WWBR

The model output for an integrated flowsheet using 4 reactor unit trains is summarised in Table 8-25, and the carbon mass balance is visualised in Figure 8-2.

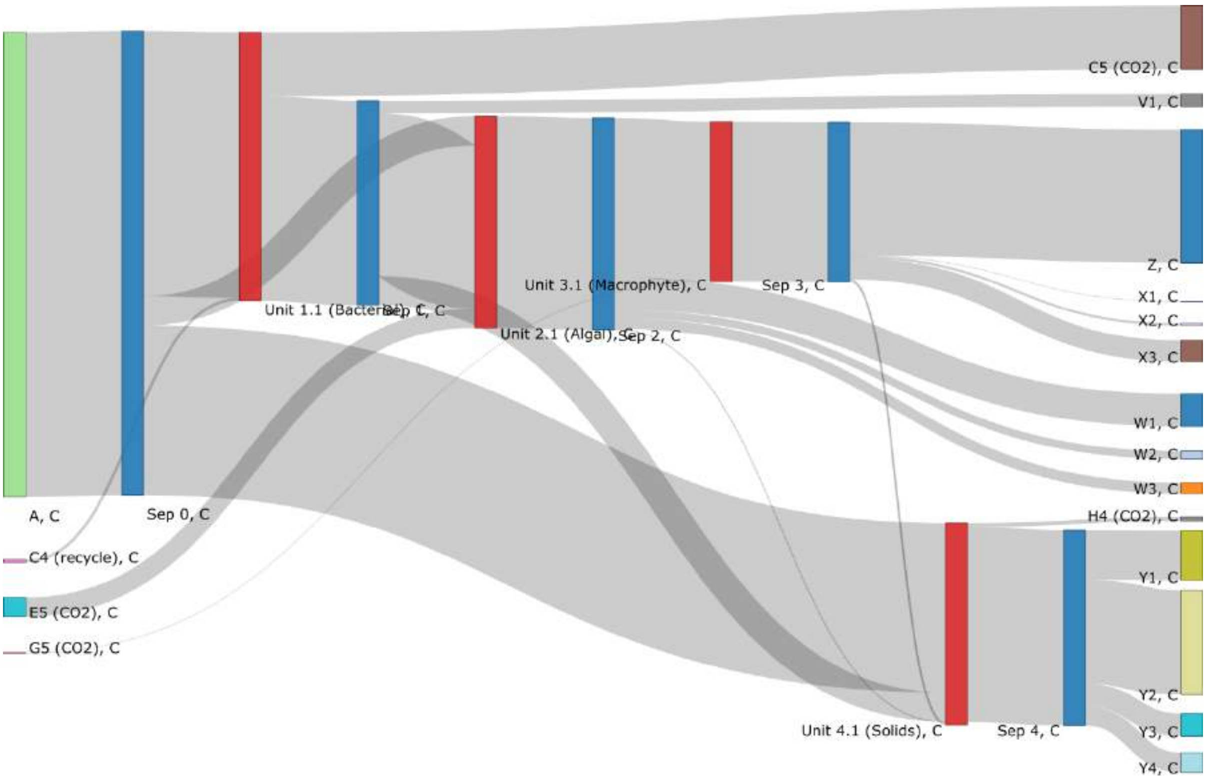


Figure 8-2: Sankey diagram of the carbon mass balance for the simulation of an integrated WWBR using municipal wastewater as feedstock

Bacterial product V1, Algal high value product W1, Algal lipid product W2, Digestible algal biomass W3, Macrophyte crust, liquor and cake-related products Y1-Y3, Compost Y4. Compliant water

Table 8-25: Inventory of carbon, nitrogen, phosphorus and water for generic WWBR using municipal wastewater

Item	Stream Description	C kg/day	N kg/day	P kg/day	W kg/day
Raw, unsettled wastewater A1 to mixing tank	domestic wastewater	580	163	37	999 062
Incoming (total)		580	163	37	999 062
CO_2 (total)		60	0	0	0
Rainfall/Evaporation (total)		0	0	0	1 172
Bacterial product V1		15	4	0.40	11 752
Algal bioproduct W1		41	7	1	5 861
Algal oil W2		10	0	0	234
Algal digestible waste W3		13	1	0.07	1
Cellulosic fibre X1		0.42	0	0	0.15
Cellulosic biomass X2		3	1	0.18	0.30
N,P rich sediment X3		26	6	2	0.05
Crust/surface related product stream Y1		62	18	8	6
Liquor related product stream Y2		130	32	1	11 721
Cake-related product stream Y3		28	3	1	53
Compost Y4		24	13	6	274
Compliant effluent Z		166	79	17	970 332
Total outgoing		580	163	37	1 000 234
Difference (should be 0)		0	0	0	0
Difference (%)		0	0	0	0
Item	Stream Description	% C of total	% N of total	% P of total	% Water of total
Raw, unsettled wastewater A1 to mixing tank	domestic wastewater	100.00	100.00	100.00	100.00
Incoming (total)		100.00	100.00	100.00	100.00
CO_2 (total)		10.32	0.00	0.00	0.00
Rainfall/Evaporation (total)		0.00	0.00	0.00	0.12
Bacterial product V1		2.65	2.54	1.10	1.17
Algal bioproduct W1		7.02	4.50	2.90	0.59
Algal oil W2		1.71	0.00	0.00	0.02
Algal digestible waste W3		2.32	0.37	0.20	0.00
Cellulosic fibre X1		0.07	0.00	0.00	0.00
Cellulosic biomass X2		0.52	0.41	0.49	0.00
N,P rich sediment X3		4.55	3.65	4.41	0.00
Crust/surface related product stream Y1		10.64	11.22	22.02	0.00
Liquor related product stream Y2		22.46	19.43	2.20	1.17
Cake-related product stream Y3		4.88	1.53	2.21	0.01
Compost Y4		4.14	8.12	17.61	0.03
Compliant effluent Z		28.72	48.24	46.85	97.01
Difference (should be 0)		0.00	0.00	0.00	0.00

Even from this early stage model using the estimate values from Section 8.1, some clear trends are evident. For example, the large difference between algal nutrient removal and macrophyte nutrient removal (averaged to a daily value) indicates that algae may be a better option in intensive production systems for nitrogen and phosphorus removal. Even in this conservative scenario, the potential for the wastewater biorefinery is significant. While increasing the bioreactor yields is an obvious route to improve productivity, the cumulative effect of imperfect separations have a greater effect overall. This reinforces the need for appropriate bioreactor design targeted specifically at product recovery. In addition, while maximising the productivity of individual reactor units could lead to better economic returns by individual products, the optimisation of overall efficiency of the integrated plant carries higher priority and will yield higher dividends. Further, the combined optimisation of products and water quality is needed through repeated iterations of refinement.

8.3.4 Evaluation of the simulation of an integrated WWBR

It becomes more apparent once the integrated WWBR is modelled that the combination of a numerical simulation with a visualisation component is a helpful tool. Already at this level of generic experimentation certain non-intuitive aspects of a scenario emerge together with the confirmation of some more expected outcomes. In Section 8.3 the trialled simulation is repeated using two different wastewaters to further explore both the application of the WWBR concept and the usefulness of the developed simulation tool.

8.4 Contextualisation of an Integrated WWBR for Possible Scenarios

In this section, different wastewaters are compared in terms of their bioproduction potential. The section is concluded with evaluation of different realistic separation and yield scenarios to inform future research required on bioproduction in integrated systems.

8.4.1 Comparison of different wastewaters in an Integrated WWBR

The domestic wastewater used to demonstrate the simulation for an integrated system is an example of a complex, dilute wastewater (Section 8.3). Two further examples are given and briefly compared in terms of bioproduction potential per 1000 m^3, using data from Chapter 4. Poultry abattoir waste (Section 4.3.3) is used as the first example representative of complex, more concentrated wastewater. The pulp and paper wastewater is used as an example of a more chemically defined process. These are both industries of high importance in South Africa. Further, they cover the two ends of the spectrum of scale of production: abattoirs are often small, scattered industries, while pulp and paper production is covered by four major producers (Section 4.3.1). The wastewater values used are listed in Table 8-26, with the range of reported values indicated in brackets.

The yield, composition and efficiency values used in the demonstration of the model (Section 8.3) were used in this section, except where noted.

Table 8-26: Summary of incoming wastewater values used to compare an integrated multi-unit process using different wastewaters

Incoming (Stream A1)	Domestic municipal	Poultry abattoir	Pulp and paper
Liquid component, total flow 1 000 m^3/day			
C (kg/m^3)	0.160	(1.3 – 7.5) 4.4	(0.7 – 1.2) 0.95
N (kg/m^3)	0.050	(0.10 – 0.25) 0.125	(0.0087 (ammonia) + 0.00152 (nitrate)) 0.00711
P (kg/m^3)	0.008	(0.10 – 0.25) 0.125	0.004
Solids component			
Solids (kg/m^3)	0.72	(0.2 – 1.2) 0.7	2.93
C (kg-C/ kg solids)	0.583	0.61	0.715
N (kg-N/ kg solids)	0.157	0.041	0.00735
P (kg-P/ kg solids)	0.04	0.06	0.0023
Reference	Section 8.3	(Molapo, 2009) (Kiepper, et al., 2008)	(Cloete, et al., 2010)
1 000 m^3 is equivalent to:	5 000 people (population equivalent (PE) = 0.2m^3/day)	80 000 birds (fairly large abattoir in SA)	11 450 000 A4 sheets (57 tonnes of office print quality 80 gsm paper)

8.4.2 Poultry abattoir wastewater as feedstock for integrated WWBR simulation

The data used for this example is sourced from Molapo (2009) who considered 34 registered and operating high-throughput poultry abattoirs, of which 26 (76.4%) were visited. In February 2006, 322 registered poultry abattoirs, 176 high-throughput, 67 low-throughput, and 79 rural abattoirs were recorded.

Abattoir solid wastes include condemned meat organs and carcass, bone, feathers and manure, while the solids settled from wastewater, mainly evisceration waste, and wash waste are transferred in wastewater streams. This wastewater normally passes through screens which remove the larger solids either for treatment or final disposal.

The industry has changed from essentially a number of farm-based operations to large commercial producers where economies of scale in rearing and processing have led to a high degree of operational efficiency. Despite legislation governing the management of waste from poultry abattoirs in South Africa, abattoirs still face serious problems of high volumes of waste, characterized by inadequate disposal technologies leading to environmental and public health implications for nearby communities. Waste material is still not being disposed of properly. Ground water is being contaminated, air pollution exists and disposal sites are health hazards to scavengers (Molapo, 2009).

Suitable methods of disposal of solid wastes include burial, incineration, composting, land application, digestion, animal feed, rendering and landfill, but some of these methods are becoming less feasible due to increasing costs and tighter regulations. Complementing existing practices with the WWBR may ease some of these pressures.

Rendering is used in 46% of the plants interviewed in Molapo's study (2009), creating a high-COD malodourous wastewater. A further 8% of plants discharge blood into the municipal system, and 35% bury the blood, showing significant potential for a WWBR system to be implemented. Feathers are valuable and not considered as available to the WWBR approach outlined here, although they may contribute to rendering wastes. Research projects targeting value from feather waste are underway in South Africa.

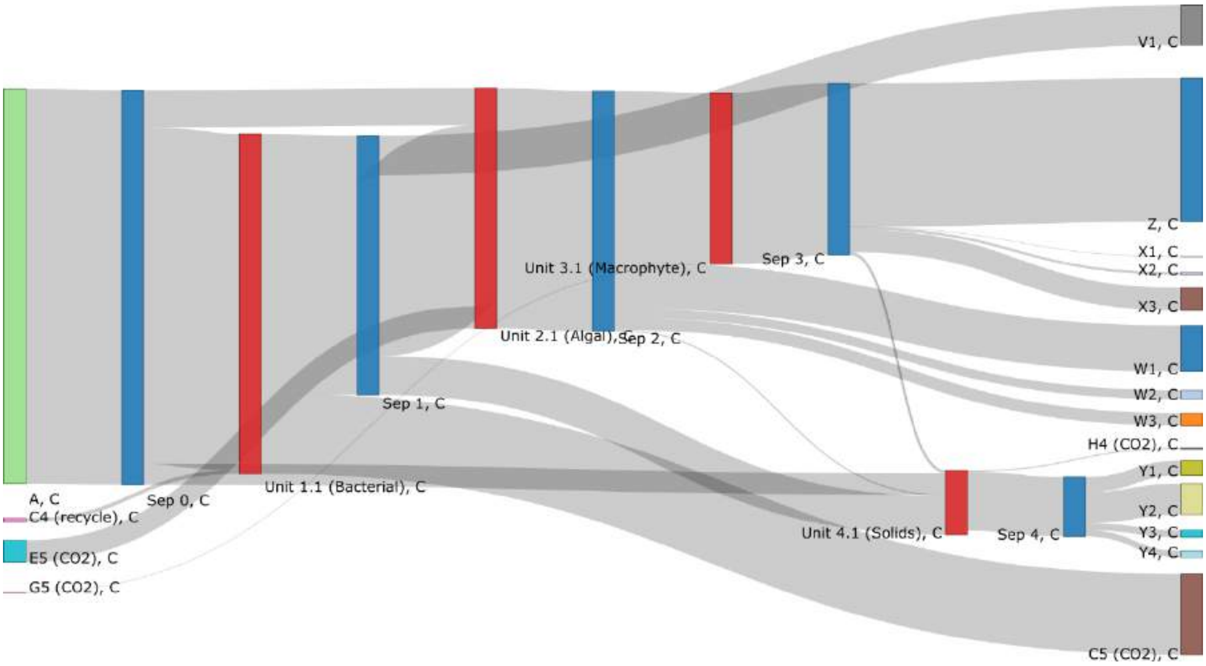


Figure 8-3: Sankey diagram of the carbon mass balance for the simulation of an integrated WWBR using poultry abattoir wastewater as feedstock
Bacterial product V1, Algal high value product W1, Algal lipid product W2, Digestible algal biomass W3, Macrophyte crust, liquor and cake-related products Y1-Y3, Compost Y4. Compliant water Z

Poultry plants today generate a product spectrum including whole birds, cut up parts, deboned meat and other further processed convenience products, which means the waste generated in processing is now more localised to the abattoir, providing opportunities in innovative integrated waste management.

Poultry abattoirs produce considerable amount of condemned meat issue, which is rich in proteins and fats, but unsuitable for human consumption. From a discussion with an industry player, the range of waste products have found use in the industry, but the manure is still a problem.

Poultry abattoirs use about 15 to 20 ℓ water per bird, with about 80 to 85% discharged as wastewater. Surface water used for cleaning, and overflow from e.g. scalding tanks seem to be the biggest factor influencing wastewater treatment in the abattoir, at an average value of 25% of the total water consumption each (Molapo, 2009). This, combined with odour and dust related air pollution suggests that a macrophyte bioreactor might be a suitable main-priority WWBR application in this context, while solids bioreactors may be suitable for the manure (Chen, et al., 2005). Indeed, 42% of abattoirs interviewed in Molapo's study (2009) discharge into a wetland or dam to be used for irrigation.

WWBR in the poultry abattoir context has potential for improved waste management especially in the lower-throughput and rural abattoirs, and ‘backyard industries’.

Table 8-27: Inventory of carbon, nitrogen, phosphorus and water for generic WWBR using poultry abattoir wastewater

Item	Stream Description	C kg/day	N kg/day	P kg/day	W kg/day
Raw, unsettled wastewater A1 to mixing tank	poultry abattoir wastewater	4 827	154	167	994 650
Incoming (total)		4 827	154	167	994 650
CO_2 (total)		733	0	0	0
Rainfall/Evaporation (total)		0	0	0	988
Bacterial product V1		484	14	17	145 763
Algal bioproduct W1		565	65	18	65 065
Algal oil W2		110	0	0	2 594
Algal digestible waste W3		149	7	1	7
Cellulosic fibre X1		0.33	0	0	0.11
Cellulosic biomass X2		31	7	2	0.24
N,P rich sediment X3		277	63	17	0.04
Crust/surface related product stream Y1		183	34	22	18
Liquor related product stream Y2		378	56	2	19 532
Cake-related product stream Y3		85	6	2	157.27
Compost Y4		78	23	18	812
Compliant effluent Z		1 754	-119	69	761 689
Total outgoing		4 827	154	167	995 632
Difference (should be 0)		0	0	0	0
Difference (%)		0	0	0	0
Item	Stream Description	**% C of total**	**% N of total**	**% P of total**	**% Water of total**
Raw, unsettled wastewater A1 to mixing tank	poultry abattoir wastewater	100.00	100.00	100.00	100.00
Incoming (total)		100.00	100.00	100.00	100.00
CO_2 (total)		15.19	0.00	0.00	0.00
Rainfall/Evaporation (total)		0.00	0.00	0.00	0.10
Bacterial product V1		10.03	8.84	9.89	14.64
Algal bioproduct W1		11.70	42.12	10.53	6.54
Algal oil W2		2.28	0.00	0.00	0.26
Algal digestible waste W3		3.09	4.37	0.50	0.00
Cellulosic fibre X1		0.01	0.00	0.00	0.00
Cellulosic biomass X2		0.64	4.53	1.14	0.00
N,P rich sediment X3		5.75	40.75	10.23	0.00
Crust/surface related product stream Y1		3.79	22.13	13.19	0.00
Liquor related product stream Y2		7.83	36.38	1.32	1.96
Cake-related product stream Y3		1.76	3.64	1.34	0.02
Compost Y4		1.61	14.96	10.54	0.08
Compliant effluent Z		36.33	-77.74	41.34	76.50
Difference (should be 0)		0.00	0.00	0.00	0.00

8.4.3 Paper wastewater as feedstock for integrated WWBR simulation

Paper mill wastewater was chosen as separate from pulp because it is more biologically suitable, complex and has more potential for bioremediation, to treat, for example, the deinking byproducts.

The solid waste generated in paper mills consist of rejects, deinking sludge, primary sludge and secondary or biological sludge (Bajpai, 2015).

Rejects are impurities and consist of lumps of fibres, staples and metals from ring binders, sand, glass and plastics and paper constituents as fillers, seizing agents and other chemicals. Rejects also have a relatively low moisture content, significant heating values, are easily dewatered and are, generally, incinerated or disposed of in landfills. Screen rejects have a high content of cellulose fibre.

Deinking sludge contains mainly short fibres or fines, coatings, fillers, ink particles (a potential source of heavy metals), extractive substances and deinking additives. It is normally reused in other industries (e.g. cement, ceramics), or is incinerated, even though it has a poor heating value. Deinking sludge is generated during recycling of paper (except for packaging production). Separation between ink and fibres is driven by a flotation process. The generated deinking sludge contains minerals, ink and cellulose fibres (that are too small to be withheld by filters). This stream is expected to be suitable for PGA production in the bacterial bioreactor.

Primary sludge is generated in the clarification of process water. The sludge consists of mostly fines and fillers and it is relatively easy to dewater. This sludge can be reincorporated into the process for board industry.

Secondary or biological sludge is generated in the clarifier of the biological units of the wastewater treatment. It is either recycled to the product (board industry) or thickened, dewatered and then incinerated or disposed of in landfill. Secondary sludge volumes are lower than those corresponding to the primary sludge. Secondary sludges are often difficult to handle (due to a high microbial protein content). These solids need to be mixed with primary sludge to permit adequate dewatering.

About 40–50 kg of dry sludge is generated in the production of 1 tonne of paper at a paper mill and of that approximately 70 % is primary sludge and 30 % secondary sludge (Bajpai, 2015). Based on the estimates of 50 kg of dry sludge per tonne paper produced, and the production of 57 tonnes of paper per 1000 m^3 of wastewater, a solids concentration of 2.94 kg/m^3 can be calculated. It is assumed that fibre is the only component of the solids fraction. Its composition was estimated based on that of macrophyte biomass N: 0.00735, P: 0.0023 and C: 0.715.

The inventory of C, N, P and water through the integrated WWBR processing paper wastewater is given in Table 8-28. A quarter of the incoming carbon remains in the complaint water with the remainder distributed to macrophyte products (37%), algal products (11%), bacterial products (5%) and compost (5%). As can be seen, the default yield values produce a deficit in the N and P streams, due to the low nutrient content in the paper mill wastewater, and the inability of the model in its current format to adjust for nutrient limitation.

Table 8-28: Inventory of carbon, nitrogen, phosphorus and water for generic WWBR using paper mill wastewater using default values

Item	Stream Description	C kg/day	N kg/day	P kg/day	W kg/day
Raw, unsettled wastewater A1 to mixing tank	paper mill wastewater	3045	29	11	996109
Incoming (total)		3045	29	11	996109
CO_2 (total)		325	0	0	0
Rainfall/Evaporation (total)		0	0	0	1073
Bacterial product V1		131	9	0	62380
Algal bioproduct W1		227	31	4	29823
Algal oil W2		50	0	0	1189
Algal digestible waste W3		68	3	0.38	3
Cellulosic fibre X1		0.37	0	0	0.13
Cellulosic biomass X2		15	3	1	0.27
N,P rich sediment X3		131	30	8	0.04
Crust/surface related product stream Y1		320	19	6	31
Liquor related product stream Y2		671	26	1	48280
Cake-related product stream Y3		147	6	1	275
Compost Y4		128	5	5	1422
Compliant effluent Z		831	-103	-16	853778
Total outgoing		3045	29	11	997182
Difference (should be 0)		0	0.01	0	0
Difference (%)		0	0.02	0	0
Item	**Stream Description**	**% C of total**	**% N of total**	**% P of total**	**% Water of total**
Raw, unsettled wastewater A1 to mixing tank	paper mill wastewater	100.00	100.00	100.00	100.00
Incoming (total)		100.00	100.00	100.00	100.00
CO_2 (total)		10.67	0.00	0.00	0.00
Rainfall/Evaporation (total)		0.00	0.00	0.00	0.11
Bacterial product V1		4.31	32.40	0.81	6.26
Algal bioproduct W1		7.45	108.15	41.50	2.99
Algal oil W2		1.65	0.00	0.00	0.12
Algal digestible waste W3		2.24	10.74	3.54	0.00
Cellulosic fibre X1		0.01	0.01	0.01	0.00
Cellulosic biomass X2		0.48	11.51	8.37	0.00
N,P rich sediment X3		4.32	103.59	75.34	0.00
Crust/surface related product stream Y1		10.51	67.24	58.07	0.00
Liquor related product stream Y2		22.02	91.84	5.81	4.84
Cake-related product stream Y3		4.84	19.49	9.27	0.03
Compost Y4		4.21	15.91	43.00	0.14
Compliant effluent Z		27.28	-360.89	-145.72	85.62
Difference (should be 0)		0.00	0.00	0.00	0.00

To eliminate the nutrient limitation (N and P), the yield values for bacterial biomass and product V1 were required to be reduced by a factor of 8, as indicated in Table 8-29. No adjustments to the other units were made, but it is likely that the algal bioreactor would be omitted altogether in this scenario, and the

VFA interim product directed to methane through anaerobic digestion. The resulting inventory is shown in Table 8-30 and the carbon mass balance is visualised in Figure 8-4. These demonstrate the major importance of the macrophyte products for this wastewater processing system.

Table 8-29: Summary of revised yield values used in generic WWBR for paper mill wastewater

Bioreactor Unit	Conversion value (Y)	
1.1. Bacterial bioreactor	Biomass: V1: Interim Product VFA: CO_2:	0.021 0.015 0.7 – 0.021 – 0.015 = 0.664 0.24
2.1. Algal bioreactor	Not used, splitter 0.2 directs all flow to B1	r_{B1} = 1
3.1. Macrophyte Bioreactor	Biomass: X1: X2: X3: CO_2:	0.000 000 0601 0.000 000 0601 * eff_{X1} 0.000 000 0601 * (1 - eff_{X1}) dependent on process -0.000 000 0601
4.1. Solids Bioreactor	Biomass: Y1: Y2: Y3: Y4: CO_2:	0.028 0.037 0.045 0.4 remainder 0.020

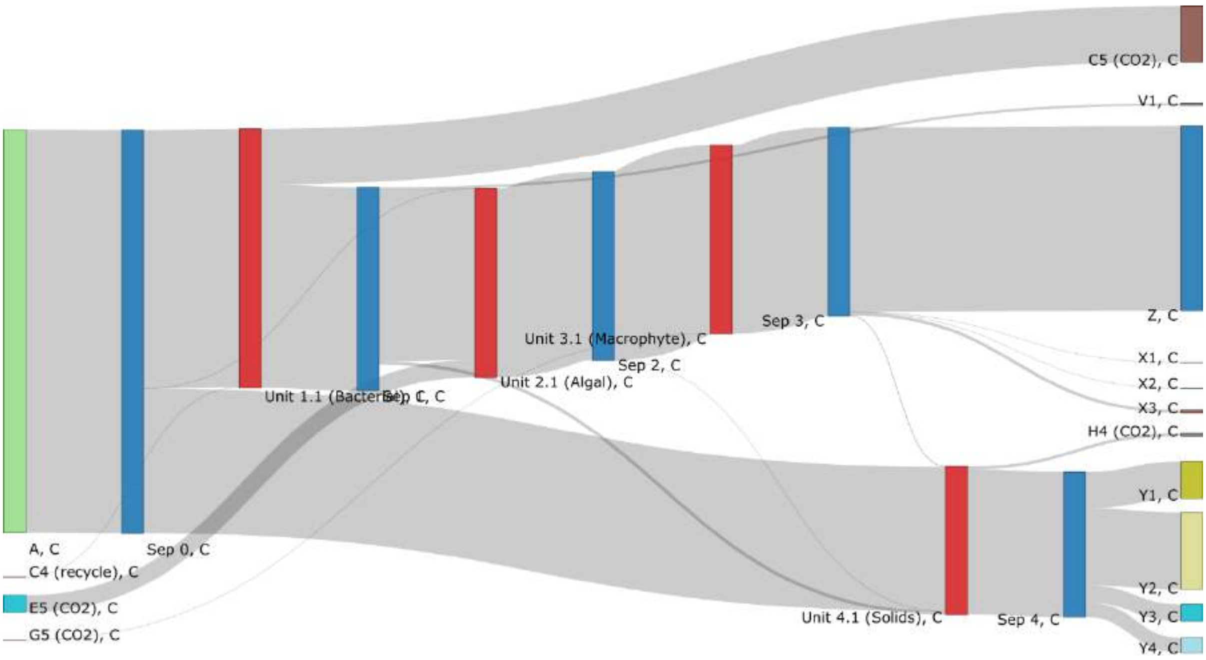


Figure 8-4: Sankey diagram of the carbon mass balance for the simulation of an integrated WWBR using paper mill wastewater as feedstock and revised yield values
Bacterial product V1, Algal high value product W1, Algal lipid product W2, Digestible algal biomass W3, Macrophyte crust, liquor and cake-related products Y1-Y3, Compost Y4. Compliant water Z

Table 8-30: Inventory of carbon, nitrogen, phosphorus and water for generic WWBR using paper mill wastewater using revised values

Item	Stream Description	C kg/day	N kg/day	P kg/day	W kg/day
Raw, unsettled wastewater A1 to mixing tank	paper mill wastewater	3045	29	11	996 109
Incoming (total)		3045	29	11	996 109
CO_2 (total)		444	0	0	0
Rainfall/Evaporation (total)		0	0	0	1 154
Bacterial product V1		19	2	0.16	7 797
Algal bioproduct W1		0	0	0	0
Algal oil W2		0	0	0	0
Algal digestible waste W3		0	0	0	0
Cellulosic fibre X1		0.40	0	0	0.14
Cellulosic biomass X2		3	1	0.16	0.30
N,P rich sediment X3		24	5	1	0.05
Crust/surface related product stream Y1		276	8	2	27
Liquor related product stream Y2		581	8	0.24	44 939
Cake-related product stream Y3		127	4	1	238
Compost Y4		110	-3	2	1 229
Compliant effluent Z		1395	4	4	943 0339
Total outgoing		2979	29	11	997 2639
Difference (should be 0)		66	0	0	0
Difference (%)		2.16	0	0.01	0
Item	**Stream Description**	**% C of total**	**% N of total**	**% P of total**	**% W of total**
Raw, unsettled wastewater A1 to mixing tank	paper mill wastewater	100.00	100.00	100.00	100.00
Incoming (total)		100.00	100.00	100.00	100.00
CO_2 (total)		14.90	0.00	0.00	0.00
Rainfall/Evaporation (total)		0.00	0.00	0.00	0.12
Bacterial product V1		0.65	7.46	1.51	0.78
Algal bioproduct W1		0.00	0.00	0.00	0.00
Algal oil W2		0.00	0.00	0.00	0.00
Algal digestible waste W3		0.00	0.00	0.00	0.00
Cellulosic fibre X1		0.01	0.01	0.01	0.00
Cellulosic biomass X2		0.09	2.07	1.50	0.00
N,P rich sediment X3		0.79	18.58	13.51	0.00
Crust/surface related product stream Y1		9.28	29.16	22.01	0.00
Liquor related product stream Y2		19.50	27.03	2.20	4.51
Cake-related product stream Y3		4.27	13.95	5.48	0.02
Compost Y4		3.68	-11.18	14.33	0.12
Compliant effluent Z		46.83	12.92	39.43	94.56
Difference (should be 0)		0.00	0.00	0.00	0.00

Pulp and paper wastes are very low in N and P. There is some potential for bioproducts to improve the production processes, for example the de-inking process, but due to the high C content and the high energy requirements of the industry, energy-generating activities through incineration and anaerobic digestion are suggested. In this case study, focus on macrophyte production has been used to overcome the nutrient shortage.

Further, while final polishing through macrophyte bioreactors may be achieved as well, irrigation to plantations on site for specialty paper (PR, marketing uses) may be an option and may provide their fertilisation.

PGA is proposed as a suitable WWBR product in this application to be used in-house for heavy metals removal, flocculation, or deinking agent. Its production may be considered using the bacterial production unit or solid state fermentation (SSF).

8.4.4 Remarks on using different wastewaters in an Integrated WWBR

The total products produced by the three wastewater investigated are summarised per 1 000m^3 in Table 8-31 and visually compared in a bar graph in Figure 8-5. In addition, the values have been normalised to 1 000 kg-C/day incoming, as summarised in Table 8-32 and Figure 8-6. While these values are not directly comparable due to the widely differing incoming nutrient loads, it does give an indication of the potential of each wastewater stream. The values were determined by dividing the total C of the product by the C fraction, with the exception of the sediment product X3 and compost product Y4, which was estimated by adding the C,N,P and water amounts, as the composition of these are dependent on the process.

From these graphs, it can be seen that the streams with higher nutrient (N and P) content are more suitable to bacterial and algal production. Carbon rich streams are well suited to energy products.

Table 8-31: Comparison of total amount of each product produced by three wastewater streams investigated, per 1 000m^3 incoming wastewater

kg/day	Domestic municipal wastewater	Poultry wastewater	Paper mill wastewater
Bacterial product V1	33	1042	41
Algal bioproduct W1	60	831	0
Algal oil W2	12	137	0
Algal digestible waste W3	26	287	0
Cellulosic fibre X1	1	0	1
Cellulosic biomass X2	4	43	4
N,P rich sediment X3 *	34	357	30
Crust/surface related product stream Y1	133	393	594
Liquor related product stream Y2	347	1007	1549
Cake-related product stream Y3	81	244	365
Compost Y4 *	318	930	1337
Compliant effluent Z C (mg/L)	0.172	2.302	1.479
Compliant effluent Z N (mg/L)	0.081	-0.157	0.004
Compliant effluent Z P (mg/L)	0.018	0.091	0.004

* estimated through mass balance

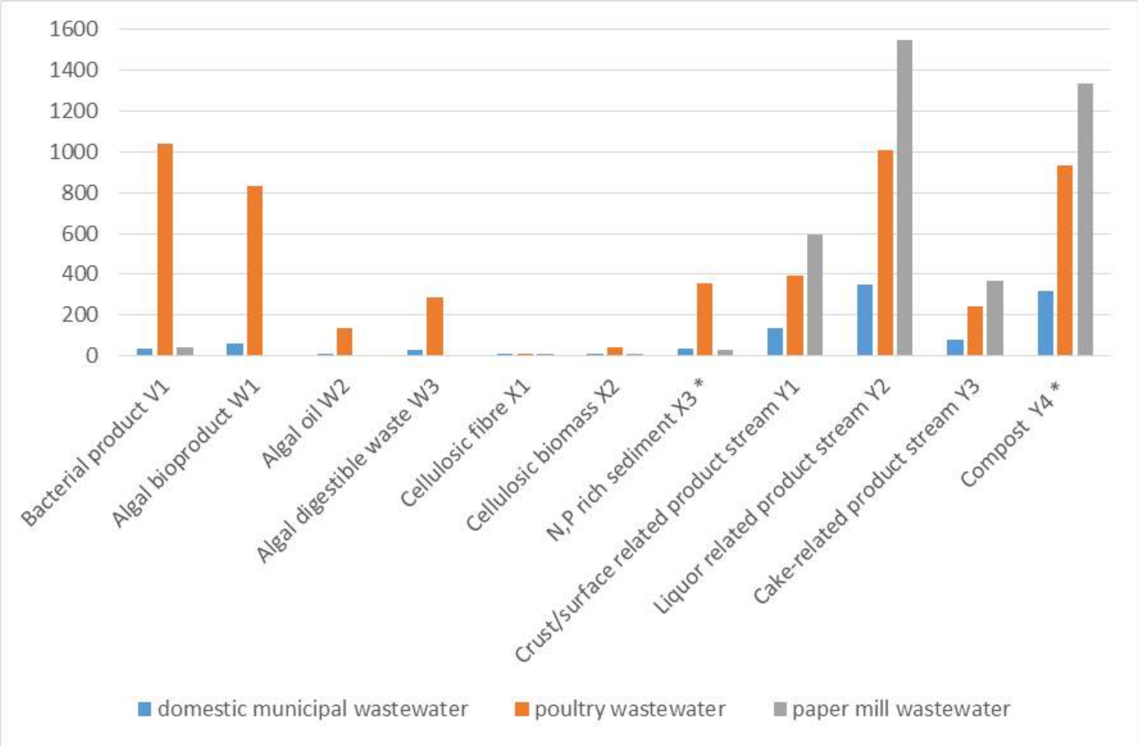


Figure 8-5: Bar graph comparing total amounts of products produced (kg/day) by each wastewater stream investigated, per 1000m^3/day incoming wastewater

Table 8-32: Comparison of total amount of each product produced by three wastewater streams investigated, per 1000 kg-C/day

kg/day	domestic municipal wastewater	poultry wastewater	paper mill wastewater
Bacterial product V1	57	216	14
Algal bioproduct W1	103	172	0
Algal oil W2	21	28	0
Algal digestible waste W3	45	59	0
Cellulosic fibre X1	1	0	0
Cellulosic biomass X2	7	9	1
N,P rich sediment X3 *	59	74	10
Crust/surface related product stream Y1	229	81	195
Liquor related product stream Y2	599	209	509
Cake-related product stream Y3	140	51	120
Compost Y4 *	548	193	440

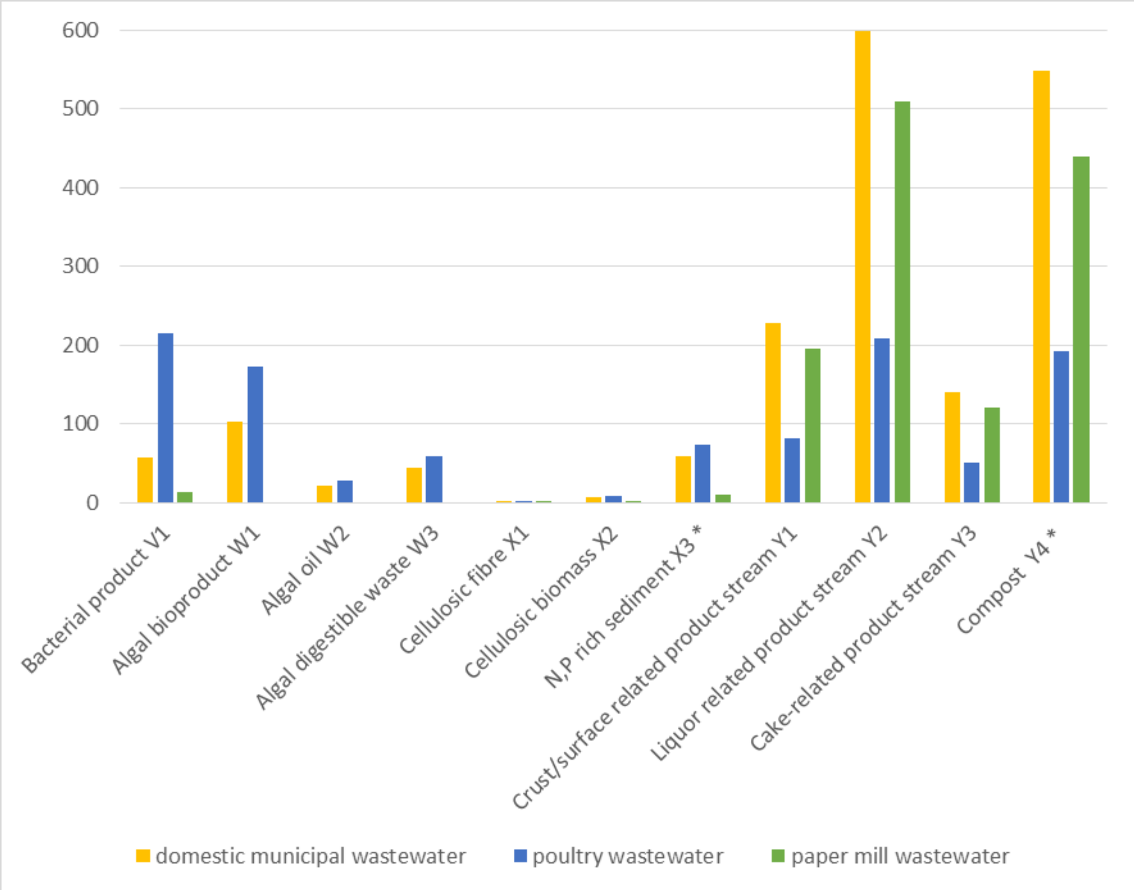


Figure 8-6: Bar graph comparing total amounts of products produced by each wastewater stream investigated, per 1000 kg-C/day incoming substrate

8.5 Future Evaluation of Potential Wastewater Biorefineries

Building on the material balance tool set up in Chapter 7 to describe the integrated wastewater biorefinery flowsheet, the model has been populated with appropriate yields, conversion factors and separation factors across the unit operations included. This has been done by drawing on literature values as well as prior work carried out within the Centre for Bioprocess Engineering Research at the University of Cape Town focused on techno-economic studies and environmental assessment studies, both requiring effective material balance inventories. In all cases, conservative estimates have been made.

Using the calibrated material balance tool, both the unit operations individually and the integrated process can be analysed in terms of the partitioning of incoming C, N and P to the product range of bacterial commodities such as biopolymers, algal products and macrophyte products, as well as compliant water. This nutrient partitioning has been visualised through use of Sankey diagrams, showing the potential of the tool.

In the final stage of the chapter, an initial assessment of different scenarios has been carried out though the modelling of the generic flowsheet containing a bacterial biopolymer reactor, algal reactor, macrophyte reactor and fungal solids reactor. Three differing substrates of varying complexity and nitrogen availability have been investigated. Here the importance of nitrogen for partitioning of carbon to the higher value products has been identified, setting the scene for the ongoing scenario analysis to inform target setting for WWBRs.

9 SOUTH AFRICAN WASTEWATER BIOREFINERIES: CONCEPTUAL APPROACH EMERGING FROM THIS STUDY

Wastewater treatment works are faced with increasing economic and environmental pressure, providing incentive for increased efficiency. This efficiency has largely focused on improved energy efficiency, but improved knowledge about engineering design and the biology involved in nutrient removal has opened up possibilities for efficiency in the nutrient resource cycle to which wastewater contributes to as well. The potential, in kg carbon, nitrogen and phosphorus as raw material for resource recovery from wastewater is massive. Cumulatively, there is 12 750 tonnes of carbon, 325 tonnes of nitrogen and 77 tonnes of phosphorus in the wastewater on record in South Africa released every day (Section 4.1.3). This potential was explored in the previous chapter which investigated different scenarios of utilising wastewater in a WWBR context.

9.1 The WWBR Arena

While a great emphasis should be placed on reducing the amount of resources ending up in waste streams and a reduction in the amount of water directed to waste streams is of paramount importance, the potential of WWBR for reducing the losses in both areas is clear. Even in a future where nutrient and water resources are well managed, the role of WWBR is still a critical one, as a link in the ecosystem to close the nutrient cycles in an integrated system. A strength, weakness, opportunity and threat ('SWOT') analysis for WWBR is shown in Table 9-1.

Table 9-1: SWOT analysis of wastewater biorefineries, adapted from the IEA Bioenergy Task 42 Biorefinery (Fava, 2012)

Strengths of WWBR	Weaknesses of WWBR
• Diversified revenue from wastewater • Contribution to environmental bioremediation of wastewater • Contribution to closing energy and material cycles • Economic incentive to improve overall efficiency • Production of a spectrum of bio-based products (food, feed, materials, chemicals) and bioenergy (fuels, power and/or heat) feeding the full bio-based economy • A bridge between, and building on, agriculture, food, and forestry industries • An alternative to land use for bioproducts (food-feed-fuels nexus)	• Broad, undefined and unclassified area requiring an integrated approach, while being highly site-specific, difficult to work with • Variable volume, quality, concentration, energy-density and composition of water feedstock. • Poor reporting on effluent composition • Multi-dimensional stakeholder engagement required • Developing in parallel to bioeconomy: uncertainty about market trends for new and existing products

Opportunities that could allow growth in WWBR as an industry platform	**Threats to WWBR**
• International consensus that water availability is limited so that the raw materials should be used as efficiently as possible – i.e. development of multi-purpose biorefineries in a framework of scarce raw materials and energy • Strengthening of the economic position of various market sectors (e.g. agriculture, forestry, chemical and energy) due to increased income from products as well as reduced costs due to waste management. • The technology focus on using dilute raw material effectively and an explicit focus on appropriate reactor design for product recovery can contribute to the development of a portfolio of possible products not previously economically feasible (e.g. PGA related products)	• Inability to cross disciplinary divides to build appropriate skillsets • Economic and political instability affecting priority of exploring the WWBR concept • Products from wastewater may have a low market pull • High investment capital for pilot and demo projects • Unfavourable implementation and interpretation of regulations at a local level • Changing water use due to e.g. climate change, water scarcity creates uncertainty about raw material inputs

9.1.1 Interrelating challenges

The main challenge in a WWBR is the diverse and indefinite nature of the wastewater entering the system. There are several ways to embrace this complexity, for example pre-treatment of the water which may include digestion or some form of sterilisation, and dosing with supplementary substrate to complement and improve the wastewater composition, but this does also add complexity and cost to the process. Where possible, this should be limited to substrates sourced in close proximity to the WWBR. Fundamentally, the most critical aspect is appropriate bioreactor design.

A secondary challenge is the potentially competing objectives of producing a regulation compliant effluent water as product, as well as other economically valuable products. Resource recovery is gaining interest globally, however, and is recognised to improve the operational efficiency of waste treatment facilities in addition to producing products of value. Considerate plant management is key to the success of the WWBR along with appropriate regulation and its interpretation, buy-in from stakeholders like upstream wastewater generators, government and members of the public potentially affected by the effective industrialisation of wastewater,.

These two challenges interrelate. While the technologies to address aspects of bioprocessing, wastewater treatment and resource recovery already exist in isolation, little knowledge is yet available about how they integrate, and little to no commercial scale integration exists. The feasibility model demonstrated in Chapter 8 facilitates early-stage investigation into the interaction of different bioreactors. The next step is to test the assumptions inherent in the model at laboratory and pilot scale.

9.1.2 Industry players

Industrial wastewaters may already be utilised to improve water and energy efficiency where feasible, and this may create an opportunity cost to implementing a WWBR. In contrast, the perceived effort and a lack of the trust required to build industrial ecologies to create WWBRs may negatively impact moving forward. However, there are some industry players who are already open to investigation of resource recovery or even more fundamental biorefinery concepts. The attractiveness may be lie in a combination of factors, such as biologically suitable waste streams, problematic waste streams, an innovative industry culture, particularly a desire to be part of the emerging bioeconomy, or a need to find new revenue streams. These are the enterprises and individuals who should be part of the development of WWBR in South Africa.

These organisations can be grouped as either part of a large or niche industry, and as being present as a large or small entity. Niche industries may be more interested in higher value, lower volume products and are likely to be more agile in entering new markets and adapting their processes. Niche products may also benefit from industrial ecosystems through sharing distribution and logistics challenges through for example cooperatives. Larger concerns are often highly price competitive, and their main driver may be reducing costs. Large companies in either of these industry groupings may have more bureaucracy and innovation may struggle to find expression, while small companies may be more responsive in adapting and exploring processes to suit their needs. These are general trends and individual companies may not fit the generalisations. There may also be a number of smaller companies who are very active in the WWBR context but are difficult to identify as obtaining reliable information is problematic, not least because there are no standardised keywords or terms to use in searches.

9.1.3 Early stage decision making

The following decision making matrix (Figure 9-1) is a very early stage attempt at facilitating choices when considering a WWBR using a specific waste stream. While it is suggested to have most, if not all of the units present for a resilient system, only one unit is likely to be optimised for bioproduct productivity. This heuristic process is intended to be a guideline only, to be further developed as more information becomes available, and for each specific scenario.

The question of desired product develops in parallel, and iteratively with the decision making matrix, and can force a decision if a product can only be produced by, for example, an algal bioreactor.

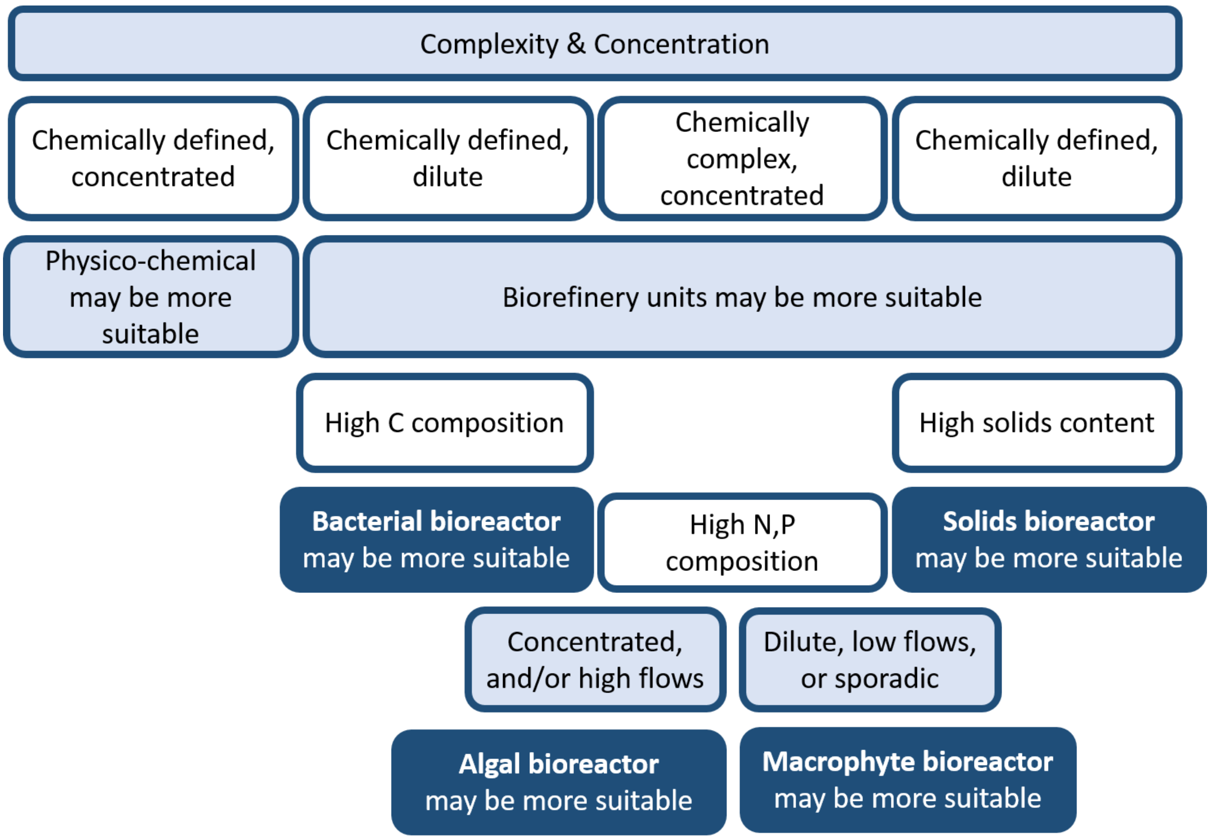


Figure 9-1: Decision making matrix to guide selection of priority bioreactor

Table 9-2 is a qualitative comparison of the aspects at play if a stakeholder has a waste stream and needs to make a decision on a product group based on the capital and operational investment required. This could be the case where a utility is considering entering the WWBR space but is coming from a culture of risk management and service delivery, or where an existing development stands to be renovated.

Table 9-2: Comparison sheet for main priority reactor unit selection in WWBR

Category	Bacterial bioreactor	Algal bioreactor	Macrophyte bioreactor	Solids bioreactor
Maintenance	4	3	1	2
Operational cost	3	4	2	1
Operational effort	4	3	1	2
Operational skill required	3	4	1	2
Capital investment required per m^3 inflow	2	4	3	1
Space requirement per m^3 inflow	1	3	4	1

9.2 The Future of the WWBR

Going forward, effort is required in three broad areas: analysis, research and cooperation. Wastewater needs to be characterised in more detail and reported with a greater frequency. Industry players are encouraged to share wastewater samples, wastewater composition, general input and pilot scale versions of their technologies with research institutions to facilitate integrated solutions that can scale. Research needs to be directed towards testing existing technologies with appropriate detailed analysis, in integrated systems at pilot scale. Scientific research on promising products should to be adapted where needed, and developed into engineering detail, including for example, yields in non-sterile systems with ecological selection, productivities and product recovery studies. Equally important, these companies and groups need to share their work, to excite the public and gain the interest of niche industries who can help grow the circular economy. Because this is a complex, interrelating framework, a continuous serving of bite-size, and well-crafted publications over an extended time period, which make full use of social media, have a better engagement than singular scientific reports alone.

9.2.1 Better wastewater analysis and characterisation

One of the key hurdles recognised in the implementation of industrial ecology is knowledge brokering with respect to waste streams. Although information was obtained from numerous companies in the industrial, food and beverage, mining and electricity generation sectors in South Africa in the study by Cloete, et al. (2010), the majority of the companies contacted did not perform analyses for the full spectrum of hazardous substances in the effluent.

From a pilot water disclosure project (CDP, 2009) key findings included that most companies have information on their direct water usage, but most companies do not have data on water use or water issues in their supply chain, and while many companies have a water management plan, it is only for their own plants. Wastewater was not even explicitly mentioned, but the majority of the respondents identified water as an opportunity.

At time of writing there is still no system in place to regulate the level of detail that metropolitan councils should go into in obtaining information on effluent production, even for conventional wastewater treatment (especially with regard to chemical composition) and as a result, the data obtainable from metropolitan councils is inconsistent and comparisons are not always possible (WRC SA, 2015; Cloete, et al., 2010). Some leading examples, such as the EThekwini Municipality, collect excellent data that can form a starting point for these investigations (Mabeer, 2015).

In order to investigate potential products from wastewater to contribute to the circular economy, a better understanding of the wastewater space and the inventory of potential raw materials is required. Focus on water as a “fit for purpose” key product is paramount. This requires better quantification of wastewater generation and understanding of the potential for reduced volumes and increased concentrations with increasingly water-wise processing, to better predict future wastewater volumes, composition and resource recovery potential. The analysis and characterisation falls in a hierarchy of groupings, as illustrated in Table 9-3.

Table 9-3: Category of analysis groupings in order of priority for WWBR

Category	Parameters
Fundamental parameters	Total Carbon Total Nitrogen Total Phosphorous COD Total Solids Content (TS) Suspended Solids Content (TSS)
Factors affecting biological growth	pH Conductivity Heavy metals Toxins Chemically reactive inorganics Recalcitrant organics (e.g. phenols) FOG Detergents
Substrate Quality	Substrate characterisation (e.g. simple sugars, VFAs, cellulose) Presence of micronutrients, essential amino acids

From a qualitative perspective process considerations can inform what is possible. For example waters with high oil content may be well suited to produce bacterial stress products, e.g. surfactants. Pretreatment may improve the internal robustness of the process, through improving the substrate quality, or to produce, for example, bioenergy. As the incoming substrates are analysed and characterised, the preferred ‘major product’ may change as all things are considered. Water is a major product and may be the only one that exits the system as a whole, but this would still be a WWBR with the other products improving the internal economics. There should be a focus on higher value bioproducts as these may bring the greatest amount of economic benefit, but the rest of the products possible should not be ignored, as these can favourably affect the overall economic feasibility. Even if these products are only used to support the internal process, this still contributes to make the overall process more robust.

9.2.2 Pilot scale integrated systems

Although the main purpose of pilot studies is to contribute technical knowledge of the integrated system, they also provide an opportunity to explore the methodology of determining wastewater data with the industries producing them, and establishing a standard for reporting wastewater that would be useful for WWBR. The pilot studies have as much of a social acceptance function as a technical one, and industry champions already active in the wastewater resource recovery sector should be encouraged to lead the charge. As such, the type of pilot systems to be studied depend on the champions willing to engage.

9.2.3 Cooperation across sectors

Desrochers (2001) opines that the most famous example of industrial ecology, Kulenborg Eco-Industrial Park in Denmark, was not a planned synergy, but rather evolved with time. The author concludes that development of an institutional framework that forces firms to internalise their externalities (by enforcing environmental regulation, for example), while leaving them the necessary freedom to develop new and profitable uses for by-products, should be given higher priority than the planning of localised industrial symbiosis. In this regard, in South Africa, there seems to be a conflicting mix of enabling and obstructing factors in attempts at creating these frameworks. Some of these perceptions are listed in Table 9-4.

Table 9-4: Factors influencing the viability of wastewater biorefineries

Enabling factors	Obstructing factors
Existing thinking considering co-siting wastewater treatment works with organic waste management, can improve logistics for industrial partnerships (e.g. Athlone solid waste management complex) (Coetzee, 2012)	Regulations may be inadequate (but not prohibitive) – e.g. classifying streams as waste legally limits their use/beneficiation
Environmental impetus to improve water quality	Poor quantification of wastewaters. A government driven system is required to regulate the level of detail that metropolitan councils should go to in obtaining information on effluent production (especially with regard to chemical composition)
Economic impetus from industry to reuse water and reduce cost of disposal	In cases of adequate regulation, interpretation by authorities may still inhibit optimal use
New biological reactor designs focusing on ecological niche enable novel routes to biological products – e.g. Nereda system and work done at University of Cape Town on phosphate and nitrogen handling	Current reactor design in existing plants may require retrofitting, current operator understanding may be inadequate
Greater focus on holistic thinking and water sensitive urban design provide potential for better integration between stakeholders.	Resistance to change in an bureaucratic environment
Greater market push for biodegradable and more environmentally friendly products provide a market demand for (biological) products from waste streams	A prevalent misunderstanding of the real market needs from the industries targeted for uptake of the products from wastewater
Environmental impetus to develop the industrial ecology and circular economy	An unrealistic expectation of the real price obtainable from intermediate products by the producer (versus the advertised price obtainable for the finished products)
	An unrealistic expectation of the purity obtainable from products from wastewater – unrealistically expecting these to reach a similar price to highly pure product equivalent
	Application/Market reach of products from wastewater may be limited due to health or religious concerns. Food applications are out of reach. These are unlikely to change even as public perception and acceptance improves.
The large amounts of biomass currently not being adequately processed before export, combined with often limited existing infrastructure that would otherwise represent an opportunity cost, while considered typical of Africa, may represent a niche opportunity that suit small and medium enterprises well	Limited understanding of how significant the impact of logistics is on realising product to market. e.g. logistics is not the core business of the industry producing the wastewater and/or producing the product, thus a logistics partner needs to be found, with concomitant costs and challenges (aka having to manage yet another partner)
The typical decentralised nature of waste and highly site specific requirements present a block for companies who need to operate at large scale with high efficiency, but presents an opportunity to smaller entrepreneurs.	

There is a perception among, especially small-scale, entrepreneurs that government policies on waste beneficiation are prohibitive. It does need to be acknowledged that government needs to manage the risk and health of the entire population, which includes its most vulnerable members. To improve policies (where needed), the interpretation of these policies and the perception of all stakeholders involved, government at all levels should be involved in the pilot integrated studies, voicing the risks and concerns from the design stages to manage these risks iteratively, and the onus on getting them involved is the responsibility of the people doing the study, be it academic or industrial researchers.

In this project, the City of Cape Town, including Mr Kevin Samson, the Manager for the Wastewater, Water and Sanitation Department, Wastewater Branch, Mr Barry Coetzee, the Manager for the Technical Strategic Support Utility Services Directorate and the Athlone wastewater treatment works, including Mr Michael Toll, were involved at various levels. They were responsive to emails and honest in their dialogue, which allowed key concerns to be incorporated, or at least, acknowledged early on in the project.

The importance of industrial bodies cannot be overstated, and these include those not directly related to WWBR. The Water Institute for South Africa (WISA) have periodic meetings, where the work could be presented, and more valuably, informally discussed with experts in industry. The African Utility Week (AUW) allowed access to several industry groupings, and the discussions with the solid waste management industry stakeholders proved relevant. The Water Research Commission's (WRC) assigned steering committee, consisting of researchers and industry stakeholders from across the country, is invaluable both for the members' direct contribution of their immense knowledge and experience, but also as a way to speedily transfer knowledge between the various research groupings represented, the research project in question and industry. These are only some examples of industry links.

For the WWBR to succeed, these networks need to be nurtured in formal and informal ways, small gains and challenges need to be continually shared. This goes further than creating another industry body, but relies on many, varied links in a healthy social ecosystem. In short, we need to play.

10 CONCLUSIONS AND RECOMMENDATIONS

Value from Waste – recognising the tension between productivity and remediation

In addressing the growing needs of a world population, growing in both number and affluence, as well as the associated growth in environmental burden associated with waste assimilation, new thinking is required to address both waste treatment and resource productivity. Strongly emerging themes are those of valorisation of waste as well as waste minimisation i.e. use the value of the resource to its full potential before classifying any part of it as waste. Application of this thinking is allowing early delivery examples to be emerging globally, based on both industrial ecology and the application of second and third generation biorefineries (Section 2.2). Application of this thinking to wastewaters creates a tension in the approach. This tension centres around the relative prioritisation of delivery of clean water and the effective utilisation of the organic loading, N, P and heat within the wastewater, to name a few, with the associated maximisation of productivity towards the selected product(s).

Towards the Wastewater Biorefinery

The development of the wastewater biorefinery concept (Section 2.3) facilitates the use of multiple unit operations to allow simultaneous multi-criteria optimisation within the overall system. To develop this wastewater biorefinery to reach its potential requires the integration of learnings from conventional wastewater treatment processes, bioprocess technology and environmental biotechnology towards implementing the principles of the circular economy, as well as process systems engineering for system optimisation.

The biorefinery concept has developed from its initial approach centred on woody biomass for largely energy-related products. The second generation biorefinery concept extended the focus to multiple products while the third generation biorefinery allows for variation in both feedstocks and major product(s) to meet varying needs for feedstock treatment and varying product demands (Section 2.2.3). The developing wastewater biorefinery concept meets the third generation biorefinery approach. The wastewater biorefinery concept was launched in 2008 (Section 2.4.1). It has growing interest with six major research groups in Europe focussed on its implementation (Section 2.4.3) and the first commercial applications emerging in Europe (Section 2.4.2). Global application has yet to be seen. In this report, we focus on refinement of the wastewater biorefinery (WWBR) concept, identification of its guiding principles and constraints, the challenges of its implementation and its applicability to South Africa.

Towards the Wastewater Biorefinery in South Africa

In South Africa, the Water Research Commission has championed substantial research into wastewater treatment (Section 2.5). While much of it is focussed on removal of pollutants alone or on cleaner production, a number of example projects do provide research on which to build the wastewater biorefinery concept (Section 2.5.6). Specifically, in systems implemented for the combined treatment of wastewaters towards clean water with simultaneous value addition in South Africa, only biogas projects have been implemented for value generation (Section 2.7), with examples of integrated water treatment and biogas generation towards heat, electricity or steam including systems using both municipal wastewater and industrial wastewaters as feedstock. The former include the Johannesburg municipality (2.7.3) while the latter extend from large scale anaerobic digestion of petrochemical wastewaters at Sasol sites and PetroSA, application on the larger breweries within the SABMiller for steam generation, treatment of abattoir effluent through to small scale anaerobic digesters distributed across a range of wastewaters (2.7.2). It is suggested that the use of anaerobic digestion for water treatment with biogas generation for conversion to electricity is under-reported in South Africa and is driven by increasing electricity prices and insecurity of electricity supply. Increasingly, research projects are seeking to extend the product spectrum from wastewater treatment beyond clean water and energy, with pilot studies being implemented on the production of algae from wastewater, production of elemental sulphur from acid mine drainage and the implementation of wetlands.

In South Africa, there are competing tensions on the implementation of WWBR for simultaneous water treatment and value creation. On the one hand, a lack of skilled personnel in the wastewater treatment arena demands the implementation of simple and robust technologies. On the other hand, the simultaneous treatment of waste with value creation can generate the resources required to sustain the treatment facilities (Section 3.1.1). Further, developing an integrated approach with the combined potential for wealth creation, upskilling and job creation in a region or community can motivate for its efficient operation by that region or community, prioritising it over the less tangible water treatment. The current low compliance in terms of wastewater treatment in South Africa makes it a target for investment (Section 3.1.2), providing opportunity for implementation of new approaches. The potential for value generation may assist to motivate the investment. Further it may drive the efficient operation of the facility to maximise value (Section 3.1.1). An additional current driver in South Africa is the growing water scarcity, necessitating new approaches to increasing available water and its governance. Sustainable water treatment services thus become a necessity to ensure water availability. This may benefit from new financing models, including public private partnerships (PPP) and other opportunities to bridge the current funding gap (Section 3.1.3).

The WWBR strives towards zero waste by valorising elements of the wastewater stream through maximising nutrient re-use and recycling through the generation of bio-based products and energy while ensuring the compliance of the resultant water stream (Section 2.3). To further evaluate the potential for WWBR in South Africa, a review of the nature of the wastewater feedstock in South Africa is presented (Section 4.1). This is followed by a rudimentary inventory of WW resources across a number of key sectors in South Africa (Sections 4.2 and 4.3). A discussion on products of interest has been presented (Chapter 5) with two polymeric commodity bioproducts discussed in more detail (Section 5.4). Together, these position the applicability of the WWBR. This has been followed by an evaluation of key criteria of the WWBR, requirements of the WWBR reactor systems (Chapter 6) and the generation and analysis of the integrated WWBR flowsheet (Section 7.1).

South Africa's wastewater feedstocks

Review of South Africa's wastewaters has demonstrated a considerable resource value. To utilise this, an inventory of the available resources is required. A number of attempts at this data collection have been made; however, it remains incomplete and much of it is dated. The data on waste streams tends to be reluctantly communicated by industry. A slow response to carbon disclosures has been evident through the Carbon Disclosure Project of 2015 and illustrates the major challenge around information brokering so essential for effective implementation of industrial ecology of which wastewater biorefineries are a subset. Further, the level of data collection presented in the reports is fragmented and incomplete.

The importance of characterising the available waste streams in terms of their volume, concentration, overall inventory and complexity is essential (Section 3.2.2). The relative availability of carbon, nitrogen and phosphorus, as well as stream complexity will drive the applicable uses of each stream. Further to this, stream variability in composition and volume, as well as seasonality is key in informing application for each stream. Through an initial data collection exercise, significant carbon availability has been estimated across the following industries (million tonne per annum): municipal wastewater 4.6 (Section 4.2), dairy industry 3.9 (Section 4.3.3), petroleum industry 1.8 (Section 4.3.2), pulp and paper industry 1.0 (Section 4.3.1) and edible oil 0.5 (Section 0) with high associated nitrogen availability in the following streams (in descending order): municipal wastewaters and, pulp and paper, dairy, abattoir, beverage industry wastewaters. This estimation is given in Chapter 4 with detail provided in Appendix C.

A common reporting framework is not in place, at the level of metropolitan councils or any other entity, resulting in incomplete data reported in inconsistent units etc. Discrete examples of state-of-the-art data collection such as that conducted by EThekwini Municipality (Appendix section C.3) demonstrate the value of such data. Development of such a reporting framework will be valuable as will be its population with up-to-date data with associated geographical details. This will both allow categorisation of wastes in terms of their complexity, concentration and volume as well as their potential for use within a location. In the collection of these data, cognisance of the dual approach i.e. both water treatment and product creation, is required. It is suggested that a careful definition of required dataset be drawn up and the form of data be specified. For example, for the purposes of WWBR determination of the available carbon in terms of elemental carbon is much more valuable than collecting the information as chemical oxygen demand (COD) (Sections 4.1.2 and 7.3.3). It is anticipated that much of this required up-to-date data will come available through the NatSurv Reports currently being compiled (Section 4.1.1).

Potential products from the wastewater biorefinery

With such an inventory available, potential exists to match products and appropriate technologies to the treatment of raw materials. The product spectrum considered should ideally be informed by the market pull and can be informed by the DST's current studies on bio-based products for South Africa's bioeconomy.

A number of factors inform product selection (Section 3.3). In the first instance, a demand for the product(s) selected is essential. It is most preferable for the product to find application within the sector from which the waste is generated, linking the market demand to the waste produced. Where this is not possible, a market within the geographic region is preferred. Secondly, where large volume wastewaters are treated, the production of commodity products, able to fully utilise the nutrient resource, are favoured owing to the competing requirements for products of value and clean water. Further, it is not desirable to target high purity products from waste feedstocks, further supporting commodity products. Finally, separation of the product from the, often dilute, wastewater stream is required. For this, products reporting to a phase other than the aqueous phase are preferred.

WWBRs incorporate multiple unit operations to ensure removal of all nutrients and the combined optimisation of multiple products, not possible from a single unit operation. Hence products must be selected to address removal of each set of nutrients. While the juxtaposition of these products and their integration through the unit operations of the WWBR has been considered in later chapters, in Chapter 5 an analysis of a number of potential products to be produced from the WWBR is presented, with initial focus on the carbon rich product. This product spectrum aligns well with those highlighted with potential for the bio-based economy, both in South Africa and abroad, including platform chemicals, bio-based plastics and polymers, biomaterials, biosurfactants, biolubricants, biosolvents, enzymes, organic acids and amino acids, animal and aqua-feeds, soil improvers and bioenergy products. Biopolymers, such as the bioplastics PLA and PHA (Section 5.4.1) as well as PGA (Section 5.4.2) used as a flocculant, for metal removal and for water retention, have been highlighted as products of interest.

Integrating bioreactor design and the wastewater biorefinery flowsheet

Through focus on the first reactor in the WWBR process flowsheet, we have explored key requirements of the bioreactor design in the WWBR (Chapter 6). The nature of the feedstock as typically dilute and potentially complex places a requirement on the bioreactor for decoupling of the biomass retention and hydraulic retention times (Section 6.1). By retention of the biomass within the reactor (increasingly important with decreasing feedstock concentration), a critical biocatalyst concentration can be achieved to allow rapid passage of the feedstock through the reactor with efficient conversion. This is augmented where the biological phase has an ecological niche. Together these remove the need for sterilisation; owing to the large flows, it is essential that no sterilisation is required to attain a robust process. De-coupling of biomass and hydraulic retention times allows continuous, or semi-continuous, operation to be ensured as wastewaters cannot be stored. As discussed above, the bioreactor system must be designed for product recovery (Section 6.1.3). Most typically, it is desirable to achieve this by partitioning of the product into a phase other than the aqueous phase, eliminating the need for the separation from the large volume aqueous phase. Thus the ease of product recovery should be considered in an integrated manner with bioreactor design.

The bioreactor selection for the WWBR is proposed to meet the above requirement whilst being drawn from those reactors already used in WWT to ensure familiarity for the operating staff and potential to retrofit the reactors (Section 6.2). Activated sludge, biological nutrient removal, packed bed, fluidised bed, trickle bed membrane, moving bed and aerobic granular sludge bioreactors were considered along with the rotating bed contactor. These were assessed against the above criteria, leading to the shortlisting of the rotating bed contactor, aerobic granulated sludge reactor and moving bed bioreactor as the most promising in terms of potential for biomass retention and product removal, listed in order of ascending preference (Section 6.4). Associated with this study, experimental investigation of the moving bed bioreactor (Appendix E) for the production of the product polyglutamic acid (Appendix D) has been initiated.

As an illustration of the WWBR, a flowsheet (Section 7.1.1) has been compiled through this study comprised of 1) solid liquid separation, 2) a bioreactor for the reduction of the organic load with simultaneous product of a polymer product, typically using a bacterial (or yeast or fungal) system (Section 7.4), 3) an algal bioreactor system for removal of trace organics, N and P (Section 7.5.1), 4) a macrophyte bioreactor for polishing of the water with respect to N and P (Section 7.5.2), and 5) a solids bioreactor (typically fungal) utilising solid state fermentation to handle the sludge (7.5.3). Potential exists to add and subtract units e.g. add an anaerobic digester for production of electricity, heat or both. Potential also exists for multiple units in each of these categories. From this generic flowsheet, the product spectrum includes the microbial bioproduct such as the biopolymer, algal oil, algal bioproduct, macrophyte fibre and biomass, compost, sludge products, bioenergy and clean water. A simplified but integrated material balance model has been established using this generic flowsheet (Chapter 7). It has been populated with typical performance data for these biological systems (Section 8.1). The generic flowsheet model forms the key tool for the exploring of WWBR scenarios to investigate the potential of this approach.

Exploring the wastewater biorefinery flowsheet through selected example processes

The generic flowsheet and material balance model assembled in this study provide a useful tool for the analysis of the performance potential of the wastewater biorefinery. Through its demonstration in terms of the bacterial bioreactor for the production of the biodegradable plastic PHA from confectionery wastewater (Section 8.2), its usefulness and potential for refinement has been demonstrated. Further flow visualisation using the Sankey diagrams is demonstrated. The use of elemental compositions of the wastewater in terms of C, N and P is preferred over the electron balance approach of COD, owing to the need for substantial additional information for the use of the latter in the material balance. The need to simultaneously optimise the compliance of the outgoing water stream and the productivities of desired products drives the motivation for the integration of multiple unit operations.

An integrated WWBR approach is demonstrated for the treatment of municipal wastewater with the generation of the polymer PGA, algal products, macrophyte products and fungal products (Section 8.3). Low productivity of the macrophyte reactor compared with the algal reactor system suggests the need for scenario analysis around their relative contribution. Further potential exists for refinement of effluent compliance, with scenario analysis proposed to address this. In the final demonstration of the material balance model, the performance of the WWBR is compared on use of different wastewater streams with differing nutrient provision (Section 8.4). The municipal wastewater was compared to a nitrogen rich poultry abattoir effluent and to a pulp and paper wastewater. The proportions of products of interest are altered to meet the differing elemental loads and concentrations within the wastewaters, demonstrating the flexibility of the WWBR. The availability of nitrogen in the wastewater favoured the bacterial and algal products while in the presence of excess carbon, more carbon reported to the lower value products. There is substantial potential to refine this partitioning through an improved understanding of the system. This can be facilitated through scenario analysis using the material balance tool.

Re-visiting and refining the wastewater biorefinery concept

Through characterisation of a range of wastewaters in South Africa, the significance of South African wastewaters as a resource for bio-based products is evident, with in excess of 12 750 tonne C, 325 tonne N and 77 tonne P available per day from the wastewaters reviewed. While a key focus of the process industries and of society is to reduce the waste streams formed, both in terms of water and organic components of the waste, the ongoing prominence of waste streams is clear. This accentuates the need for the closure of water and nutrient cycles, both to maximise resource productivity and to address water scarcity in nations such as South Africa. Through this, the importance and potential of wastewater biorefineries is highlighted.

In order to realise the potential benefit of these waste streams in terms of both water and bioproducts, the challenge presented by these wastewaters must be acknowledged. This includes the complexity and variability of many waste streams. Their effective use requires rigorous analysis and characterisation of the wastewater streams as well as the effective communication of this information. Further, information on the magnitude of the resource and its complexity on a geographic basis is necessary for the application of the WWBR concept.

Based on the resources available, meta-research on products of interest, their market demand and suitability and their production systems through microbial, algal or plant systems is required. In this analysis, the relevant bioreactor design for application in the WWBR, addressing the provision of a niche environment for desired biocatalysts to avoid sterilisation is required. Further, bioreactor design should address the de-coupling of hydraulic and biomass residence times as well as design for product recovery, preferably into a different phase. The success of this approach stands to benefit from the integration of traditional bioprocess engineering approaches and environmental bioprocess approaches used in remediation systems. The application of these bioreactors and associated product systems require demonstration at both laboratory and pilot scale, as unit operations and as integrated systems.

Pilot scale demonstration of integrated systems is required for the validation from a technical perspective both of the unit operations and of the integration of the complex processes. Through these data, the meeting of the dual aims of achieving compliance of water for re-use and closing nutrient cycles to enhance resource productivity can be considered. Further, the social value of the system requires demonstration, contributing to the acceptance and desirability of the WWBR approach. Such holistic communication leads to cooperation and incentivisation of investment, as well as social acceptability.

Recommendations

The potential of wastewater biorefineries in South Africa is clearly demonstrated through this study. This is seen through the availability of a substantial feedstock with potential for bioconversion, the significant capacity for value addition, the opportunity for focus on innovation in water treatment and the potential for improved performance in water treatment and standards compliance through the incentivisation through value addition inherent to the WWBR. In addition to drawing attention to this potential, it is recognised that considerable development of the concept is required to facilitate its application. In this section, a number of areas for further work are highlighted with accompanying recommendations.

The review of WWBRs worldwide has illustrated that this concept is nascent globally and that South Africa is well positioned to contribute substantially. South Africa has a well-developed research community on water treatment and it is recommended that a number of aspects of completed research can be harnessed towards the WWBR by the continued engaging of our research capability and the development of consortia. Further, owing to the investment currently required in our WWT industry even in terms of traditional treatment options, it is timely to integrate treatment with valorisation with the aim that simultaneous treatment and value creation may incentivise compliance. The simultaneous quality water treatment and production of products of value may thrust South Africa and its infrastructure providers into the forward thinking arena of the circular economy.

The following specific recommendations are made:

1. It is proposed that a framework for data collection be compiled and an improved inventory across the industry be gathered, as the knowledge base on South African wastewaters was incomplete at the time of compiling this report. In this inventory, information on volume, concentration and complexity of the wastewater should be reported. Further, for material balancing, it is proposed that the basis of elemental composition is used and that geographic information is incorporated, in addition to industry averages.

2. It is suggested that a rigorous set of preferred products be identified that are robust and suitable for production from specified waste resources, through the integration of current research on the preferred bioproducts for South Africa's bio-based economy and the development of the inventories proposed above. This selection should be informed by market research and a clear understanding of the customer for the product. A distinct advantage of developing products for use in the same industry from whence the waste came is recognised, securing product market.

3. It is suggested that targeted research on the relevant product spectrum from the solids and macrophyte bioreactor systems be conducted, with a specific focus on indigenous species and consortia. Limited research has been conducted on these unit operations, and they form a key requirement for water compliance.

4. In this study, focus has been placed on the bacterial bioreactor design for the WWBR. Testing is required in the laboratory and on the plant for implementation of the concepts proposed for these bacterial bioreactors.

5. The bioreactor design studies conducted as part of this project should be extended to the other bioreactor units: algal bioreactors, macrophyte bioreactors, sludge digesters and solid state fermentation bioreactors for sludge utilisation.

6. The flow-sheet approach and material balance model provides a good framework for analysis of varied scenarios for value creation from waste using the WWBR. Analysis of the current scenarios suggests value in process refinement to enable a larger partitioning of the major resources to the products of most value. This refinement should be undertaken and the material balancing tool applied to varied scenarios. Through refinement of the material balancing tool and of the process flow sheets proposed, progress towards this goal will be achieved. Following the refinement of the flowsheeting and model, its further use in scenario analysis is proposed to identify the most promising approaches for further study.

7. It is proposed that the identification of promising wastewater streams for resource recovery using the rudimentary inventory presented in Chapter 4 be undertaken. These are recommended to form the subject of case studies around which to further develop the thinking behind, technology supporting and implementation of the wastewater biorefinery. This has been initiated in the sugar, abattoir, paper and pulp, beverage and domestic wastewater sectors at a preliminary level, through this project and should be extended. It is through detailed case study research and scenario analysis that an in depth understanding of controlling factors will be derived.

8. Following implementation of recommendations (6) and (7), it is proposed that environmental analysis through e.g. LCA and techno-economic analysis be carried out for specific and promising scenarios.

9. It is suggested that the impact of WWBRs be interrogated in terms of their potential for social benefit as well as acceptability. This action will need to be well integrated with the role of communication in the understanding of social benefit and buy-in from the community. It is proposed that this be explored through a pilot study.

REFERENCES

Adav, S., Lee, D., Show, K. & Tay, J., 2008. Aerobic Granular Sludge: recent advances. *Biotechnology Advances,* 26(5), pp. 411-423.

Ahmad, M., Jamil, N. & Saeed, M., 1982. Nitrogen, lignin, wax and ash contents in flax (Linum usitatissimum). *Pakistan Journal for Agricultural Research,* 3(2), pp. 78 - 83.

Annuar, M., Tan, I. & Ibrahim, S., 2008. A kinetic model for growth and biosynthesis of medium-chain-length poly-(3-hydroxyalkanoates) in Pseudomonas putida. *Brazilian Journal of Chemical Engineering,* 25(2), pp. 217-228.

Antonie, R. L., 1976. *Fixed Biological Surfaces - Wastewater Treatment.* Ohio: CRC Press.

Arifin, Y. & Yusuf, Y., 2013. Mycelium Fibers as New Resource for Environmental Sustainability. *Procedia Engineering,* Volume 53, p. 504–508.

Asano, T. & Cotruvo, J., 2011. Groundwater recharge with reclaimed municipal wastewater: health and regulatory considerations. *Water Research,* 38(8), pp. 1941-1951.

ASSAf, 2014. *The State of Energy Research in South Africa,* Pretoria: Academy of Science of South Africa.

Atlow, S., Bobadonna-Aparo, L. & Klibanov, A., 1984. Dephenolisation of industrial wastewaters catalysed by polyphenol oxidase. *Biotechnology and Bioengineering,* Volume 26, pp. 599-603.

Babu, R., O'Connor, K. & Seeram, R., 2013. Current progress on bio-based polymers and their future trends. *Progress in Biomaterials,* 2(1), pp. 1-16.

Bailey, J. & Ollis, D., 1986. *Biochemical Engineering Fundamentals.* New York: McGraw-Hill.

Bajaj, I. & Singhal, R., 2009. Enhanced production of poly (γ-glutamic acid) from bacillus licheniformis NCIM 2324 by using metabolic precursors. *Applied Biochemistry and Biotechnology,* 159(1), pp. 133-141.

Bajaj, I. & Singhal, R., 2011. Flocculation properties of poly(cglutamic acid) produced from Bacillus subtilis isolate. *Food Bioprocess Technology,* Volume 4, pp. 745-752.

Bajpai, P., 2015. Chapter 2 Generation of Waste in Pulp and Paper Mills. In: *Manangement of pulp and paper mill waste.* s.l.:Springer.

Barclay, S. & Buckley, C., 2004. *The regional treatment of textile and industrial effluents,* Pretoria: Water Research Commission, SOuth Africa.

Beal, L. & Raman, D., 2000. Sequential Two-Stage Anaerobic Treatment of Confectionery Wastewater. *Journal of Agricultural Engineering Research,* Volume 76, pp. 211- 217.

Bechtold, T. & Mussak, R., 2010. *Handbook of Natural Colorants.* s.l.:Wiley.

Behera, S. et al., 2012. UV-Visible spectrophotometric method development and validation assay of tablet formulation. *Analytical and Bioanalytica techniques,* 3(6), pp. 1-6.

Bellprat, O. et al., 2015. Unusual past dry and wet rainy seasons over Southern Africa and South America from a climate perspective. *Weather and Climate Extremes,* Volume 9, p. 36–46.

Bending, C., Kraxenberger, T. & Romer, L., 2014. Shaping the future with industrial biotechnology-New and efficient production processes for biopolymers. *Biotechnology & biomedical engineering,* 2(1), pp. 1-9.

Bharathiraja, B. et al., 2014. Biofuels from sewage sludge - A review. *International Journal of ChemTech Research,* 6(9), pp. 4417-1127.

Bhattacharyya, D. et al., 1998. Novel poly-glutamic acid functionalized microfiltration membranes for sorption of heavy metals at high capacity. *J Membr Sci* , Volume 141, p. 121–135.

Billund BioRefinery, 2014. *Billund BioRefinery.* [Online]
Available at: http://www.billundbiorefinery.dk/en/
[Accessed 14 October 2014].

Binnie and Partners, 1987. *Water and Wastewater Management in the Fruit and Vegetable Processing Industry,* Pretoria: Water Reaseach Commission.

Binnie and Partners, 1987. *Water and Wastewater Management in the Fruit and Vegetable Processing Industry,* Pretoria: Water Research Commission.

Binnie and Partners, 1987. *Water and Wastewater Management in the Malt Brewing Industry (NatSurv 1),* Pretoria: Ater Resaerch Commission, South African.

Bioeconomy in Action, n.d. *bioeconomy.dk.* [Online]
Available at: www.bioeconomy.dk/news/besides-eu-usa-and-germany-several-countries-have-published-bioeconomy-strategies.
[Accessed 11 May 2014].

bio-plastics.org, 2013. [Online]
Available at: http://www.bio-plastics.org/en/information--knowledge-a-market-know-how/basics/what-are-bioplastics
[Accessed 10 August 2014].

Bio-plastics, 2014. *Production of PHA.* [Online]
Available at: http://www.bio-plastics.org/en/information--knowledge-a-market-know-how/bioplastic-types/polyhydroxyalkanoates
[Accessed 31 October 2014].

Biorefine2g.eu, 2013. *Biorefine2g.eu.* [Online]
Available at: http://www.biorefine2g.eu/
[Accessed 11 May 2014].

BIO-TIC, 2014. *Overcoming hurdles for innovation in industrial biotechnology in Europe Biobased Chemical Building Blocks summary,* s.l.: European Union.

Birrer, G., A-M, C. & Gross, R., 1994. Gamma-poly(glutamic acid) formation by Bacillus licheniformis 9945a: physiological and biochemical studies. *International Journal of Biology and Macromolecules,* 16(5), pp. 265-275.

Bizcommunity, 2015. *Veolia contracted to install waste water treatment facility at Distell.* [Online]
Available at: http://www.bizcommunity.com/Search/196/11/s-veolia+distell.html
[Accessed 26 February 2016].

Bledzki, A. & Gassan, J., 1999. Composites reinforced with cellulose based fibres. *Progress in Polymer Science,* Volume 24, p. 221–274.

Blonskaja, V., Menert, A. & Vilu, R., 2003. Use of two-stage anaerobic treamtent for distillery. *Advances in Environmental Reasearch,* 7(3), pp. 671-678.

Bohlmann, G. M., 2006. *Biorefinery Process Economics.* [Online]
Available at:
http://dc.engconfintl.org/cgi/viewcontent.cgi?article=1030&context=bioenergy_i
[Accessed 03 06 2016].

Borghei, S. & Hosseini, S., 2002. The treatment of phenolic wastwater using a moving bed biofilm reactor. *Process Biochemistry,* Volume 39, pp. 1177-1181.

Bostock, M., 2012. *Sankey Diagrams.* [Online]
Available at: https://bost.ocks.org/mike/sankey/
[Accessed 01 05 2016].

Bozkurt, H., van Loosdrecht, M., Gernaey, K. & Sin, G., 2016. Optimal WWTP process selection for treatment of domestic wastewater – A realistic full-scale retrofitting study. *Chemical Engineering Journal,* Volume 286, p. 447–458.

Brand South Africa, 2008. *South Africa's farming sectors.* [Online] Available at: http://www.southafrica.info/business/economy/sectors/542547.htm#.V02y1_l96Um [Accessed 05 04 2016].

Bremner, A. & Johnston, M., 1996. *Poultry Meat Hygiene and Inspection.* London: WB Saunders Company Ltd.

Brito, A. et al., 2007. Brewery and winery wastewater treatment: some focal points of design and operation. In: V. Oreopoulus & W. Russ, eds. *Utilisation of byproducts and treatment of waste in the food industry.* new York: Springer.

Brouckaert, C., Mhlanga, F. & Mashava, A., 2013. *Quantitative Assessment of Industrial effluents for discharge to sewer,* Pretoria: Water Research Commission, South Africa.

Buisman, C. J., 1995. *Method for removing sulphur compounds from water.* USA, Patent No. US 5474682 A.

Bumbak, F. et al., 2011. Best practices in heterotrophic high-cell-density microalgal processes: achievements, potential and possible limitations. *Applied Microbiology & Biotechnology,* Volume 91, p. 31–46.

Burton, S. et al., 2009. *Energy from wastewater - A feasibility study technical report,* Gezina: Water Research Commission.

Cai, T., Park, S. & Li, Y., 2013. Nutrient recovery from wastewater streams by microalgae: Status and prospects. *Renewable and Sustainable Energy Reviews,* Volume 19, pp. 360-369.

Candela, T. & Fouet, A., 2006. Poly-gamma-glutamate-in bacteria. *Molecular biology,* 60(5), pp. 1091-1098.

Carvajal-Zarrabal, O. et al., 2011. Treatment of vinasse from tequila production using polyglutamic acid. *Environmental management,* Volume 95, pp. 66-70.

CAWP, 2003. *Declaration of the Coalition against Water Privatisation.* [Online] Available at: http://www.saha.org.za/apf/declaration_of_the_coalition_against_water_privatisation_cawp.htm [Accessed 16 March 2016].

CDP, 2009. *The Case for Water Disclosure.* [Online] Available at: https://www.cdp.net/CDPResults/CDP-Case-for-Water-Disclosure-2009.pdf

CDP, 2015. *Accelerating action: CDP Global Water Report 2015,* s.l.: s.n.

CeBER, 2014. *Centre for Bioprocess Engineering Research Laboratory Methods Manual (unpublished).* s.l.:University of Cape Town.

Cerrillo, M., Oliveras, J., Viñas, M. & Bonmatí, A., 2016. Comparative assessment of raw and digested pig slurry treatment in bioelectrochemical systems. *Bioelectrochemistry,* Volume 110, p. 69–78.

Chavan, A. & Mukherji, S., 2010. *Effect of co-contaminant phenol on performance of a laboratory-scale RBC with algal-bacterial biofilm treating petroleum hydrocarbon-rich wastewater,* Mumbai: Society of Chemical Industry.

Chemical Engineering, 2014. Economic Indicators. *Chemical Engineering,* 121(3), pp. 71-72.

Chen, G., 2009. A microbial polyhydroxyalkanoates (PHA) based bio- and materials industry. *Chemical Society Reviews,* Volume 38, pp. 2434-2446.

Chen, H., 2013. *Modern Solid State Fermentation: Theory and Practice.* Dordrecht: Springer.

Chen, H.-G. & Zhang, Y.-H., 2015. New biorefineries and sustainable agriculture: Increased food, biofuels, and ecosystem security. *Renewable and Sustainable Energy Reviews,* Volume 47, pp. 117-132.

Chen, M. et al., 2015. Bioremediation of soils contaminated with polycyclic aromatic hydrocarbons, petroleum, pesticides, chlorophenols and heavy metals by composting: Applications, microbes and future research needs. *Biotechnology Advances: Part 1,* 33(6), pp. 745-755.

Chen, X., Chen, S., Sun, M. & Yu, Z., 2005. High yield of poly-γ-glutamic acid from Bacillus subtilis by solid state fermentation using swine manure as the basis of a solid substrate. *Bioresource Technology,* Volume 96, pp. 1872-1879.

Chernick, I., 2016. *IOL: Worse water crisis looms in 2035.* [Online]
Available at: http://www.iol.co.za/news/south-africa/worse-water-crisis-looms-in-2035---iss-1991425
[Accessed 03 March 2016].

Chevron, n.d. *Chevron: Environment - Maintaining Clean Water.* [Online]
Available at:
http://capetown.chevron.com/home/environmentandsafety/environment/maintainingcleanwater.aspx
[Accessed 23 February 2016].

Chojnacka, K. & Marquez-Rocha, F.-J., 2004. Kinetic and Stoichiometric Relationships of the Energy and Carbon Metabolism in the Culture of Microalgae. *Biotechnology,* Volume 3, pp. 21-34.

Chowdhury, P., Viraraghavan, T. & Srinivasan, A., 2010. Biological treatment processes for fish processing wastewater-a review. *Bioresource Technology,* Volume 101, pp. 439 - 449.

Christofoletti, C. et al., 2013. Sugarcane vinasse: Environmental implications of its use. *Waste Management,* 33(12), pp. 2752-2761.

Chua, A. S. M., Takabatake, H., Satoh, H. & Takashi, M., 2003. Production of polyhydroxyallkanoates (PHA) by activated sludge treating municipal wastewater: effect of pH, sludge retention time (SRT), and acetate concentration in influent. *Water Research,* 37(15), pp. 3602-3611.

City of Johannesburg, n.d. *City of Johannesburg: Wastewater to Energy,* s.l.: s.n.

Cloete, T., Gerber, A. & Maritz, L., 2010. *A first order inventory of water use and effluent production by SA industrial, mining and electricity generation sectors,* Pretoria: Water Research Commission.

CoCT, 2010. *City of Cape Town: Water and Sanitation Department FAQ Issue1.* [Online]
Available at:
https://www.capetown.gov.za/en/Water/Documents/FAQIssue1_17May2010.pdf
[Accessed 10 March 2016].

Coetzee, B., 2012. *Technical Strategic Support Manager, Utility Services Directorate, City of Cape Town. Personal communication..* s.l.:s.n.

Cohen, B., Mason-Jones, K., Rambaran, N. & Lewis, Y., 2014. *Doing business in South Africa: Water in Industrial Processing.* [Online]
Available at: http://tgh.co.za/wp-content/uploads/2.-Industrial-VIEWING.pdf
[Accessed 2015].

Cohen, J., 2006. *A Life Cycle Assessment into Energy Recovery From Organic Waste: A Case Study Of The Water Treatment Facility Of Sabmiller Newlands Brewery. MSc Thesis..* s.l.:s.n.

Cornell Waste Management Institute, 1996. *Cornell Composting.* [Online]
Available at: http://compost.css.cornell.edu/monitor/monitormoisture.html
[Accessed 06 06 2016].

Corpuscoli, 2016. *Officina Corpuscoli Work.* [Online]
Available at: http://www.corpuscoli.com/

Cortez, L., 2010. R&D Needs in the Industrial Production of Vinasse. In: L. Cortez, ed. *Sugarcane bioethanol: R&D for Productivity and Sustainability.* Sao Paolo, Brazil: Blucher, pp. 619-636.

Cortez, L. & Brossard Perez, L., 1997. Experiences on Vinasse Disposal:. *Brazilian Journal of Chemical Engineering,* 13(1).

Costley, S. & Wallis, F., 2001. Bioremediation of Heavy Metals in a synthetic wastewater using a rotating biological contactor. *Water Research,* 35(15), pp. 3715-3723.

Couto, S. et al., 2002. Performance of a solid-state immersion bioreactor for ligninolytic enzyme production: evaluation of different operational variables. *Process Biochemistry,* 38(2), pp. 219-227.

Couto, S. & Sanromán, M., 2006. Application of solid-state fermentation to food industry - A review. *Journal of Food Engineering,* 76(3), pp. 291-302.

Cowan, A., Mambo, P., Westensee, D. & Render, D., 2016. *Evaluation of integrated algae pond systems for municipal wastewater treatment,* Pretoria: Water Research Commission, South Africa.

Cromwick, A.-M., Birrer, G. & Gross, R., 1995. Effects of pH and Aeration on y -Poly(glutamic acid) Formation by Bacillus licheniformis in Controlled Batch Fermentor Cultures. *Biotechnology and Bioengineering,* Volume 50, pp. 222-227.

Crous, L. & Britz, P., 2010. *The use of constructed wetland technology in the treatment and beneficiation of brewery effluent for aquaculture. F. Masi, J. Nivala (Eds.), ,.* Italy, IWA, pp. 1255-1259.

CSIR SA, 2010. *A CSIR Perspective on Water in South Africa.* [Online] Available at: http://www.csir.co.za/nre/docs/CSIR%20Perspective%20on%20Water_2010.PDF

CTBE, n.d. *Integrated Evaluation of Biorefineries.* [Online] Available at: http://ctbe.cnpem.br/en/research/integrated-evaluation-biorefineries/ [Accessed 18 March 2016].

DAFF SA, 2011. *A profile of the South African Mutton Market Value Chain.* [Online] Available at: http://www.daff.gov.za/docs/amcp/muttonmvcp11-12.pdf [Accessed 13 05 2016].

DAFF SA, 2012. *A profile of the South African Pork Market Vlaue Chain.* [Online] Available at: http://www.nda.agric.za/docs/AMCP/Pork2012.pdf [Accessed 13 05 2016].

DAFF SA, 2013. *DAFF news.* [Online] Available at: http://www.daff.gov.za/docs/agrinews/daffnewsjan2013.pdf [Accessed 10 06 2016].

DAFF SA, 2014. *Status of the South African Marine Fishery Resources 2014.* [Online] Available at: http://www.nda.agric.za/doaDev/sideMenu/fisheries/03_areasofwork/Resources%20Research/STATUS%20OF%20THE%20SOUTH%20AFRICAN%20MARINE%20FISHERY%20RESOURCES%202014%20WEB.pdf [Accessed 12 06 2016].

Dammer, L., Carus, M., Raschka, A. & Scholz, L., 2013. *Market Developments of and Opportunities for biobased product and chemicals,* The Netherlands: nova-Institute for Ecology and Innovation.

Das, K. & Mukherjee, A., 2007. Comparison of lipopeptide biosurfactants production by Bacillus subtilis strains in submerged and solid state fermentation systems using a cheap carbon source: Some industrial applications of biosurfactants. *Process Biochemistry,* Volume 42, pp. 1191-1199.

De Bruin, L. et al., 2004. Aerobic granular sludge technology: an alternative to activated sludge?. *Water Science and Technology,* 49(11-12), pp. 1-7.

De Kreuk, M. K., Heijnen, J. J. & van Loosdrecht, M. C., 2005. *Simaltaneous COD, Nitrogen and Phosphate Removal by Aerobic Granular Sludge,* Netherlands: Delf Univercity of Technology.

De la Fuente, T., 2014. *A level playing field for renewable nutrients - Revision of the fertilisers regulation and end-of-waste criteria for biowaste..* Valladolid: s.n.

Dedekind, Z., Engelbrecht, F. & van der Merwe, J., 2016. Model simulations of rainfall over southern Africa and its eastern escarpment. *Water SA,* 42(1), pp. 129 - 143.

DelftX , 2014. *TBP01x Technology for Biobased Products (course notes).* Delft : TU Delft .

DELTA, 2013. *Living on water from Mars.* [Online]
Available at: http://delta.tudelft.nl/artikel/living-on-water-from-mars/26740
[Accessed 08 October 2014].

Desrochers, P., 2001. Cities and industrial symbiosis: Some historical perspectives and policy implications. *Journal of Industrial Ecology,* 5(4), pp. 29-44.

Desrochers, P. & Sautet, F., 2008. Entrepreneurial Policy: The Case of Regional Specialization vs. Spontaneous Industrial Diversity. *Entrepreneurship Theory and Practice ,* 32(5), pp. 813-832.

Dhull, N., Gupta, K. & Soni, S., 2014. Heterotrophic and mixotrophic cultivation of Chlorella pyrenoidosa and the enzymatic hydrolysis of its biomass for the synthesis of third generation bioethanol.. *PeerJ PrePrints ,* Volume 2:e483v1.

DiGregorio, B., 2009. Biobased Performance Bioplastic: Mirel. *Chemistry and Biology,* 16(1), pp. 1-2.

Diwani, G. et al., 2000. Treatment of Confectionery and Gum Factory Wastewater Effluent. *Adsorption Science and Technology,* 18(9), pp. 813-821.

Diya'uddeen, B., Daud, W. & Azia, A., 2011. Treatment technologies for petroleum refinery effluents: A review. *Process Safety and Environmental Protection,* 89(2), p. 95 – 105.

Doble, M. & Kumar, A., 2005. *Biotreatment of industrial effluents.* 1 ed. Oxford, UK: Elsevier Butterworth-Heunemann.

Dobson, R., Gray, V. & Rumbold, K., 2011. Microbial utilization of crude glycerol for the production of value-added products. *Joournal of Industrial Microbiology and Biotechnology,* 39(2), pp. 217-226.

Dodkins, I. & Mendzil, A., 2014a. *Floating Treatment Wetlands,* Swansea, Wales: Sustainable Expansion of the Applied Coastal And Marine Sectors.

Dodkins, I. & Mendzil, A., 2014b. *Floating treatment wetlands (FTWs) in water treatment: Treatment efficiency and potential benefits of activated carbon,* s.l.: s.n.

Dolnicar, S., Hurlimann, A. & Grün, B., 2011. What affects public acceptance of recycled and desalinated water?. *Water Research,* 45(2), pp. 933-943.

Donnelly, L., 2015. *Mail & Guardian: Industry can ease the water crisis.* [Online]
Available at: http://mg.co.za/article/2015-11-15-industry-can-ease-the-water-crisis
[Accessed 07 March 2016].

Doran, P., 1995. *Bioprocess Engineering Principles.* 7th ed. s.l.:Elsevier Academic Press.

Dragone, G., Fernandes, B., Vicente, A. & Teixeira, J., 2010. *Third generation biofuels from microalgae,* Braga, Portugal: Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar.

DST SA, 2013. *Bioeconomy Strategy.* Pretoria: Department of Science and Technology, South Africa.

DST SA, 2013. *The Bio-economy Strategy,* Pretoria: Department of Science and Technology, South Africa.

Du Preez, J., 2010. *Treatment of typical South African milking parlour wastewater by means of anaerobic sequencing batch reactor technology (MSc thesis).* s.l.:University of Stellenbosch.

Dubber, D. & Gray, N. F., 2010. Replacement of chemical oxygen demand (COD) with total organic carbon (TOC) for monitoring wastewater treatment performance to minimize disposal of toxic analytical waste. *Journal of environmental science and health. Part A, Toxic/hazardous substances & environmental engineering,* 45(12), pp. 1595-600.

DWA SA, 2001. *Guidelines for the Handling, Treatment and Disposal of Abattoir Waste.* [Online]
Available at: https://www.dwa.gov.za/Documents/Policies/WDD/AbattoirWasteHandling_Disposal.pdf
[Accessed 30 04 2016].

DWA SA, 2009. *Executive Summary: Municipal Wastewater Treatment – Base Information for Targeted Risk-based Regulation. Western Cape Province..* [Online]
Available at: https://www.dwa.gov.za/dir_ws/wsam/vdfileload/file.asp?val=20&tablename=AsetFiles&fld=ID
[Accessed 10 March 2016].

DWA SA, 2013. *Revision of General Authorisations in Terms of Section 39 of the National Water Act.* [Online]
Available at: http://www.gpwonline.co.za/Gazettes/Gazettes/36820_6-9_WaterAffairs.pdf
[Accessed 10 08 2016].

DWS SA, 2014. *Green Drop Progress Report,* Pretoria: Department of Water and Sanitation, South Africa.

DWS SA, 2015. *Department of Water and Sanitation Annual Report 2014/2015.* [Online]
Available at: http://www.gov.za/sites/www.gov.za/files/DWA-annual-report-2014-15.pdf
[Accessed 17 March 2016].

DWS SA, 2015. *Water and Sanitation.* [Online]
Available at: http://www.gcis.gov.za/sites/www.gcis.gov.za/files/docs/resourcecentre/WATER%20AND%20SANITATION2015.pdf
[Accessed 05 March 2015].

Ebnesajjad, S., 2013. *Handbook of Biopolymers and Biodegrdable plastics.* Oxford: Elsevier.

Ecovative, 2016. *Ecovative Design.* [Online]
Available at: http://www.ecovativedesign.com/

Ekama, G., Wilsenach, J. & Chen, G., 2011. Saline sewage treatment and source separation of urine for more sustainable urban water management. *Water Science & Technology,* 64(6), pp. 1307 - 1316.

El-Gohary, F., Nasr, F. & Aly, H., 1999. Cost-Effective Pre-treatment of FoodProcesing Industrial Wastewater. *Water Science and Technology,* 40(7), pp. 17-24.

Elsevier, n.d. *Scopus.* [Online]
Available at: https://www.elsevier.com/solutions/scopus
[Accessed 2016].

Erasmus, D., 2012. *Fewer dairy farmers produce more milk.* [Online]
Available at: http://www.farmersweekly.co.za/news.aspx?id=31892
[Accessed 10 06 2016].

Ersahin, M., Ozgun, H., Dereli, R. & Ozturk, I., 2011. Anaerobic Treatment of Industrial Effluents: An Overview of Applications. In: F. S. G. Einschlag, ed. *Waste Water - Treatment and Reutilization.* s.l.:Istanbul Technical University.

eThekwini City, n.d. *Durban Water Recycling Project.* [Online]
Available at:
http://www.durban.gov.za/City_Services/water_sanitation/Services/Pages/durban-recyling.aspx
[Accessed 23 February 2016].

EuropaBio, 2011. *Biorefinery feasibility study.* [Online]
Available at:
http://www.europabio.org/sites/default/files/report/europabio_and_partners_biorefinery_feasibility_study.pdf
[Accessed 06 06 2016].

European Bioplastics, 2013. *Frequently asked questions on bioplastics,* Berlin: European Bioplastics.

European Bioplastics, 2014. *Production capacity,* s.l.: European Bioplastics.

European Bioplastics, 2015. *Global bioplastics production capacities continue to grow despite low oil price.* [Online]
Available at: http://www.european-bioplastics.org/pr_151104/
[Accessed 22 05 2016].

European Bioplastics, 2016. *European Bioplastics.* [Online]
Available at: http://www.european-bioplastics.org/
[Accessed 12 September 2015].

European Commission, 2009. *Taking bio-based from promise to market- Measures to promote the market introduction of innovative bio-based products,* s.l.: European Commission.

European Plastics Converters, n.d. *BIOPLASTICS & Biodegradibility Questions & Answers,* Brussels: European Plastics Converters.

exchange-rates.org, 2015. *South African Rands (ZAR) to 1 US Dollar (USD).* [Online]
Available at: http://www.exchange-rates.org/history/ZAR/USD/G/180
[Accessed 21 January 2015].

FAO UN, 1996. *Management of Waste from Animal Product Processing: Slaughterhouses.* [Online]
Available at: http://www.fao.org/wairdocs/lead/x6114e/x6114e04.htm
[Accessed 04 04 2016].

Fava, F. e. a., 2012. *Biowaste biorefinery in Europe: opportunities and R&D needs,* Barcelona: European Federation of Biotechnology.

Fernández-Dacosta, C. et al., 2015. Microbial community-based polyhydroxyalkanoates (PHAs) production from wastewater: Techno-economic analysis and ex-ante environmental assessment. *Bioresource Technology,* Volume 185, pp. 368-377.

Fernando, S., Adhikari, S., Chandrapal, C. & Murali, N., 2006. Biorefineries: Current Status, Challenges, and Future Direction.. *Energy & Fuels,* Volume 20, p. 1727 – 1737.

Ferry, A. & Giljova, S., 2015. *Biogas Potential in Selected Waste Water Treatment Plants,* s.l.: Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH.

Floating Island International, 2016. *Biohaven Technology.* [Online]
Available at: http://www.floatingislandinternational.com/products/biohaven-technology/
[Accessed 10 March 2016].

Food Stuff South Africa, 2011. *The sweet fortunes of SA's confectionery market.* [Online]
Available at: http://www.foodstuffsa.co.za/news-stuff/latest-sa-news/1315-the-sweet-fortunes-of-sas-confectionery-market
[Accessed 10 05 2016].

Fosso-Kankeu, E. & Mulaba-Bafubiandi, A., 2014. Implication of plants and microbial metalloproteins in the bioremediation of polluted waters: A review. *Physics and Chemistry of the Earth, Parts A/B/C,* Volume 67-69, pp. 242-252.

Franks, L., Euston-Brown, m., Ferry, A. & Giljova, S., n.d. *Municipal WWTW: Biogas to Energy (Co-Generation),* Pretoria: GIZ & SALGA.

Froud, M., 2016. *South Africa now 8th biggest wine producer in the world – 2016 update.* [Online] Available at: http://www.topwinesa.com/?p=7833 [Accessed 12 06 2016].

Gademan, G., Gaydon, P. & de Bruin, B., 2010. *Granular Aerobic Activated Sludge,* Pietermaritzburg: SSI Engineers and Environmental Consultants.

Gasim, H., Kutty, S., Isa, M. & Isa, M., 2012. Treatment of petroleum refinery wastewater by using UASB reactors. *International Journal of Chemical and Biological Engineering,* Volume 6, pp. 174-177.

GCIS SA, 2013. *South Africa Yearbook 2012/2013.* [Online] Available at: http://www.gcis.gov.za/sites/www.gcis.gov.za/files/docs/resourcecentre/yearbook/2012/03%20Agriculture.pdf [Accessed 10 06 2016].

Gerardo, M. et al., 2015. Harvesting of microalgae within a biorefinery approach: A review of the developments and case studies from pilot-plants. *Algal Research,* Volume 11, pp. 248-262.

Germany. Federal Government, 2012. *Biorefineries Roadmap,* Berlin: German Federal Ministries.

Ghanbarzadeh, B. & Almasi, H., 2013. *Biodegradable polymers,* s.l.: INTECH.

Ghatak, H., 2011. Biorefineries from the perspective of sustainability: Feedstocks, products, and processes.. *Renewable and Sustainable Energy Reviews,* 15(8), pp. 4042-4052.

GIZ, RECORD & SANEDI, 2014. *The State of Waste to Energy Research in South Africa,* Pretoria: Deutsche Gesellschaft für Internationale.

GIZ, n.d. *GIZ Office South Africa.* [Online] Available at: https://www.giz.de/en/worldwide/312.html [Accessed 23 February 2016].

Global Lamp Index , 2011. *The Kalundborg Symbiosis: A model of progressive resource exchanges.* [Online] Available at: http://www.lampindex.com/2011/10/the-kalundborg-symbiosis/ [Accessed 08 October 2014].

Global Utility Week Series, 2016. *African Untility Week.* [Online] Available at: http://www.african-utility-week.com/uploads/Pages/site152_34275_en_file1.pdf [Accessed 07 06 2016].

Goto, A. & Kunioka, M., 1992. Biosynthesis and hydrolysis of poly (γ-glutamic acid) from Bacillus subtilis IFO 3335. *Bioscience, Biotechnology and Biochemistry,* 56(7), pp. 1031-1035.

Gouma, S., Fragoeiro, S., Bastos, A. & Magan, N., 2014. Bacterial and Fungal Bioremediation Strategies. In: S. Das, ed. *Microbial Biodegradation and Bioremediation.* s.l.:Elsevier, pp. 301-323.

Grady, C., Daigger, G., Love, N. & Filipe, C., 2011. *Biological Wastewater Treatment.* 3rd ed. London: IWA Publishing.

Grady, J. C. L., Daigger, G. T., Love, N. G. & Filipe, C. D. M., 2011. *Biological Wastewater Treatment.* 3rd ed. London: IWA Publishing.

Graedel, T. & Allenby, B., 2010. *Industrial ecology and sustainable engineering.* London. UK: Pearson.

Gratziou, M. K., Tsalkatidou, M. & Kotsovinos, N. E., 2006. Economic Evaluation Of Small Capacity Sewage Processing Units. *Global NEST Journal,* 8(1), pp. 52-60.

Gray, K. A., Zhao, L. & Emptage, M., 2006. Bioethanol. *Current opinion in chemical biology,* 10(2), pp. 141-146.

GreenCape, n.d. *Supporting the Business of Clean.* [Online]
Available at: http://greencape.co.za/
[Accessed 22 February 2016].

Griffiths, M., Harrison, S., Smit, M. & Maharaj, D., 2016. Major commercial products from micro- and macro-algae. In: F. Bux & Y. Chisti, eds. *Algal Biotechnology, Green Energy and Technology.* s.l.:Springer, pp. 269-300.

Grotkjær, T., 2016. Commercial Development of Fermentation Processes. In: *Fundamental Bioengineering.* s.l.:Wiley, pp. 499-546.

Hagelqvist, A., 2013. *Sludge from pulp and paper mills for biogas production-Strategies to improve energy performance in wastewater treatment and sludge management (Dissertation).* s.l.:Karlstad University.

Hallenbeck, P. C. & Benemann, J. R., 2004. Biological hydrogen porduction: fundamentals and limiting processes. *International Journal of hydrogen energy,* 29(2), pp. 173-185.

Hamelinck, C. N., van Hooijdonk, G. & Faaij, A. P., 2005. Ethanol from lignocellulosic biomas: techno-economic performance in short-, middle, and long-term. *Biomass and bioenergy,* 28(4), pp. 384-410.

Hampannavar, U. & Shivayogimath, C., 2010. Anaerobic treatment of sugar industry wastewater by upflow anaerobic sludge blanket reactor at ambient temperature. *nternational Journal of Enviromental Science,* 1(4), pp. 631-639.

Harding, K., 2009. *A generic approach to environmental assessment of microbial bioprocesses through Life Cycle Assessment (LCA). PhD Thesis.* s.l.:University of Cape Town.

Harding, K., Dennis, J., von Blottnitz, H. & Harrison, S., 2007. nvironmental analysis of plastic production processes: Comparing petroleum-based polypropylene and polyethylene with biologically-based poly-β-hydroxybutyric acid using life cycle analysis. *Journal of Biotechnology,* 130(1), pp. 57-66.

Harding, K., Dennis, J., von Blottnitz, H. & Harrison, S., 2008. A life-cycle comparison between inorganic and biological catalysis for the production of biodiesel. *Journal of Cleaner Production,* 16(13), pp. 1368-1378.

Harper, H., Daniel, H. & Murphy, H., 1933. The total Nitrogen, Phosphorous and Calcium content of common weeds and native grasses in Oklahoma. *Academy of Science,* Volume 36 - 44.

Harrison, S. et al., 2014. *Addressing the Challenges Facing Biological Sulphate Reduction as a Strategy for AMD Treatment: Reactor stage - raw materials, products and process kinetics,* Pretoria: Water Research Commission, SA.

Haughn, M., 2015. *WhatIs.com.* [Online]
Available at: http://whatis.techtarget.com/definition/bioplastic
[Accessed 25 February 2015].

Heidrich, E., Curtis, T. & Dofling, J., 2011. Determination of the internal chemical energy of wastewater. *Environmental Science and Technology,* 45(2), pp. 827 - 832.

Henze, M., Harremoës, P., la Cour Jansen, J. & Arvin, E., 2002. *Wastewater Treatment: Biological and Chemical Processes.* 3rd ed. Dordrecht: Springer.

Henze, M., van Loosdrecht, M. C., Ekama, G. A. & Brdjanovic, D., 2008. *Biological Wastewater Treatment.* London: IWA Publishing.

Higson, A., 2013. *Global trends in the Bioeconomy,* Trondheim: NNFCC: The Bioeconomy Consultants.

Holmgren, K., Li, H., Verstraete, W. & Cornel, P., 2015. *State of the art compendium report on resource recovery from water,* s.l.: International Water Association (IWA).

Holtzkampf, E., 2012. *Liquor Consumption Patterns in South Africa.* [Online]
Available at:
http://www.sawis.co.za/info/download/Liquor_consumption_article_eh_nov_2012.pdf
[Accessed 05 12 2015].

Holtzkampf, E., 2016. *Liquor Consumption Patterns in South Africa 2015.* [Online]
Available at:
http://www.sawis.co.za/info/download/Liquor_consumption_patterns_in_South_Africa_2015_eh_V1_-_final.pdf
[Accessed 04 06 2016].

iBert, 2015. *ibert (Pty) Ltd Linked In Page.* [Online]
Available at: https://www.linkedin.com/company/ibert-pty-ltd
[Accessed 25 February 2016].

IEA Bioenergy, n.d. *IEA Bioenergy Task 42 Biorefining.* [Online]
Available at: http://www.iea-bioenergy.task42-biorefineries.com/en/ieabiorefinery.htm
[Accessed 03 October 2014].

IEA, 2014. *Annual Report IEA Bioenergy,* Paris: International Energy Agency.

IfBB, 2016. *Institute for Bioplastics and Biocomposites.* [Online]
Available at: http://ifbb.wp.hs-hannover.de/downloads/index.php?site=Statistics&nav=5-1-0-0-0
[Accessed 03 06 2016].

Imran, M. et al., 2015. Microbial biotechnology for decolorization of textile wastewaters. *Rev Environ Sci Biotechnol,* Volume 14, pp. 73-92.

Inbaraj, B. & Chen, B., 2012. In vitro removal of toxic heavy metals by poly(c-glutamic acid)-coated superparamagnetic nanoparticles. *International Journal of Nanomedicine,* Volume 7, p. 4419–4432.

Inbaraj, B. et al., 2006. Removal of cationic dyes from aqueous solution using an anionicpoly-c-glutamic acid-based adsorbent. *Journal of Hazardardous Material,* Volume 137, p. 226–234.

Inbaraj, B. et al., 2011. The synthesis and characterization of poly(c-glutamic acid)-coated magnetite nanoparticles and their effects on antibacterial activity and cytotoxicity. *Nanotechnology,* Volume 22, pp. 75-101.

Index Mundi, 2010. *South Africa Animal Numbers, Cattle Cow Slaughter by Year.* [Online]
Available at:
http://www.indexmundi.com/agriculture/?country=za&commodity=cattle&graph=cow-slaughter
[Accessed 13 05 2016].

Industry SA, 2014. *Industry SA: Innovation - Sasol & GE.* [Online]
Available at: http://www.industrysa.co.za/sasol-ge/
[Accessed 25 February 2016].

Inglesby, A., Griffiths, M., Harrison, S. & van Hille, R., 2015. Anaerobic digestion of Spirulina sp. and Scenedesmus sp.: a comparison and investigation of the impact of mechanical pre-treatment. *Journal of Applied Phycology,* Volume 27, pp. 1891-1900.

Isafiade, A. et al., 2015. Optimal synthesis of heat exchanger networks for multi-period operations involving single and multiple utilities. *Chemical Engineering Science,* Volume 127, pp. 175-188.

IWA Resource Recovery Cluster, 2015. *State of the Art Compendium Report on Resource Recovery from Water,* s.l.: International Water Association.

IWA, 2009. *IWA Water Wiki.* [Online]
Available at:

http://www.iwawaterwiki.org/xwiki/bin/view/Articles/IndustrialWastewaterTreatment
[Accessed 06 04 2016].

IWARR2015, 2015. [Online]
Available at: http://www.iwarr2015.org/
[Accessed 10 February 2016].

Jahren, S. J., Rintala, J. A. & Odegaard, H., 2001. Aerobic moving bed biofilm reactor treating thermomechanical pulping whitewater under thermophilic conditions. *Water Research,* Volume 36, pp. 1067 - 1075.

Jang, Y. et al., 2012. Bio-based Production of C2-C6 Platform Chemicals. *Biotechnology and Bioengineering,* 109(10), pp. 2437-2459.

JavaScript, n.d. [Online]
Available at: https://d3js.org/
[Accessed 01 05 2016].

Jiang, Y., 2011. *Polyhydroxyalkanoates production by bacterial enrichments, PhD thesis,* Delft: TU Delft.

Jiang, Y. et al., 2012. Waste to resource: converting paper mill wastewater to bioplastic. *Water Research,* Volume 46, pp. 5517-5530.

Jovanovic, N., Mu, Q., Bugan, R. & Zhao, M., 2015. Dynamics of MODIS evapotranspiration in South Africa. *Water SA,* 41(1).

Kadlec, R. & Wallace, S., 2009. *Treatment Wetlands.* 2nd ed. Boca Raton, FL: CRC Press.

Kakuru, W., Turyahabwe, N. & Mugisha, J., 2013. Total Economic Value of Wetlands Products and Services in Uganda. *The Scientific World Journal,* pp. Article ID 192656, 13 pages.

Kalogeris, E. et al., 2003. Performance of an intermittent agitation rotating drum type bioreactor for solid-state fermentation of wheat straw. *Bioresource Technology,* Volume 86, p. 207–213.

Kamm, B., Gruber, P. & Kamm, M. eds., 2006. *Biorefineries – Industrial Processes and Products: Status Quo and Future Directions.* Weinheim: Wiley VCH.

Kemacheevakul, P., Polprasert, C. & Shimizu, Y., 2011. Phosphorus Recovery from Human Urine and Anaerobically Treated Wastewater Through pH Adjustment and Chemical Precipitation. *Environmental Technology,* 32(7), pp. 693 -– 698.

Khan, N. et al., 2015. Fruit waste streams in South Africa and their potential role in developing a bio-economy. *South African Journal of Sciences,* 111(5/6), pp. 1-11.

Kiepper, B., Merka, W. & Fletcher, D., 2008. *Proximate Composition of Poultry Processing Wastewater Particulate Matter from Broiler Slaughter Plants,* s.l.: Poultry Science Association Inc.

Kim, S., Park, J.-e., Cho, Y.-B. & Hwang, S.-J., 2013. Growth rate, organic carbon and nutrient removal rates of Chlorella sorokiniana in autotrophic, heterotrophic and mixotrophicconditions. *Bioresource Technology,* Volume 144, pp. 8-13.

Kings, S., 2014. *Mail & Guardian: SA may need to turn to private sector to solve its water crisis.* [Online]
Available at: http://mg.co.za/article/2014-07-29-sa-may-need-to-turn-to-private-sector-to-solve-its-water-crisis
[Accessed 03 March 2016].

Klazinga, R., 2009. *Bioplastics A sustainable solution?,* Enschede: University of Twente.

Kleerebezem, R., Joosse, B., Rozendal, R. & van Loosdrecht, M., 2015. Anaerobic digestion without biogas?. *Reviews in Environmental Science and Biotechnology,* Volume 99, pp. 1-15.

Kleerebezem, R. & van Loosdrecht, M., 2007. Mixed culture biotechnology for bioenergy production. *Current Opinion in Biotechnology,* 18(3), pp. 207 - 212.

Klein, J. & Lee, D., 1978. *Biological treatment of aqueous water from usual conversion processes.* s.l., s.n., pp. 379-390.

Kong, Q.-x.et al., 2010. Culture of Microalgae Chlamydomonas reinhardtii in Wastewater for Biomass Feedstock Production. *Applied Biochemistry and Biotechnology,* Volume 160, pp. 9-18.

Kubota, H., Nambu, Y. & Endo, T., 1995. Convenient esterification of poly(c-glutamic acid) produced by microorganism with alkyl halides and their thermal properties. *J Polym Sci A Polym Chem,* Volume 33, p. 85–88.

Kumar, A., Srivastava, A., Galaev, I. & Mattiasson, B., 2007. Smart Polymers: Physical Forms & Bioengineering Applications. *Progress in Polymer Science,* 32(10), pp. 1205-1237.

Kumar, R., Vikramachakravarthi, D. & Pal, P., 2014. Production and purification of glutamic acid: A critical review towards process intensification. *Chemical Engineering and Processing,* Volume 81, pp. 57-79.

Kymäläinen, H.-R. & Pasila, A., 2000. Equilibrium moisture content of flax/linseed and fibre hemp straw fractions. *Agricultural and Food Science in Finland,* Volume 9, pp. 259 - 268.

Lassen, B., 2012. *Dairy production in South Africa - Impressions.* [Online]
Available at: http://www.agribenchmark.org/fileadmin/Dateiablage/B-Dairy/Country_Report/12_country_information_SA.pdf
[Accessed 05 04 2016].

Lee, S., 1996. Bacterial polyhydroxyalkanoates. *Biotechnology and Bioengineering,* 49(1), pp. 1-14.

Levin, D. B., Pitt, L. & Love, M., 2004. Biohydrogen productionL prospects and limitations to practical application. *International journal of hydrogen energy,* 29(2), pp. 173-185.

Lewis, A. & Randall, D., 2011. Value Recovery from Wastewater Using Eutectic Freeze Crystallization. *Water Conditioning & Purification.*

Lim, S. et al., 2012. Effect of poly-c-glutamic acids (PGA) on oil uptake and sensory quality in doughnuts. *Food Science and Biotechnology,* Volume 21, p. 247–252.

Lindsey, K. & Hirt, H., 1999. *Use Water Hyacinth! A Practical Hanbook of uses for the Water Hyacinth from Across the World.* Winnenden: Anamed.

Li, Q., Du, W. & Liu, D., 2008. Perspectives of microbial oils for biodiesel production. *Applied Microbilogy and Biotechnology,* 80(5), pp. 749-756.

Liu, Y. & Tay, J., 2004. State of the art of biogranulation technology for wastewater treatment. *Biotechnology Advances,* 22(7), pp. 533-563.

Li, Y.-C.et al., 2012. Techno-economic evaluation of biohydrogen production from wastewater and agricultural waste. *International Journal of Hydrogen Energy,* Volume 37, pp. 15704-15710.

Loo, C. & Sudesh, K., 2007. Polyhdroxyalkanoates: Bio-based microbial plastics and their properties. *Malaysian Polymer Journal,* 2(2), pp. 31-57.

Lopar, M. et al., 2014. Study of metabolic network of Cupriavidus necator DSM 545 growing on glycerol by applying elementary flux modes and yield space analysis. *Journal of industrial microbiology & biotechnology,* 41(6), pp. 913-930.

Lopez, J., Burgos, A. & Rodriguez, P., 2015. *Technology fact sheets for effluent treatment plants on textile industry. Primary treatment series: Primary Clarifier (FS-PRIM-002).* [Online]
Available at: https://www.wateractionplan.com/documents/186210/186352/INDITEX-FS-PRIM-002-Primary+clarifier.pdf/eebaccea-ce77-4898-8d20-a3e39a6957a1

Louw, T., Griffiths, M., Jones, S. & Harrison, S., 2016. Techno-economics of algal biodiesel. In: F. Bux & Y. Chisti, eds. *Algal Biotechnology, Green Energy and Technology.* s.l.:Springer, pp. 111-141.

Mabeer, V., 2015. *Personal communication.* s.l.:WRC steering committee meeting.

Mac Donald, C., 2004. *Water usage in the South African pulp and paper industry (MSc Thesis).* Durban: University of KwaZulu-Natal.

Maclean, M., n.d. *D3 Tips and Tricks Interactive Data Visualization in a Web Browser (updated continuously).* [Online]
Available at: https://leanpub.com/D3-Tips-and-Tricks
[Accessed 01 05 2016].

Madonsela, Z., 2013. *Selection of bacterial species from wastewater for potential production of PGA: isolation, characterisation and growth kinetics.* Cape Town: University of Cape Town.

Ma, F. & Hanna, M. A., 1999. Biodiesel production: a review. *Bioresource technology,* 70(1), pp. 1-15.

Mahlakahlaka, S. & Maseko, S., 2014. *Treatment of vinasse with concomitant value creation – energy and fertilizer (Unpublished Project Report).* s.l.:Department of Chemical Engineering, University of Cape Town.

Malandra, L., Wolfaardt, G., Zietsman, A. & Viljoen-Bloom, M., 2003. Microbiology of a biological contactor for winery wastewater treatment. *Water Research,* Volume 37, pp. 4125-4134.

Malandra, L., Wolfaardt, G., Zietsman, A. & Viljoen-Bloom, M., 2003. Microbiology of a biological contactor for winery wastewater treatment. *Water Research,* Volume 37, pp. 4125 - 4134.

Margaritis, R. A., 2003. Characteristics of Poly(glutamic acid) Fermentation by Bacillus subtilis. *Biotechnology and Bioengineering,,* Volume 82, pp. 299-305.

Marques, G., Rencoret, J., Gutiérrez, A. & del Río, J., 2010. Evaluation of the Chemical Composition of Different Non-Woody Plant Fibers Used for Pulp and Paper Manufacturing. *The Open Agriculture Journal ,* Volume 4, pp. 93-101.

Mata-Alvarez, J., Mace, S. & Llabres, P., 2000. An overview of research achievements and perspectives. *Bioresource Technology,* 74(1), pp. 3-16.

McLean, R., Beauchemin, D., Clapham, L. & Beveridge, T., 1990. Metal-binding characteristics of the gamma glutamyl capsular polymer of Bacillus lichenformis ATCC 9945. *Applied Environmental Microbiology,* Volume 56, pp. 3671-3677.

Meissner, L. et al., 2015. Influence of nitrogen source and pH value on undesired poly(γ-glutamic acid) formation of a protease producing Bacillus licheniformis strain. *Journal of Industrial Microbiology & Biotechnology,* Volume 42, pp. 1203-1215.

Melamane, X., Strong, P. & Burgess, J., 2007. Treatment of wine distillery wastewater: A review with emphasis on anaerobic membrane reactors. *South African Journal of Enology and Viticulture,* 28(1), p. 25–36.

Menendez, M. & Black-Veatch, P., 2011. *How we use energy at wastewater plants...and how we can use less.* [Online]
Available at:
http://www.ncsafewater.org/Pics/Training/AnnualConference/AC10TechnicalPapers/AC10_Wastewater/WW_T.AM_10.30_Menendez.pdf
[Accessed 05 November 2014].

Mitchell, D., Krieger, N. & Beroviéc, M. (., 2010. *Solid-state fermentation bioreactors: fundamentals of design and operation.* New York, USA: Springer Press.

Mohanty, A., Misra, M. & L, D., 2005. *Natural fibres, biopolymers and biocomposites,* s.l.: CRC Press.

Molapo, N., 2009. *Waste Handling Practices in the South African High-Throughput Poultry Abattoirs, unpublished Msc Thesis.* s.l.:Central University of Technology, Free State .

Mooibroek, H. et al., 2007. Assessment of technological options and economical feasibility for cyanophycin biopolymer and high-value amino acid production. *Applied Microbiology and Biotechnology,* 77(2), pp. 257-267.

Mooij, P. R., Stouten, G. R., van Loosdrecht, M. C. & Kleerebezem, R., 2015. Ecology-based selective environments as solution to contamination in microalgal cultivation.. *Current Opinion in Biotechnology,* Volume 33, pp. 46-51.

Mooij, P. R., Stouten, G. R., van Loosdrecht, M. C. & Kleerebezem, R., 2015. Ecology-based selective environments as solution to contamination in microalgal cultivation.. *Current Opinion in Biotechnology,* Volume 33, pp. 46-51.

Moosbrugger, R., Wentzel, M. & Ekama, G., 1993. Treatment of wine distillery waste in UASB systems—feasibility, alkalinity requirements and pH control. *Water Science & Technology,* 28(2), pp. 45-54.

Moraes, B., Zaiat, M. & Bonomi, A., 2015. Anaerobic digestion of vinasse from sugarcane ethanol production in Brazil: Challenges and perspectives. *Renewable and Sustainable Energy Reviews,* Volume 44, pp. 888-903.

Moraes, L., Brito, P. & RM, A., 2013. The Existing Studies on Biosynthesis of Poly(ɣ-glutamic acid) by Fermentation. *Food and Public Health,* 3(1), pp. 28-36.

Moral, M., 2013. *Process to obtain Potassium Sulfate from Vinasse.* USA, Patent No. 0089478 A1.

Mothes, G., Schnorpfeil, C. & Ackermann, J. U., 2007. Production of PHB from crude glycerol. *Engineering in Life Sciences,* 7(5), pp. 475-479.

Mourtos, N., 2015. *Charles W. Davidson College of Engineering.* [Online]
Available at: http://www.engr.sjsu.edu/nikos/courses/AE160/pdf/VIS-disk.E.pdf
[Accessed 20 April 2015].

MPO, 2016. *Dairy Market Trends.* [Online]
Available at: http://www.mpo.co.za/images/downloads/kms201603.pdf
[Accessed 01 04 2016].

Murray, S., 2013. *Interactive Data Visualization for the Web An Introduction to Designing with D3.* [Online]
Available at: http://oreil.ly/interactive_data_visualization_web
[Accessed 01 05 2016].

Mussig, J. (., 2010. *Industrial applications of natural fibres: structure, properties and technical applications.* s.l.:Wiley.

Naidoo, C., 2013. Johannesburg Water's Foray into Renewable Energy. *Civil Engineering,* Volume June.

Nasir, I. M., Mohd Ghazi, T. I. & Omar, R., 2012. Anaerobic digestion technology in livestock manure treatment for biogas production: a review. *Engineering in Life Sciences,* 12(3), pp. 258-269.

Nasseri, A., Rasoul-Amini, S., Morowvat, M. & Ghasemi, Y., 2011. Single Cell Protein: Production and Process.. *American Journal of Food Technology,* Volume 6, pp. 103-116.

National biodiesel board, 2008. *Comprehensive survey on energy use for biodiesel production.* [Online]
Available at: http://www.biodiesel.org/docs/ffs-production/comprehensive-survey-on-energy-use-for-biodiesel-production.pdf?sfvrsn=4
[Accessed 05 November 2014].

Ndaba, D., 2011. *Durban wastewater partnership marks ten-year milestone.* [Online]
Available at: http://www.engineeringnews.co.za/article/the-dwr-plant-hailed-as-a-successful-publicprivate-partnership-project-2011-09-02
[Accessed 24 February 2016].

Neethling, G., 2014. *Waste Management: Red Meat Abattoirs (Presentation).* s.l.:Red Meat Abattoir Association.

News 24, 2015. *News 24: Power crisis biggest risk to SA growth - IMF.* [Online]
Available at: http://www.fin24.com/Economy/Eskom/Power-crisis-biggest-risk-to-SA-growth-IMF-20150623
[Accessed 09 March 2016].

Nicolella, C., van Loosdrecht, M. & Heijnen, J., 2000. Wastewater treatment with particulate biofilm reactors. *Journal of Biotechnology,* 80(1), pp. 1-33.

Nieddu, M. & Vivien, F.-D., 2013. *Collective Productive Patrimonies (patrimoines productifs collectifs) and Sustainability Transitions..* s.l.:REGARDS.

Niero, M., Pizzol, M., Bruun, H. G. & Thomsen, M., 2014. Comparative life cycle assessment of wastewater treatment in Denmark including sensitivity and uncertainty analysis. *Journal of Cleaner Production,* Volume 68, pp. 25-35.

Nijhuis Water Technology, 2014. [Online]
Available at: http://www.nijhuis-water.com/default.aspx?taal=true
[Accessed 08 October 2014].

Nkadimeng, L. S., 2015. *Maximising energy recovery from the brewery wastewater treatment system: a study evaluating the anaerobic digestion wastewater treatment plant at SAB's Newlands brewery. MSc Thesis.* s.l.:University of Cape Town.

Novaes-Ledieu, M., Jimenez-Martinez, A. & Villanueva, J., 1967. Chemical Composition of Hyphal Wall of Phycomycetes. *Journal of General Microbiology,* Volume 47, pp. 237-245.

Novozymes , 2013. *Novozymes utilizes wastewater to produce biogas.* [Online]
Available at: http://novozymes.com/en/news/news-archive/Pages/novozymes-utilizes-wastewater-to-produce-biogas.aspx
[Accessed 08 October 2014].

Nuengjamnong, C., Chiarawatchai, N., Polprasert, C. & Otterpohl, R., 2011. Treating Swine Wastewater by Integrating Earthworms into Constructed Wetlands. *Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering,* 46(7), pp. 800-804.

Obruca, S. et al., 2015. Biotechnological conversion of spent coffee grounds into polyhydroxyalkanoates and carotenoids. *New Biotechnology.*

Obruca, S. et al., 2014. Utilization of oil extracted from spent coffee grounds for sustainable production of polyhydroxyalkanoates. *Applied Microbiology and Biotechnology,* Volume 98, p. 5883–5890.

Ogunleye, A. et al., 2014. Poly-γ-glutamic acid: production, properties and applications. *Microbiology,* Volume 161, pp. 1-17.

Olguín, E. J., 2012. Dual purpose microalgae–bacteria-based systems that treat wastewater and produce biodiesel and chemical products within a Biorefinery. *Biotechnology Advances,* 30(5), pp. 1031-1046.

Olguín, E. J., 2012. Dual purpose microalgae–bacteria-based systems that treat wastewater and produce biodiesel and chemical products within a Biorefinery. *Biotechnology Advances,* 30(5), pp. 1031-1046.

Oliveira, D., 2015. Sasol to complete design of biogas plant by year-end. *Engineering News,* 24 April.

Orhon, D., Yıldız, G., Cokgor, E. & Sozen, S., 1995. Respirometric Evaluation of the Biodegrability of Confectionery Wastewaters. *Water Science and Technology,* 32(12), pp. 11-19.

Otani, Y., Tabata, Y. & Ikada, Y., 1999. Sealing effect of rapidly curable gelatin-poly(L-glutamic acid) hydrogel glue on lung air leak. *Ann Thorac Surg,* Volume 67, p. 922–926.

Ozturk, I. & Altinbas, M., 2008. *The Project of Process Improvement in the Treatment Plant of Kent Food Factory,* s.l.: Istanbul Technical University.

Packaging strategies, 2012. *Adventures in the Bio-based Plastics Market: New Pga Polymer Developments From Findland.* [Online]
Available at: http://www.packstrat.com/articles/84823-adventures-in-the-bio-based-plastics-market-new-pga-polymer-developments-from-finland
[Accessed 23 May 2015].

Pagliaro, M. & Rossi, M., 2008. *The future of glycerol: new uses of a versatile raw material.* s.l.:Royal Society of Chemistry.

PAMSA, 2012. *Pulp and Paper Summary 2011.* [Online]
Available at: http://www.thepaperstory.co.za/wp-content/uploads/2011/09/PAMSA_Pulp-Paper-Summary-findings-2011-FINAL_AUGUST2012.pdf
[Accessed 29 03 2016].

PAMSA, 2012. *The Paper Story.* [Online]
Available at: http://www.thepaperstory.co.za/wp-content/uploads/2011/09/PAMSA_Pulp-Paper-Summary-findings-2011-FINAL_AUGUST2012.pdf
[Accessed 29 03 2016].

PAMSA, 2015. *South African Pulp and Paper Industry Summary 2014.* [Online]
Available at: http://www.thepaperstory.co.za/wp-content/uploads/2011/09/PAMSA-2014-stats-report-final_Aug2015.pdf
[Accessed 06 05 2016].

Pandey, A., Soccol, C. & Larroche, C. eds., 2010. *Current Developments in Solid-State Fermentation.* Dordrecht: Springer press.

Park, J., Craggs, R. & Shilton, A., 2011. Wastewater treatment high rate algal ponds for biofuel production. *Bioresource Technology ,* 102(1), p. 35–42.

Park, J., Craggs, R. & Shilton, A., 2011. Wastewater treatment high rate algal ponds for biofuel production. *Bioresource Technology ,* 102(1), p. 35–42.

Parsons, R., Krugell, W. & Keeton, G., 2015. *Quoted in: mybroadband - South African electricity prices – 1994 to 2015.* [Online]
Available at: http://mybroadband.co.za/news/energy/130320-south-african-electricity-prices-1994-to-2015.html
[Accessed 03 March 2016].

Patni, N. & Jui, P., 1987. Changes in Solids and Carbon Content of Dairy-Cattle Slurry in Farm Tanks. *Biological Wastes,* Volume 20, p. 11–34.

Pearce, K. & Whyte, D., 2005. *Water and wastewater management in the oil refining and re-refining industry (NAtSurv 15) ,* Pretoria: Water Research Commission, South Africa.

Peccia, J., Haznedaroglu, B., Gutierrez, J. & Zimmerman, J. B., 2013. Nitrogen supply is an important driver of sustainable microalgae biofuel production. *Trends in Biotechnology,* 31(3), p. 134–138.

Pelletier, M. et al., 2013. An evaluation study of mycelium based acoustic absorbers grown on agricultural by-product substrates. *Industrial Crops and Products,* Volume 51, p. 480–485.

Pesticide Research Institute, 2013. *Vinasse.* [Online]
Available at: http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5105742
[Accessed 09 September 2014].

Petroleum Africa, 2008. *Chevron Opens largest Wastewater Treatment Plant in SA.* [Online]
Available at: http://www.petroleumafrica.com/chevron-opens-largest-wastewater-treatment-plant-in-sa/
[Accessed 23 February 2016].

Pitman, A. & Boyd, L., 1999. Transforming local government wastewater departments - From adversary to industrial partner. *Water Science and Technology,* 39(10-11), pp. 39-45.

Pollution Research Group, 2015. *Water and Wastewater Management in the Soft Drink Industry (NatSurv 3 Ed 2),* Pretoria: Water Research Commision, South Africa.

Polprasert, C., 2007. *Organic waste recycling: technology and management.* 3rd Ed ed. London: IWA Publishers.

Pott, R., Howe, C. & Dennis, J., 2013. Photofermentation of crude glycerol from biodiesel using Rhodopseudomonas palustris: Comparison with organic acids and the identification of inhibitory compounds. *Bioresource Technology,* Volume 130, pp. 725-730.

Prado, F. et al., 2005. Citric acid production by solid-state fermentation on a semi-pilot scale using different percentages of treated cassava bagasse. *Brazilian Journal of Chemical Engineering,* 22(4), pp. 547-555.

Prado, R., Caione, G. & Campos, N., 2013. Filter Cake and Vinasse as Fertilizers Contributing to Conservation Agriculture. *Appled and Environmental Soil Science,* pp. 1-8.

Prasad, M. (., 2015. *Bioremediation and Bioeconomy.* 1 ed. s.l.:Elsevier.

Project Assignments, n.d. *Project Assignments: News and latest projects.* [Online]
Available at: http://www.projass.co.za/?PageID=63
[Accessed 25 February 2016].

Quiroz, M. et al., 2013. Fish processing wastewater as a platform of the microalgal biorefineries. *Biosystems Engineering,* Volume 115, pp. 195-202.

Rajagopal, R. et al., 2013. Sustainable agro-food industrial wastewater treatment using high rate anaerobic process. *Water,* Volume 5, pp. 292-311.

Ramaswamy, S., Ramarao, B. & Huang, H.-J., 2013. *Separation and purification technologies in biorefineries.* Chichester: John Wiley.

Rand Water, n.d. *Water Situation in South Africa.* [Online]
Available at: http://www.waterwise.co.za/site/water/environment/situation.html
[Accessed 16 05 2016].

Randall, D., Nathoo, J. & Lewis, A., 2011. A case study for treating a reverse osmosis brine using Eutectic Freeze Crystallization—Approaching a zero waste process. *Desalination,* 266(1-3), p. 256 – 262.

Rawat, I., Bhola, V., Kumar, R. & Bux, F., 2013. Improving the feasibility of producing biofuels from microalgae using wastewater. *Environmental Technology,* 34(13-14), pp. 1765-1775.

Rawat, I., Ranjith Kumar, R., Mutanda, T. & Bux, F., 2011. Dual role of microalgae: Phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. *Applied Energy,* 88(10), pp. 3411-342.

RECORD, n.d. *Renewable Energy Center of Research and Development.* [Online]
Available at: http://www.record.org.za/
[Accessed 29 February 2016].

Reddy, C., Ghai, R., Reashmi, V. & Kalia, 2003. Polyhydroxyalkanoaes: an overview. *Bioresource technology,* 87(2), pp. 137-46.

Reinsel, M. A., n.d. *WaterWorld: Floating Wetlands help boost nitrogen removal in lagoons.* [Online]
Available at: http://www.waterworld.com/articles/print/volume-28/issue-6/editorial-features/floating-wetlands-help-boost--nitrogen-removal-in-lagoons.html
[Accessed 10 March 2016].

Rencken, G. & van Aartsen, J., 2008. *Secondary wastewater treatment plant for Mondi bussiness paper in Richards Bay,* Modderfontein, SOuth Africa: VWS Envig (Pty) Ltd.

Reneseng, n.d. [Online]
Available at: http://reneseng.com/
[Accessed 10 February 2016].

Reuters, 2016. *SA must admit national drought crisis to help farmers.* [Online] Available at: http://www.sabc.co.za/news/a/ef105a004b8cea139e89fe77bc6a42c4/P-South-Africa-must-admit-national-drought-crisis-to-help-farmers-20160302 [Accessed 10 06 2016].

Richardson, C., 2011. *Investigating the role of reactor design for maximum environmental benefit of algal oil for biodiesel. Unpublished Masters Thesis.* s.l.:Department of Chemical Engineering, University of Cape Town.

Rittmann, B. & McCarty, P., 2001. *Environmental biotechnology: principles and applications.* Boston: McGraw-Hill.

RMRD SA, n.d. *Scope of the Industry.* [Online] Available at: http://www.rmrdsa.co.za/Redmeatindustry/Scopeoftheindustry.aspx [Accessed 13 05 2016].

Roels, J., 1983. *Energetics and kinetics in biotechnology.* s.l.:Elsevier Biomedical Press.

Rose, P. et al., 2007. *HAPS and the treatment of domestic and industrial wastewaters Part 4: System performance and tertiary treatment operations,* Pretoria: Water Research Commission, South Africa.

Roux-Van der Merwe, M., Badenhorst, J. & Britz, T., 2005. Fungal treatment of an edible oil containing industrial effluent. *World Journal of Microbiology and Biotechnology,* Volume 21, pp. 947-953.

Rumjeet, S., 2016. *Title (Unpublished MSc Thesis).* s.l.:University of Cape Town.

Saadi, I., Laor, Y., Raviv, M. & Medina, S., 2007. Land spreading of olive mill wastewater: effect on soil microbial activity and potential phytotoxicity. *Chemosphere,* Volume 66, pp. 75-83.

SAB, 2013. *SAB Stories: Sustainability.* [Online] Available at: http://www.sabstories.co.za/sustainability/working-towards-zero-waste-operations/ [Accessed 24 February 2016].

SAB, 2014. *SAB Stories: Sustainablity - Making More Beer Using Less Water.* [Online] Available at: http://www.sabstories.co.za/sustainability/making-more-beer-using-less-water/ [Accessed 24 February 2016].

SABC, 2016. *SABC - Lower tariff increase due to Eskom's failure to meet deadlines.* [Online] Available at: http://www.sabc.co.za/news/a/6d445e804bf4adca8fc4bf1f0c850adb/LowerundefinedtariffundefinedincreaseundefineddueundefinedtoundefinedEskomsundefinedfailureundefinedtoundefinedmeetundefineddeadlines-20160803 [Accessed 09 March 2016].

SABIA, 2014. *Projects Data Base.* [Online] Available at: http://biogasassociation.co.za/website/wp-content/uploads/2014/08/SABIA-Projects-Data-base-May-2014.pdf [Accessed 24 February 2016].

SABIA, n.d. *South African Biogas Industry Association.* [Online] Available at: http://www.biogasassociation.co.za/ [Accessed 23 February 2016].

SAB, n.d. *SAB: Sustainable Development Projects - Water Resources.* [Online] Available at: http://www.sab.co.za/sablimited/content/all-sustainable-development-projects?oid=2668&sn=Detail&pid=2574 [Accessed 24 February 2016].

SAB, n.d. *Sustainable Development: Projects.* [Online] Available at: http://www.sab.co.za/sablimited/content/all-sustainable-development-

projects?oid=2547&sn=Detail&pid=2574
[Accessed 24 February 2016].

SADISTA, 2013. *South African Deep Sea Trawling Association: Facts and Figures.* [Online]
Available at: http://www.sadstia.co.za/the-fishery/facts-and-figures
[Accessed 21 05 2016].

SALGA, n.d. *South African Local Government Association.* [Online]
Available at: http://www.salga.org.za/
[Accessed 23 February 2016].

Salminen, E. & Rintala, J., 2002. Anaerobic digestion of organic solid poulty slaughterhouse waste - A review. *Bioresource Technology,* 83(1), pp. 13-26.

SAMPRO, 2014. *Dairy Industry Review 2014.* [Online]
Available at: http://www.sampro.co.za/images/stories/pdf/reviewapr14.pdf
[Accessed 10 06 2016].

SANEDI, 2014. *The State of Waste to Energy Research in South Africa: A Review,* Pretoria, South Africa: SANEDI; RECORD; GIZ.

SANEDI, n.d. *South African National Energy Development Institute.* [Online]
Available at: http://www.sanedi.org.za/
[Accessed 23 February 2016].

SAPIA, 2014. *South African Petroleum Industry Association 2014 Annual Report,* s.l.: SAPIA.

SAPPI, 2014. *Southern Africa Sustainability Report.* [Online]
Available at: http://www.sappi.com/group/Sustainability/2014-Sappi-Southern-Africa-Sustainability-Report.pdf
[Accessed 25 February 2016].

SASA, 2016. *South African Sugar Industry Directory 2014-2015.* [Online]
Available at: http://www.sasa.org.za/publications/IndustryDirectory.aspx
[Accessed 07 06 2016].

Satoh, H., Mino, T. & Matsuo, T., 1999. PHA production by activated sludge. *International production by activated sludge,* 25(1-3), pp. 105-109.

Satyawali, Y. & Balakrishnan, M., 2008. Wastewater treatment in molasses-based alcohol distilleries for COD and color removal: a review. *Journal of Environmental Management,* 86(3), pp. 481-497.

SAWIS, 2016. *SA Wine industry Statistics 2016, nr 40.* [Online]
Available at: www.sawis.co.za/info
[Accessed 04 06 2016].

Scharathow, R., 2012. *Driving the evolution of plastics-bioplastics markets and framework,* Berlin: Germany: European Bioplastisc.

Schenk, P. et al., 2008. Second generation biofuels: high efficiency microalgae for biodiesel production. *Bioenergy research,* 1(1), pp. 20-43.

Schneider, K., 2016. *News 24: Drought pushes South Africa to water, energy, and food reckoning.* [Online]
Available at: http://www.fin24.com/Opinion/drought-pushes-south-africa-to-water-energy-and-food-reckoning-20160124
[Accessed 03 March 2016].

Schutze, E., n.d. *Liquid fuel from coal.* [Online]
Available at:
http://www.mediaclubsouthafrica.com/component/content/article?id=123:liquid-fuel-from-coal
[Accessed 18 03 2016].

Seggie, E., 2011. *Wastewater treatment pilot making green-brewing strides at PE plant.* [Online] Available at: http://www.engineeringnews.co.za/article/environmentally-friendly-plant-treats-brewery-effluent-2011-04-15
[Accessed 24 February 2016].

Seghezzo, L. et al., 1998. A review: the anerobic treatment of sewage in UASB and EGSB reactors. *Bioresource Technology,* 65(3), pp. 175-190.

Selena Oil and Gas, n.d. *Glossary & Unit conversion.* [Online] Available at: http://www.selenaoil.com/eng/glossary--unit-conversion/
[Accessed 23 03 2016].

Sheik, A., Muller, E. & Wilmes, P., 2014. A hundred years of activated sludge: time for a rethink. *Frontiers in Microbiology,* 5(MAR), p. Article 47.

Shen, L., Haufe, J. & Patel, M., 2009. *Product overview and market projection of emerging bio-based plastics,* Utrecht: Netherlands: European Polysaccharide Network of Excellence.

Shih, I.-L. & Wu, J.-Y., 2009. Biosynthesis and application of poly (γ-glutamic acid). In: B. Rehm, ed. *Microbial production of biopolymers and polymer precursors: Applications and perspectives.* Norfolk: Caister Academic Press.

Shivajirao, P., 2012. Treatment of Distillery Wastewater Using Membrane Technologies. *International Journal of Advanced Engineering Research and Studies,* 1(3), pp. 275-283.

Shizas, I. & Bagley, D., 2004. Experimental determination of energy content of unknown organics in municipal wastewater streams. *Journal of Energy Engineering,* 2(45-53), p. 130.

Shuler, M. & Kargi, F., 1992. *Bioprocess Engineering: Basic Concepts.* New Jersey: Prentice-Hall Inc.

Shyu, Y.-S., Hwang, J.-Y. & Hsu, C.-K., 2008. Improving the rheological and thermal properties of wheat dough by the addition of c-polyglutamic acid. *LWT – Food Sci Technol,* Volume 41, p. 982–987.

Siebrits, R., Winter, K. & Jacobs, I., 2014. Water research paradigm shifts in South Africa. *South African Journal of Science,* 110(56), pp. 2013-0296.

Singhania, R., Patel, A., Soccol, C. & Pandey, A., 2009. Recent advances in solid-state fermentation. *Biochemical Engineering Journal,* Volume 44, p. 13–18.

Singh, B. et al., 2015. Sustainable production of biofuels from microalgae using a biorefinery approach. In: G. Kaushik, ed. *Applied Environmental Biotechnology: Present Scenario and Future Trends.* New Delhi: Springer , pp. 115-128.

South Africa. Department of Science and Technology, 2001. *A National Biotechnology Strategy for South Africa,* s.l.: s.n.

SSP PVT Limited, n.d. *Zero Liquid Discharge Solution for Distillery Effluent.* [Online] Available at: http://www.industrialeffluenttreatment.com/molasses-baseddistilleries.
[Accessed 27 September 2014].

Stafford, W. et al., 2013. Technologies for recovery of energy from wastewaters: Applicability and potential in South Africa.. *Journal of Energy in Southern Africa,* 24(1), pp. 15 - 26.

Stamets, P., 1993. *Growing gourmet and medicinal mushrooms.* s.l.:Ten speed press.

Steffan, Robertson and Kirsten Inc, 1989a. *Water and Wastewater Management in the Dairy Industry (NatSurv 4),* Pretoria: Water Research Commission, South Africa.

Steffen Robertson and Kirsten Inc, 1989. *Water and Wastewater Management in the Red Meat Industry (NatSurv 7),* Pretoria: Water Research Commission, South Africa.

Steffen, Robertson & Kirsten Inc, 1989d. *Water and Watewater Management in the Edible Oil Industry (NatSurv 6),* Pretoria: Water Research Commission, South Africa.

Steffen, Robertson and Kirsten Inc, 1990. *Water and Wastewater Management in the Sugar Industry (NatSurv 11),* Pretoria: Water Research Commission, South Africa.

Steffen, Robertson and Kirsten Inc, 1993. *Water and Wastewater Management in the Textile Industry (NatSurv 13).* Pretoria, Water Research Commission, South Africa.

Steffen, Robertson and Kristen Inc, 1989b. *Water and Wastewater Management in the Poultry Industry (NatSurv 9),* Pretoria: Water Research Commission, South Africa.

Stensel, H. & Burton F, 2003. *Wastewater Engineering: Treatment and Reuse.* New York: Mc Graw Hill.

Stephenson, T., Judd, S., Jefferson, B. & Brindle, K., 2000. *Membrane Bioreactors for Wastewater Treatment.* London: IWA Publishing.

Strande, L., Ronteltap, M., Brdjanovic, D. & (eds), 2015. *Faecal Sludge Management: Systems Approach for Implementation and Operation.* s.l.:IWA Publishing.

Strydom, J., Britz, T. & Mostert, J., 1997. Two-phase anaerobic digestion of three different dairy effluents using a hybrid bioreactor. *Water SA,* 23(2), p. 151–156.

Sugama, S. & Okazaki, N., 1979. Growth estimation of Aspergillus oryzae cultured on solid media. *Journal of Fermentation Technology,* Volume 57, pp. 408-412.

Sumardiono, S., Syaichurrozi, I. & Sasong, S., 2013. The effect of COD/N ratios and pH control on biogas production from vinasse. *International Journal of Biochemistry Research & Review,* 3(4).

Surujlal, S., Tivchev, G., Kasan, H. & Bux, F., 2004. *Development of biological treatment technology for the remediation of edible oil effluent,* Pretoria: Water Research Commission, South Africa.

Surujlal, S., Tivchev, G., Kasan, H. & Bux, F., 2004. *Development of biological treatment technology for the remediation of edible oil effluent,* Pretoria: Water Research Commission, South Africa.

SWPN, 2013. *Strategic Water Partners Network: Closing the water gap by 2030.* [Online] Available at: http://library.constantcontact.com/download/get/file/1107312969272-337/SWPN-SA+brochure+2013.pdf
[Accessed 22 February 2016].

SWPN, 2015. *Strategic Water Partners Network: Creating Shared Value through Innovative Partnerships.* [Online]
Available at: http://www.sabmiller.com/docs/default-source/stories-files/keeping-sa's-taps-running/about-the-strategic-water-partners-network.pdf
[Accessed 22 February 2016].

Tamis, J. et al., 2014. Enrichment of Plasticicumulans acidivorans at pilot-scale for PHA production on industrial wastewater PHA production on industrial wastewater. *Journal of Biotechnology,* Volume 192, p. 161–169.

Tanimoto, H. et al., 2007. Acute effect of poly-cglutamic acid on calcium absorption in post-menopausal women. *J Am Coll Nutr,* Volume 26, p. 645–649.

Tawfik, A., Klapwijk, B., el-Gohary, F. & Lettinga, G., 2001. Treatment of anaerobically pre-treated domestic sewage by a rotating biological contactor. *Water Research,* Volume 36, pp. 147-155.

Tchobanoglous, G., Burton, F. & Stensel, H., 2003. *Wastewater Engineering: Treatment & Reuse.* 4th Ed ed. Columbus: McGraw Hill Education.

Theobald, D., 2015. *How is solids-separation controlled in primary wastewater treatment.* [Online] Available at: http://www.processingmagazine.com/magazine/columns/how-is-solids-separation-controlled-in-primary-wastewater-treatment/

Thompson, J. C. & He, B. B., 2006. Characterisation of crude glycerol from biodiesel production from multiple feedstocks. *Applied engineering in Agriculture,* 22(2), p. 261.

Tiepelt, M., 2015. *Biogas in South Africa (SAIREC 2015).* [Online]
Available at: http://www.sairec.org.za/wp-content/uploads/2015/10/M-Tiepelt-SAIREC-Conference.pdf
[Accessed 24 February 2016].

Tokiwa, Y., Calabia, B., Ugwu, C. U. & Aiba, S., 2009. Biodegradability of plastics. *International Journal of Molecular Sciences,* 50(3), pp. 3722-3742.

Tomszak-Wandzel, R., Górniaczyk, J. & Mędrzycka, K., n.d. *Anaerobic treatment of distillery wastewaster.* s.l.:Gdansk University of Technology.

Toprak, H., 2006. *Wastewater Treatment Plant Design.* [Online]
Available at: http://web.deu.edu.tr/atiksu/ana52/wdesign06.html
[Accessed 03 04 2016].

Tshwarisano, L., 2016. *Sasol builds water treatment pilot plant at Sasol One site.* [Online]
Available at:
http://www.tshwarisano.co.za/index.php?option=com_content&view=article&id=133:sasol-builds-water-treatment-pilot-plant-at-sasol-one-site&catid=35:current-newsletter&Itemid=57
[Accessed 25 February 2016].

Turpie, J. & Malan, H., 2010. *Wetland valuation. vol III - a tool for the assessment of the livelihood value of wetlands.,* Pretoria: Water Research Commission, South Africa.

Uchrin, C., Hunter, J., Park, S. & Vadas, T., 2005. In situ Measurement of Macrophyte Photosynthesis and Respiration in Shallow Lakes. *Journal of Environmental Engineering,* 131(2), pp. 315 - 319.

Uotani, K., Hidetoshi, K., Endou, H. & Tokita, F., 2011. *Sialogogue, oral composition and food product containing the same.* USA, Patent No. US Patent 7910089 B.

Vadivel, R. et al., 2014. Significance of vinasses waste management in agriculture and environmental quality-Review. *African Journal of Agricultural Research,* 9(38), pp. 2862-2873.

Van den Berg, M., 2009. *The South African Wastewater Market - Business opportunities and export promotion for Dutch companies. MSc Thesis.* Netherlands: University of Twente.

Van Hille, R., Marais, T. & Harrison, S., 2015. *Biomass retention and recycling to enhance sulfate reduction kinetics.* s.l., s.n.

Van Schie, P. & Young, L., 1998. Isolation and characterization of phenol-degrading denitrifying bacteria. *Applied Environmental Microbiology,* Volume 64, pp. 2432-2438.

Van Zyl, P., 2008. *Anaerobic Digestion of Fischer-Tropsch Reaction Water: Submerged Membrane Anaerobic Reactor Design, Performance Evaluation & Modeling. PhD Thesis.* s.l.:University of Cape Town.

Veolia, n.d. *Veolia News & Media: Case Studies - Chevron Refinery.* [Online]
Available at: http://www.veoliawaterst.co.za/medias/case-studies/ImproChem_Chevron_Refinery_CapeTown_SouthAfrica_Case_Study.htm
[Accessed 23 February 2016].

Veolia, n.d. *Veolia News & Media: Case Studies - Shell and BP.* [Online]
Available at: http://www.veoliawaterst.co.za/medias/case-studies/Shell_BPSouthAfrican_Petroleum_Refineries_SAPREF_Durban_SouthAfrica_Case_Study.htm
[Accessed 23 February 2016].

Veolia, n.d. *Veolia: News & Media - Veolia's first Biothane Biobulk CSTR installation converts effluent to energy.* [Online]
Available at: http://www.veoliawaterst.co.za/medias/Newsletter-Articles/Distell-Biobulk-News.htm
[Accessed 26 February 2016].

Veolia, n.d. *Water Solutions: Wastewater Treatment Systems.* [Online]
Available at: http://www.veoliawaterst.co.za/water-solutions-south-africa/wastewater-treatment/
[Accessed 26 February 2016].

Verlinden, R. et al., 2007. Bacterial synthesis of biodegradable polyhydroxyalkanoates. *Journal of Applied Mircobiology,* 102(6), pp. 1437-1449.

Verrecht, B. et al., 2010. The cost of a large-scale hollow fibre MBR. *Water Research,* 44(18), p. 5274–5283.

Verster, B., 2016. *Susanna Forum.* [Online]
Available at: http://forum.susana.org/forum/categories/98-resource-recovery-from-excreta-faecal-sludge-or-wastewater/17470-solid-substrate-fermentation-ssf-for-faecal-sludge

Verster, B. et al., 2013. *Introducing the wastewater biorefinery concept: A scoping study of poly-glutamic acid production from a Bacillus-rich mixed culture using municipal waste water,* Pretoria: Water Research commission.

Verster, B. et al., 2014. *Introducing the wastewater biorefinery concept.TT 587/13.,* University of Cape Town: Water research commission.

Vroman, I. & Tighzert, L., 2009. Biodegradable Polymers. *Materials,* Volume 2, pp. 307-344.

Vymazal, J., 2014. Constructed wetlands for treatment of industrial wastewaters: A review. *Ecological Engineering,* Volume 73, p. 724–751.

Wackernagel, M. et al., 2002. Tracking ecological overshoot of human ecology. *Proceedings of the National Academy of Sciences of the United States of America,* 99(14), pp. 9266-9271.

Wang, Q. et al., 2008. Coproducing lipopeptides and poly-c-glutamic acid by solid-state fermentation of Bacillus subtilis using soybean and sweet potato residues and its biocontrol and fertilizer synergistic effects. *Bioresource Technology,* Volume 99, p. 3318–3323.

Wang, X. et al., 2005. Nutrients removal from municipal wastwater by chemical precipitation in a movng bed biofilm reactor. *Process Biochemistry,* Volume 41, pp. 824-828.

Water21, 2007. *Cutting consumption on the Gold coast.* [Online]
Available at: http://www.eu-neptune.org/Publications%20and%20Presentations/Werker_-_Water21_Dec_2007.pdf
[Accessed 20 May 2015].

WEF FD-16, 2010. *Natural systems for wastewater treatment: WEF manual of practice no. FD-16.* Alexandria, VA : Water Environment Federation, USA.

WEF, 2005. Chapter 5 - Solids thickening and dewatering systems. In: *National manual of good practice for biosolids.* s.l.:Water Environment Foundation.

WEF, 2010. *Biofilm Reactors by WEF.* s.l.:Mc Graw Hill.

Weidmann-Marscheider, F., Schleich, J., Husing, B. & Angerer, G., 2005. *Techno-economic Feasibility of Large-scale Production of Bio-based Polymers in Europe,* Utrecht: European commission.

Welz, P. et al., 2015. *Treatment of winery wastewater in unplanted constructed wetlands,* Pretoria: Water Research Comission, South Africa.

Werker, A., 2008. *Production of biopolymers as a by-product of industrial wastewater treatment.* s.l., s.n.

Western Cape Business News, 2015. *ENERGY: Energy from Effluent in Anaerobic Waste Water Treatment Facility to Open in Stellenbosch, Western Cape.* [Online]
Available at:
http://www.wcbn.co.za/articles/dailynews/4579/Energy+from+Effluent+in+Anaerobic+Was

te+Water+Treatment+Facility+to+Open+in+Stellenbosch%2C+Western+Cape.html [Accessed 26 February 2016].

Wijekoon, K., Visvanathan, C. & Abeynayaka, A., 2011. Effect of organic loading rate on VFA production, organic matter removal and microbial activity of a two-stage thermophilic anaerobic membrane bioreactor. *Bioresource Technology,* Volume 102, p. 5353–5360.

Wikimedia, n.d. *Polyglutamins.* [Online] Available at: https://upload.wikimedia.org/wikipedia/commons/5/59/Polyglutamins%C3%A4ure.svg [Accessed 02 06 2016].

Wikipedia, n.d. *Sankey diagram.* [Online] Available at: https://en.wikipedia.org/wiki/Sankey_diagram [Accessed 01 06 2016].

William, B., McMullan, J. & McCahey, S., 2001. An initial assessment of spent mushroom compost as a potential energy feedstock. *Bioresource Technology,* Volume 79, pp. 227-230.

Winpenny, J., Heinz, I. & Koo-Oshima, S., 2010. *The Wealth of Waste: The economics of wastewater use in agriculture,* Rome: Food and Agriculture Organisation of the United Nations.

WISA, 2009. *Green Drop Certification.* [Online] Available at: http://www.ewisa.co.za/misc/BLUE_GREENDROPREPORT/GreenDrop_certification.htm [Accessed 01 October 2014].

Witkamp, G., Vrijenhoef, J., De Graauw, J. & van der Ham, F., 2006. *Crystillization of Materials from Aqueous Solutions.* USA, Patent No. 7 127 913.

Wolkers, H. et al., 2011. *Microalgae: the green gold of the future? Large-scale sustainable cultivation of microalgae for the production of bulk commodities.* [Online] Available at: http://www.groenegrondstoffen.nl/downloads/Boekjes/12Microalgae_UK.pdf [Accessed 25 05 2016].

Worcester Standard, 2016. *RCL Foods going green.* [Online] Available at: http://www.netwerk24.com/ZA/Worcester-standard/Nuus/rcl-foods-going-green-20160217-2 [Accessed 22 02 2016].

World Dairy Summit, 2012. *SA produces more milk, but producer numbers drop.* [Online] Available at: https://worlddairysummit2012.wordpress.com/2012/11/06/sa-produces-more-milk-but-producer-numbers-drop/ [Accessed 03 06 2016].

WOSA, 2015. *South Africa Wine Industry Stantistics.* [Online] Available at: http://www.wosa.co.za/The-Industry/Statistics/SA-Wine-Industry-Statistics/ [Accessed 10 06 2016].

WRC SA, 2015b. *Water Research Commision: Knowledge Hub.* [Online] Available at: http://www.wrc.org.za/Pages/KH_AdvancedSearch.aspx?k=natsurv&ww=1& [Accessed 18 March 2016].

WRC SA, 2015. *Private Correspondence.* s.l.:Water Reseach Commission, South Africa.

WRC SA, 2015. *South Africa's Water Research, Development and Innovation (RDI) Roadmap: 2015-2025,* Pretoria: Water Research Commission, South Africa.

WRC SA, 2016a. *NatSurv Reports.* [Online] Available at: http://www.wrc.org.za/Pages/KH_AdvancedSearch.aspx?k=NatSurv&ww=1& [Accessed 23 May 2016].

WRC SA, 2016b. *Knowledge Hub: Mine Water Category.* [Online]
Available at: http://www.wrc.org.za/Pages/KH_DocumentsList.aspx?su=c7&ms=2;77
[Accessed 23 May 2016].

WRC SA, October 2015. *Correspondence with Water Research Commission (South Africa).* s.l.:s.n.

Wu, T., Zivanovic, S., Draughon, F. & Sams, C., 2004. Chitin and chitosan--value-added products from mushroom waste. *Journal of Agricultural and Food Chemistry,* 52(26), pp. 7905-7910.

Wu, W., 2015. *Development of a Plant-Wide Steady-State WastewaterTreatment Plant Design and Analysis Program (Masters Thesis).* s.l.:University of Cape Town.

X-rates, 2015. *Monthly Average.* [Online]
Available at: http://www.x-rates.com/average/?year=2005
[Accessed 21 January 2015].

Xu, J., Chen, S. & Yu, Z., 2005. Optimization of process parameters for poly-glutamate production under solid state fermentation from Bacillus subtilis CCTCC202048. *Process Biochemistry,* 40(9), pp. 3075-3081.

Yasuzawa, M. et al., 2011. Highly selective needle-type glucose sensors prepared by the immobilization of glucose oxidase on gamma-polyglutamic acid film. *Anal Sci,* Volume 27, p. 337–340.

Yong, X. et al., 2011. Optimization of the production of poly- gamma- glutamic acid by Bacillus amyloliquefaciens C1 in solid-state fermentation using dairy manure compost and monosodium glutamate production residues as basic substrates. *Bioresource Technology ,* Volume 102, pp. 7548-7554.

Young, T., Muftugil, M., Smoot, S. & Peeters, J., 2012. MBR vs. CAS: capital and operating cost evaluation. *Water Practice & Technology,* 7(4).

Youssef, N. et al., 2004. Comparison of methods to detect biosurfactant production by diverse microorganisms. *Journal of Microbiological Methods,* 56(3), pp. 339-347.

Zeng, W. et al., 2012. Studies on the UV spectrum of poly(-glutamic acid) based on development of a simple quantitative method. *International Journal of Biological Macromolecules,* Volume 51, pp. 83-90.

ZERI, n.d. *Zero Emmissions Research Initiatives.* [Online]
Available at: http://www.zeri.org/ZERI/Beer.html
[Accessed 03 06 2016].

Zhang, D. et al., 2012. Economical production of poly(gamma-glutamic acid) using untreated cane molasses and monosodium glutamate waste liquor by Bacillus subtilis NX-2. *Bioresource Technology,* Volume 114, pp. 583-588.

Zhang, Z.-Q.et al., 2007. Production and application of a novel bioflocculant by multiple-microorganism consortia using brewery wastewater as carbon source. *Journal of Environmental Sciences,* Volume 19, p. 667–673.

Zhuwakinyu, M., 2012. *Creamer Media's Water Repor.* [Online]
Available at: http://pmg-assets.s3-website-eu-west-1.amazonaws.com/120904review_0.pdf
[Accessed 07 March 2016].

A WRC WASTEWATER REPORTS

From the 2410 WRC reports provided in the database and discussed in Section 2.5 232 reports were selected (based on title and a brief overview of executive summary, in most cases) for potential relevance to wastewater biorefineries. These 232 reports, spanning 31 years of research from 1984 to 2014, are listed here. The reports are sorted on category first, then date of publication. An electronic copy of the table can be found at https://www.dropbox.com/s/m2padaunrdsi1cc/WRC_reports_12mar15.xls?dl=0

Table: A-1: Analysis of WRC wastewater report references

Category	Ref No	Authors & Title of Report	Year of publication	Value of research in context of wastewater biorefineries	Shortcoming of research in context of wastewater biorefineries / more work required
A	1	2085/1/14: Mitchell SA ,de Wit MP, Blignaut JN, Crookes D. Wastewater treatment plants: the financing mechanisms associated with achieving green drop rating	2014	Financing mechanisms of wastewater treatment plants	Improve the performance of WWTWs through providing an incentive to the works in the form of a scoring system. Limited applicability to WWBR, except as an operational incentive mechanism.
A	2	TT 588/13: Armitage N, Fisher-Jeffes L, Carden K, Winter K, Naidoo V, Spiegel A, Mauck B, Coulson D. Water Sensitive Urban Design (WSUD) for South Africa: Framework and guidelines	2014	Biological and chemical treatment of associated contaminants, drainage and the management of industrial effluents. Water-Energy-Food Nexus. Wastewater re-use and minimisation.	Big picture of WWBR and beyond.
A	3	1826/1/13: Armitage NP, Vice M, Fisher-Jeffes L, Winter K, Spiegel A, Dunstan J. Alternative Technology for Stormwater Management	2013	Consider storm water as part of the urban water cycle	Integrated WWBR in urban environments. Using the sustainable drainage as (macrophyte) reactors.
A	4	1941/1/13: Naidoo N, Longondjo C, Vrdoljak M. Investigating operations and indigenous knowledge of water use and waste management, and establishing ways to integrate them into water services management	2013	This research was aimed at introducing communities, municipalities, practitioners, etc across South Africa to alternative ways of managing water and to allow indigenous knowledge to inform future policies. The report finds in line with general consensus that indigenous practices are environmental sustainable.	Adapting Indigenous water knowledge (IWWM) practice to suit current conditions requires that planners understand the full local environmental implication of the technology before it can be implemented. It was concluded that IWWM could assisting in addressing various challenges currently facing the water sector, and in the WWBR context how the system could fit together.

Category	Ref No	Authors & Title of Report	Year of publication	Value of research in context of wastewater biorefineries	Shortcoming of research in context of wastewater biorefineries / more work required
A	5	2087/1/P/1: SG Hosking SG, Jacoby KT, Trends in the Insight into the Growing South African Municipal Water Service Delivery Problem	2013	The study investigates the setting of water tariffs that cover costs and satisfy demand. An analysis of efficiency in the mix of water service output is one that aims to match demand to the service produced.	Evidence of failures in water service delivery are mounting, due to lack of political will, funding, low skill capacity. Study contextualizes and analyses this situation. Applicable to WWBR in that WWBRs and WWTWs would be run by a similar system, and so curreny weaknesses would likely carry over to WWBRs.
A	6	TT 518/12: Schulze RE, A 2011 perspective on climate change and the South African Water Sector	2012		The effect of climate change on hydrological responses. Predictive scenarios, indicating risk levels, for the biophysical changes associated with projected climatic change for climatically divergent catchments in South Africa were then developed.
A	7	1921/1/12: Malan HL, Day JA, Water Quality and Wetlands: Defining Ecological Categories and Links with Land-use	2012		Possible application to WWBR macrophyte bioreactor.
A	8	1840/1/11: Jeleni A, van Rooyen PG, Behrmann D, Nyland G, Hatting L, Sussens H, Integrating Water Resources And Water Services Management Tools	2011	An approach for integrating Water Resources and Water Services management tools, and to develop a Generic Integrated Framework, which can incorporate relevant and appropriate water management tools that are used both in water resources and water services	Water resource tools could be used in WWBRs, in a similar way to their current use.
A	9	1839/1/10: Braid S, Görgens A, (editors). Towards the development of IWRM implementation indicators in South Africa	2010	The meeting with the Municipality raised some very pertinent points about integration vs. co-ordination, and notification vs. engagement between the government institutions, both across sectors and across spheres of government, highlighting the 'edge' effects in the institutional structure. The examples provided by the Municipality (although one-sided), suggest that IWRM is understood, but that the hurdle lies with the administration and implementation.	Only partially relevant as a way to establish the understanding of WWBR by different stakeholders.
A	10	TT 395/09: Oosthuizen NL, Bell J, Managing your wastes to achieve legal compliance: An industry guide (and TT 396/09 ,TT 397/09 TT, 398/09)	2009	Managing waste to achieve legal compliance	Legal compliance of wastes management for industry, that WWBR should also comply with.
A	11	1449/1/07: Nogni EV, Musvoto, Ramphao MC, Characterisation of Wastewater from Low Income High Density residential	2007	Characterisation of Wastewater from Low Income High Density residential areas	Need more work to fully characterize WW for bioprocess applications. WW found to be within assumptions for municipal WW.
A	12	TT 310/07: Duncker LC, Matsebe GN, Nancy, The social/cultural acceptability of using human excreta (Faeces and Urine) for	2007	The social/cultural acceptability of using human excreta (Faeces and Urine) for food production in rural settlements in South Africa	General overview of human consideration of waste – social aspect. Further work required on where this acceptance boundary lies, with e.g. bulk chemicals

Category	Ref No	Authors & Title of Report	Year of publication	Value of research in context of wastewater biorefineries	Shortcoming of research in context of wastewater biorefineries / more work required
		food production in rural settlements in South Africa			
A	13	1479/1/06: Murphy OK, A scoping study to evaluate the fitness-for-use of greywater in urban and peri-urban agriculture	2006	An investigation of re-use of greywater – including volume assessment, composition and characteristics.	More work required to contextualize (modular) wastewater biorefinery units in the urban and peri-urban context – starting with a feasibility study
A	14	1344/1/06: Banister S, Zhao B, Coetser SE, Pulles W, The assessment and classification of inorganic manganese containing wastes.	2006	Including biological treatment of Mn	Not only for Mn recovery, but may be a good supplement for Mn-requiring biocatalysts (PGA producer)
A	15	1548/1/06: Brice J, Sevitz J, Cornelius J, Guidance for the classification, rating and disposal of common hazardous waste streams	2006	Hazardous solid waste (chemcial, electronics, medical)	Limited application to WWBR, may apply when these streams are used in WWBR context, when knowledge of their management becomes necessary.
A	16	KV 166/05: Muller JR, Approaches to abattoir effluent treatment.	2005	This report details the full scale use of a modified Sequential Batch Reactor process for the pre-treatment of abattoir effluent and for protein production. A costing and pay-back analysis is also presented.	The technology presented could be very useful in a WWBR dealing with abattoir wastewater. The report demonstrates the full scale operation of an economically sound process.
A	17	1430/1/05: Schulze RE, Climate change and water resources in Southern Africa	2005	Management of scarce water resources with climate change implications	Context
A	18	1467/2/05: Cullis J, Rossouw JN, Gorgens AHM, First order estimate of the contribution of agriculture to non-point source pollution in three SA catchments: Salinity, Nitrogen and Phosphorus.	2005	Agriculture, in its broadest sense, appeared to have a major impact on salinity loads, particularly in areas with a high degree of irrigation and natural saline geology. It was also found that the net agriculture non-point source (ANPS) load was greatest during the wet season and in some cases, such as in the Breede, there appeared to be a "first flush" impact at the start of the wet season.	Contextual information, argument for more integrated nutrient cycles.
A	19	KV 151/04: Murray K, du Preez M, Lebone M; Pearson IA, Understanding the sustainable management of small water treatment plants in rural communities: a systems thinking study	2004	Besides technical issues, a number of social and institutional issues were noted as having received inadequate attention in the past. It was believed that this was often responsible for lack of sustainability. This report investigates a "systems thinking" approach to a better understanding of these issues and their inter-relationships in this challenging context. The objectives of this project were as follows: To test the use of a systems approach for analysing the issues affecting sustainable management of small water treatment plants in	Methodology of systems thinking. WWBR needs to give attention to four potential barriers to information flow: Community articulation of needs and supplier receptiveness to those needs, and, Supplier articulation of potential solutions and community receptiveness to those solutions.

Category	Ref No	Authors & Title of Report	Year of publication	Value of research in context of wastewater biorefineries	Shortcoming of research in context of wastewater biorefineries / more work required
				rural communities. To test the use of a systems approach for developing generic process guidelines that will complement existing technical guidelines and facilitate sustainable management in future.	
A	20	456/1/04: Barclay S; Buckley CA , The regional treatment of textile and industrial effluents	2004	Regional relevance	
A	21	1033/1/04: Pillay VL, Caustic management and reuse in the beverage bottling industry	2004	The report details the use of membrane separations in order to recycle used caustic solution.	Limited direct applicability of caustic recycling to WWBRs, but membrane separations technology will be important in WWBRs and so the methods used to evaluate membranes in this study could be adapted for use in WWBRs.
A	22	1184/1/04: Freese SF, Trollip DL, Nozaic DJ, Manual for testing of water and wastewater treatment chemicals	2004	Manual of standard procedures for wastewater authorities to use for evaluation of the chemicals used in water and wastewater treatment.	The methodologies set out in this manual will be important to use in WWBR reagent testing.
A	23	1191/1/03: Cloete TE, Thantsha M, Microbial characterization of activated sludge mixed liquor suspended solids	2003	An important design parameter in activated slude WW treatment is active biomass. The objective of this investigation was to use ATP as a method to determine the active biomass fraction in activated sludge, from commercial plants.	The methodology shown in this report could be very useful in WWBRs, however, further work is required to demonstrate that the method works accurately in other systems.
A	24	820/1/00: Naidoo V, Buckley CA, Municipal wastewater characterization: Application of denitrification batch tests	2000	Municipal wastewater characterization. Wastewater is a complex substrate consisting of compounds of differing biodegradability. The organic matter is discussed in terms of chemical oxygen demand (COD). Biokinetically, these compounds have been divided into readily biodegradable (RBCOD), slowly biodegradable (SBCOD) and unbiodegradable substrate groups. Compounds with intermediate biodegradability i.e. compounds which fall between the RBCOD and SBCOD groups, have been termed readily hydrolyzable organic substrates (RHCOD). The readily biodegradable and readily hydrolyzable COD fractions of wastewater can be determined by respirometric tests such as the oxygen utilization rate (OUR) and nitrate-N utilization rate (NUR) tests.	Limited application to WWBR, gives somewhat of an overview of municipal wastewater composition. Also perhaps more up to date reports exist.
A	25	201/1/99: Buckley CA, Research into the treatment of inorganic brines and concentrates	1999		
A	26	241/1/98: Pillay VL, Research on the filtration of compressible cakes	1998	Filtration is widely employed in the water industry, for the clarification of suspensions, the concentration of suspensions and the dewatering of sludges. In most instances, the cakes formed are compressible, i.e. it undergoes changes to its structure and properties during the	Applicable to WWBRs for product recovery and unit operation.

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				filtration process. This can significantly affect the performance of the filter, as well as introduce seemingly spurious system behaviours. The objectives of this project are as follows : (i) to investigate the mechanisms responsible for compressible cake behaviour, (ii) to investigate the effects that compressible cakes have on filtration systems, (iii) to investigate methods to characterise cake compressibility, (iii) to identify and develop models and equations to predict filtration performance for compressible cake systems.	
A	27	708/1/98: Du Pisani JE,The operation and maintenance of settled sewerage (SS) systems in South Africa	1998	the operation and maintenance requirements of settled sewerage systems in South Africa.	Design requirements of equipment. Social understanding and acceptance
A	28	239/1/98: Cowan JAC, The transfer of waste-water management technology to the meat processing industry	1998	This report details the deployment of a pilot plant equipped with ultrafiltration and reverse osmosis to be used by a major inndustrial abattoir, to test the system's capabilities at no significant financial or technical risk to the industrial partner.	The technology implemented here at pilot scale could be of use in a WWBR, for the concentration of high COD wastewaters. However, applicability in a number of industrial wastewaters needs to be demonstrated.
A	29	161/1/94: Gubb & Inggs Ltd, University of Natal, Research into the treatment of wool scouring effluents	1994	the liquid wastes emanating from the commercial scouring of such wool have long been regarded as highly polluting and difficult to treat. Includes a techno-economic feasilibilty study	further work be carried out on the use of dynamic membranes for the treatment of wool scouring effluent,
A	30	TT 45/90: SRK, A guide to water and waste-water management in the red meat abattoir	1990		
A	31	TT 46/90: SRK, A guide to water and waste-water management in the poultry abattoir industry	1990	Poultry abattoirs are graded according to their maximum permissible throughput into five grades namely AP (> 10 000 birds/day) to EP (maximum 50 birds/day). AP-grade poultry abattoirs constitute only a small fraction (13%) by number of the total number (149) of abattoirs in the RSA (1990 values) but carry out the bulk of the production, namely more than 93% of the total number of broilers processed annually. Opportunities for reclaiming and recycling water are identified, and the potential for water saving in the Industry is indicated to be around 1 600 Mi/a, which is equivalent to 29% of current consumption by the Industry. In large, modern, AP-grade abattoirs, the specific effluent volume (SEV) is typically around 15 i/bird and the specific pollutant load (SPL) in terms of chemical oxygen demand (COD) is typically around 27 g COD/bird. The	Effective usage of water in the poultry industry to comply to meat hygiene regulations as well as devising treatment methods to reduce pollutant load in the wastewater. Can this wastewater be used in a WWBR? How out of date is this information?

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				principal contributors to SEV and SPL are the operations carried out for evisceration (33% of SEV, 48% of COD SPL), washdown (22% of SEV, 35% of COD SPL) and scalding (17% of SEV, 11%of COD SPL). Nationally, pollutant loads estimated for the Industry are an effluent volume of 4 900 Mi/a and mass pollutant discharges of 10 255 t/a of COD, 2 450 t/a of SS and 4 970 t/a of TDS.	
A	32	TT 48/90: University of Natal, A guide for the planning waste-water treatment plants in the textile industry Part 3: Closed-loop treatment/recycle options for textile scouring bleaching and mercerising effluents	1990	The project focussed on textile wastewaters; characterising effluents, developed possible treatment options for each effluent, assessed each system at laboratory and pilot-scale and developed basic design criteria for the implementation and installation of selected systems.	The methods developed in this report could be applied to WWBRs which treat WWs from textile plants.
A	33	75/1/90: Beekman HG, Klopper DN, Fawcett KS, Construction and operation of the Cape Flats water reclamation plant and the surveillance of the reclaimed water quality	1990	The project developed a large scall process to produce potable water from the Cape Flats wastewater treatment facility, and integrate this water with current water delivery.	The technology deployed in this report is one which could be utilised by WWBRs in their final production of potable water.
A	34	106/3/87: University of Natal, Investigations into water management and effluent treatment in the fermentation industry	1987	An investigation into the water management and effluent treatment in the processing of (i) Pulp and Paper, (ii) Metals, (iii) Fermentation Products and (iv) Pharmaceutical products	Survey on wastewater management and effluent treatment/control in the fermentation industry
A (SOCIAL)	35	TT 564/13: Wall K, Ive O, Social Franchising Partnerships for Operation and Maintenance of Water Services: Lessons and Experiences from an Eastern Cape Pilot	2013	An investigation of the business model that could occur in the sanitation sector	The project is aimed at a more social responsiveness and community level. It would be interesting to see if this can be extended to a bioproduction facility context.
A AGRIC	36	1497/1/07: Holl MA, Gush MB, Hallowes J, Versveld DB, Jatropha curcas in South Africa: An Assessment of its Water Use and Bio-Physical Potential.	2007	The water use of Jatropha curcas grown for biodiesel production	Limited applicability to WWBRs, although the wastewater byproducts of biodiesel production would be of interest.
A ANAL	37	1283/1/04: Snyman HG, Herselman JE, Kasselman G, A metal content survey of South	2004	A metal content survey of South African sewage sludge	More work required to determine the impact of these metals on the bioprocesses to be used in a wastewater biorefinery context

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		African sewage sludge and an evaluation of analytical methods for their determination in sludge			
A ANALYSIS	38	1339/1/07 Jaganyi D, Methodology and survey of organic pollutants in South African sewage sludges: Volume 1	2007	An organic pollutant content survey of South African sewage sludge	More work required to adapt these to monitor and analyse the components in the bioprocesses
A ANALYSIS	39	1286/1/07: Pillay B, Dechlan, Development and application of prokaryotic biosensor systems for the evaluation of toxicity of environmental water samples.	2007		Potential way to evaluate incoming wastewater to prevent system failure
A ANALYSIS	40	1459/1/07: Wolfaardt JF, Grant R-M, Kock MM, Characterisation of planktonic microbial populations in paper-mill water	2007	Integrated water management plans for paper mills include strategies to reduce water consumption by closure of water circuits to reuse water. Closure, however, directly and indirectly results in an increase in populations of microorganisms. Comprehensive characterisation and identification of microbial populations could result in improved control and will extend the limits for mill closure. Microbiological data could also aid in the prevention of biofilm formation and minimise corrosion and furthermore be useful to minimise health risks and improve efficiency of water treatment processes.	It is recommended that the database and key software be distributed as widely as possible and not only within the paper industry, but also to other industries where bacterial control and identification play a role. Environmental parameters in water systems influence microbial numbers and parameters such as temperature and oxidation-reduction potential should be used to predict microbial levels. These data will be invaluable for integrated water management and especially when water systems are to be closed.
A ANALYSIS	41	TT 180/05: CSIR Water and wastewater management in the oil refining and re-refining industry: NATSURV 15	2005	Determine the volume of water intake and discharge in oil refineries and re-refining industry	A breakdown of water usage and the pollutant loads were presented and recommendations were made for water and wastewater management
A ANALYSIS	42	TT 240/05: Van Zyl HD, Premlall K, Water and waste-water management in the power generating industry (NATSURV 16)	2005	Investigation of water consumed and how to minimise it in power generating industries. Twenty nine power stations situated countrywide collectively produces approximately 192 000 GW of electricity per annum. To achieve this, approximately 245 000 Ml of water is consumed. The effluent produced is much less than this, as up to 80% of this water is lost through evaporation in cooling towers. The average raw water intake / unit sent out (RWI) is dependent upon the type of power generating process, whether open or closed loop cycles are used, the type of cooling and ashing processes utilized, as well as the quality of raw water. The average RWI was found to be 1.95 l/kWh for recycling wet-cooled coal-fired plants, 6.5 l/kWh for once-through wet cooled coal-fired plants, 0.09 l/kWh for dry-cooled coal fired plants and 0.073 l/kWh for nuclear plants. Improvements in the RWI can be achieved through the use of dry-	Valuable for input estimates.

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				cooled systems and water recycling in the case of municipal plants. It is suggested that target RWI's are set at a maximum of 2.5 l/kWh for wet-cooled coal-fired processes and 0.8 l/kWh for dry-cooled power generating processes.	
A ANALYSIS	43	1121/1/04: Whitcutt JM, Emmett RA, Ramajwe T, Mbatha Z, Humphries P, Wittekindt E Biomonitoring of wastewater	2004	Develop a rapid, low-cost human cell toxicity test that could be used for the universal monitoring of complex effluents.	Process control and analysis of WWBR, or effluent thereof
A ANALYSIS	44	961/1/99: Genthe B, Franck M, A tool for assessing microbial water quality in small community water supplies: an H2S strip test	1999	The project developed a small H2S strip test for determining water quality or contamination, aimed at small communities.	The methods developed in this paper are unlikely to be applicable to WWBRs, as more robust techniques will likely be used for water quality assessment at WWBRs.
A ANALYSIS	45	TT 405/09: Leopold P, Freese SD, A Simple guide to the chemistry, selection and use of chemicals for water and wastewater treatment.	2009	Chemistry textbook relating to water and wastewater treatment	Reference guide
A ANALYSIS / TECHNIQUE	46	KV 249/10: Garcin CJ, Nicolls F, Randall B, Fraser M, Griffiths M, Harrison STL, Development of LED-photodiode-flow cell for online measurement of dissolved substances in liquids	2010	This report describes the development of an LED-photodetector device for continuous on-line monitoring using optical flow cells as a low cost alternative to conventional spectrophotometry. Conventional spectrophotometers generally use tungsten or deuterium incandescent light sources, and have diffraction gratings, mirrors, filters and various other components that make up complex and expensive instruments. The development of light emitting diodes that emit at specific wavelengths in a narrow bandwidth offer several advantages for replacing the conventional technology: LEDs are robust, inexpensive, longer lasting, smaller, and stabilise within milliseconds. The versatility of the system developed was demonstrated in two different applications: measurement of phenolic compounds in the UV light range (280 nm) during a chromatographic purification process, and monitoring of algal cell culture density in the visible light range (465 and 760 nm) during growth in a photobioreactor. The system was controlled and monitored using Labview software, and by using flow-through optical cells, it was possible to take continuous on-line measurements as opposed to periodic sampling and external measurement. The electronic components of the system have subsequently been transferred onto printed circuit boards (PCBs) to make the system more compact. The PCBs are to be incorporated	May be of excellent use for WWBR process control and analysis. Limitations of the system were primarily that it is not possible to perform spectral scans or measure at multiple wavelengths as with conventional spectrophotometers; an LED is required to illuminate at each specific wavelength of interest. However, the use of multiple LEDs in one device can overcome this limitation. Future work will include: Multiplexing several detectors to run on one platform Dual and triple wavelength functionality Design of a low cost optical flow cell that incorporates the LEDs Low-cost fluorescence measurement Development of the system into a hand-held probe for in-situ measurements Signal telemetry for remote monitoring. Besides for the applications described above, future applications of the system could include: Wastewater treatment Surface water quality Diverse chemical and industrial processes.

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				into a new design that will be thoroughly waterproofed and will be rolled out in our lab for general bioprocess monitoring.	
A ECON	47	1568/1/12: Graham PM, Blignaut JN, de Villiers L, Mostert DJ, Sibande RX, Gebremedhin SK, Harding WR, Rossouw JN, Freese SD, Ferrer SRD, Browne M, Development of a generic model to assess the costs associated with eutrophication	2012	A generic first order model of the direct and indirect costs of eutrophication in South Africa and apply it to the Vaal Dam. The modelling was applied to estimate costs to agriculture, water treatment, property, and recreation.	Resource economics – reasoning to utilize wastewater urgently.
A ECON	48	KV 267/11: Winter D, Power Outages and their Impact on South Africa's Water and Wastewater	2011	Pumping is the most vulnerable activity in the water supply chain, but the use of gravity feeds can reduce this impact in many cases.Wastewater treatment plants with back-up power supply and overflow dams are generally not impacted by power outages... There is no doubt that power outages have had a direct impact on water and wastewater service delivery in South Africa.	Wastewater biorefineries fit with an ideology of renewable resources. This report can be expanded to look at alternative ways to provide power, in context of the biorefinery. It is unclear from the abstract if measures to reduce the impact of power outages are discussed.
A ECON	49	TT 462/10: Ginsburg AE, Crafford JG, Harris KG, Framework and manual for the evaluation of aquatic ecosystems services for the resource directed measures	2010	The National Water Resource Strategy aims to strike a balance between the use of resources for livelihoods and conservation of the resource. This process invariably requires negotiation of trade-offs. These trade-offs are principally between the resource quality on the one hand and the beneficial use of water on the other. The framework developed through this project to achieve this is explicitly congruent with methods used by DWA in the determination of Resource Directed Measures and Source Directed Controls. Definition of the benefits yielded by an ecosystem have been based on the Millennium Ecosystems Assessment framework and comparative risk assessment methodology is used to develop the causal chains linking ecological production to the defined ecosystem services. Two case studies have been developed to illustrate the framework. This Framework and Manual explores how these scenarios and their associated trade-offs should be evaluated.	Ecosystem economics, of significant relevance to WWBR, both in terms of their findings and the methodology employed.
A ECON	50	TT 442/09: Turpie JK, Wetland Valuation Volume Iii: Assessment Of The Livelihood Value Of Wetlands (TT 441/09 – 444/09)	2009		Compare wetland value with the macrophyte bioreactor
A ECON	51	KV 224/09: Musee N, Lorenzen L, Market Analysis for UASB	2009	Market analysis in South Africa of upflow anaerobic sludge blanket (UASB) technology, including suppliers, industrial users, international	This technology is potentially an integral part of any WWBR, and so analysis of the market will aid WWBR development.

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		Seeding Granules: Local and International Markets		suppliers, the South African market size, and new technologies competing with UASB.	
A ECON	52	KV 193/07: Swanepoel CM; Barnard RO, Discussion paper: Wetlands in Agriculture	2007		Possible application to WWBR macrophyte bioreactor
A ECON	53	1252/1/06: Friedrich E, Buckley CA, Pillay S, Leske A, A life cycle assessment of a secondary water supply.	2006	This study shows that a system approach as well as a process approach is needed for the integral assessment of the environmental performance in the provision of water and wastewater services. From the LCAs of individual processes involved in the provision of water and wastewater in the eThekwini Municipality, it emerged that the process with the highest contribution is the activated sludge process - used in the treatment of wastewater. However, when considering the entire system and including the losses in the distribution network for potable water, the process with the highest contribution became the distribution itself. An improvement analysis was performed and is presented. It takes into account a series of possible interventions and their consequences. Most notably, one conclusion of this study is that recycling as currently undertaken in Durban, has positive environmental impacts.	A useful approach to justify and evaluate WWBR.
A ECON	54	KV 159/04: van Zyl H, Leman A, Jansen, The costs and benefits of urban river and wetland rehabilitation projects with specific reference to their implications for municipal finance: case studies in Cape Town	2004	The document details three cost benefit case studies to evaluate the economics of rehabilitation of three wetland and river systems in the Cape Town area.	This work is not directly applicable to WWBRs, however, the methodologies used to evaluate the costs and benefits associated with rehabilitation projects could be applied to WWBRs.
A ECON	55	1383/1/04: Palmer Development Group, Economic regulation of water services in South Africa	2004	This project sought to answer how a regulatory authority determine if the average water price level is appropriate, what investment level is appropriate and how the governance model effects these two questions.	The pricing of water, as examined in this document, effects the economics of WWBRs, and so is implrtant to take into account.
A ECON	56	1077/1/02: Friedrich E, Buckley CA, The use of life cycle assessment in the selection of water treatment	2002	LCA study comparing conventional technology and membrane technology	In this study the main difficulties were experienced in the data gathering stage and they have been overcome by employing overseas data and by using calculations.
A ECON	57	TT 185/02: Palmer Development Group: So you think you want to corporatise? A guide for	2002	For the purpose of this guideline we regard the corporatisation of municipal water services as entailing the creation of a separate, legal, 'corporatised' entity, owned and governed by one or more	Limited application to WWBR, can include some insight on business model considerations for WWBR, and things to caution against, e.g. High levels of managerial autonomy can lead to over-engineering or gold-plating, especially where staff are strongly engineering-oriented.

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		municipalities considering corporatised water entities		municipalities, with the explicit objective of providing water services to some or all of the municipality's water users. The corporatised entity may enter into a range of contracts with private or public partners to facilitate service delivery.	
A ECON	58	854/1/02: Kerdachi D, The review of industrial effluent tariff structures in SA and guidelines on the formulation of an equitable effluent tariff structure	2002	...there is a need for a guideline document that provides a systematic methodology on how to formulate and implement a tariff structure that allows for an equitable proportion of finance to be provided by industry for their contribution towards the cost of effluent treatment to the required liquid and solid phase standards and guidelines of the Department of Water Affairs and Forestry (hereafter referred to as DWAF) and the Department of Health (hereafter referred to as DH) respectively and for the installation of an adequate sewerage system. This mode of operation is essential and preferable to the system of punitive measures that are not easily enforceable, nor understood by the legal fraternity usually ending up in a no win situation after months and years of protracted legal proceedings.	Minimal application to WWBR, but may be useful in justifying the WWBR approach, as one could do a cost comparison of investment vs just paying the fines. Find out if there is a more up to date document.
A ECON	59	631/1/01: Van Ryneveld MB, Marjanovic PD, Fourie AB, Sakulski D, Assignment of a financial cost to pollution from sanitation systems, with particular reference to Gauteng. (Please enclose erratum)	2001	Reference to Gauteng but can be extrapolted to use with other provinces.	Cost comparison
A ECON	60	1042/1/01: Louw GJ, Development of a solar-powered reverse osmosis plant for the treatment of borehole water	2001	This project investigated the use of a Reverse Osmosis unit, powered by solar energy, capable of producing potable water from brackish borehole feed, for rural households or small communities and demonstrated its use in field trials.	This work is not directly applicable to WWBRs, however, a comparison of the cost of producing potable water using this system versus a WWBR may inform the economics of both processes.
A HEALTH	61	1561/1/11: Roos C, Pieters R, Genthe B, Bouwman H, Persistent Organic Pollutants (POPs) in the water environment	2011	Of the 23 sites tested for dioxin-like compounds (DLCs), 77% was of industrial or semi-industrial origin, 15% was industrial-residential combinations, 6% was high-density low-income residential areas and 2% was residential-agricultural combinations.	Health concerns or unexpected factors to address in WWBR
A HEALTH	62	TT 469/11: Rodda N, Carden K, Armitage N, Sustainable Use of Greywater – Guidance Report	2011	The focus of the Guidance Document is to minimise the risks of • illness in handlers of greywater and greywater-irrigated produce, or consumers of greywater-irrigated produce. • reduction in growth or yield of plants/crops irrigated with greywater. • environmental degradation, especially reduction in the ability of soil irrigated with greywater to support plant growth.	Good guidelines for general approach in WWBR.

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A HEALTH	63	1749/1/09: Genthe B, Steyn M, Aneck-Hahn NH, van Zijl C, The feasibility of a health risk assessment framework to derive guidelines for oestrogen activity in treated drinking water	2009	A previous WRC report KV 206/08 proposed a framework to deal with endocrine disrupting chemicals for drinking water in South Africa. The framework suggests a tiered approach to screening and testing of chemicals in the water environment rather than testing for specific target chemicals and recommended the use of a trigger value for oestrogen activity.	A multidisciplinary team would need to be assembled to look at the possible sources such as industry, agriculture, waste streams etc. and follow-up samples would need to be taken to identify the specific chemicals responsible, before remedial action could be taken.
A HEALTH	64	TT 322/08: Priya Moodley P, Archer C, Hawksworth D, Leibach L, Standard methods for the recovery and numeration of Helminth Ova in wastewater, sludge, compost and urine-diversion waste in South Africa.	2008	Health concerns when dealing with wastewater and related materials as substrate	Need more work and recommendations of addressing potential health impacts of wastewater biorefineries
A HEALTH	65	1774/1/08: Burger AEC, Nel A, Scoping study to determine the potential impact of agricultural chemical substances (Pesticides) with endocrine disruptor properties on the water resources of South Africa.(EDCs)	2008	Preliminary study on endocrine disruptor pesticide contamination in SA water systems.	Report informs on the prevalence of EDSs in SA water, which could guide on the presence of EDC's in WWBR. These chemicals can affect bioconversion or final product quality or applicability.
A HEALTH	66	TT 298/07: Genthe B, Knoetze M, Management of water-related microbial diseases: Volume 4: How dangerous is the problem?-Communicating the risk	2007	This guideline presents how best South Africans can protect themselves from water-related microbial diseases and provides a framework of principles and guidelines for the communication of health risks, specifically for water service providers.	If WWBRs are to be potable water producers, then this dicument will inform how best to communicate the potential health risks associated with recovered water.
A HEALTH	67	1439/1/06: Austin LM, Phasha MC, Cloete TE, Pathogen destruction in urine diversion sanitation systems: Vol 1	2006	This document discusses ecological sanitation by means of a literature review and examines processes taking place in the vault of a urine-diversion (UD) toilet focussing on pathogen destruction parameters as well as appropriate practices for faeces collection and disposal.	Limited applicability in WWBRs, however, the pathogen destruction process described could inform parallel processes in WWBRs.
A META	68	2199/1/12: Pouris, Anastassios, A Pulse Study on the State of Water Research and Development in South Africa	2013	A quantitative account of key R&D trends in the water sector. The analysis identifies that the field is performing above expectation in comparison with the country's research size.	Overview of the research landscape in South Africa with regards water. However, little attention has been paid to the increasingly important water reuse/recovery/beneficiation concepts.
A META	69	TT 503/11: Winter D, Bangure K, Water-related research projects in Agriculture undertaken in South Africa	2011	develop a database of all water-related research projects in agriculture being undertaken in South Africa during 2010	General overview of agriculture specific water research in 2010.

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A META	70	TT 513/11: Keith BA, Water Resources of South Africa, 2005 Study (WR2005): User's Guide (Version 2, November 2011)	2011	A revised appraisal of the Water Resources of South Africa. Enhancements to the WR2005 system, using the information from existing WR2005 calibrations to patch and calibrate streamflows for all 19 water management areas.	General overview of water resources. Gives an estimate of the water supply problem, which is a reason for this research.
A META	71	KV 277/11: Pollard S, Du Toit D, Biggs H, A guide to complexity theory and systems thinking for integrated water resources research and management	2011		Possible overview angle
A META	72	TT 488/11: Balfour F, Hanlie Badenhorst H, Trollip D, A gap analysis of water testing laboratories in South Africa	2011	Developed a database of existing laboratories that undertake water quality testing and, through a survey, obtained information on their capability and credibility to determine capacity gaps.	Water analysis laboratories in SA provide a crucial capacity, to analyse the inputs and outputs of potential WWBRs, particularly with regards potable water production. This database provides information on this.
A META	73	TT 514/11: Claassen M, Funke N, Nienaber S, The Water Sector Institutional Landscape by 2025	2011	Project to build knowledge about key drivers and uncertainties related to SA water sector institutions, with a focus on water resource management.	WWBRs can feed into water resource management as a water source, thus this report outlines some key stake holders in the space.
A META	74	1547/1/10: Cloete TE; Gerber A, Maritz L, Inventory of water use and waste production by industry, mining and electricity generation	2010	The overall objective of this project was to compile a first order inventory of the amount of water used and effluent produced by the South African industrial, mining and power generation sectors, and to assess the impact these might have on water quality, but existing data sets were of limited value and outdated.	It is of great concern that many of the surveyed industries do not conduct any chemical analyses on the effluents that they produce and that where chemical analyses are done, they very seldom go beyond a few basic parameters like COD, phosphate and nitrate. The current data therefore merely indicates a trend rather than enabling the user of the data to determine the exact pollution load to the environment. As there are currently no standard requirements in place for municipal councils with regard to effluent monitoring, it is recommended that such a standard be developed and implemented in order to obtain more accurate information on the chemical composition of the effluent.
A META	75	TT 450/10: Boyd LA, Tompkins RL, Heath RGM, Integrated water quality management: a new mindset	2010	Integrated water management	Contextualize.
A META	76	TT 417/09: Malzbender D, Earle A, Deedat H*, Hollingworth* B, Palesa, Review of Regulatory Aspects of Water Services Sector	2009	Review of water regulations. Covering international best practice and theory, local legislature, and costing models. Legislature seeks to control potability of water, and prevent non-compliance (ie release of sewage etc).	Legislature will apply to WWBRs, and so this document would inform operating conditions and comliance.
A META	77	TT 267/08: Pott AJ, Benadé N, Pieter van Heerden P, Grové B, Annadale JG, Steyn M,	2008	Technology transfer and integrated implementation of water management models for agriculture and water managers.	Some of these models could inform WWBR flows and aid inunderstanding WWBR systems. However, it is likely some adaptation of existing models would be required.

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		Technology transfer and integrated implementation of water management models in commercial farming.			
A META	78	TT 366/08: Frost & Sullivan, Membrane-related Water Research Impact Assessment	2008	Assessment of research relating to membranes used in water treatment. List of WRC funded projects on membrane-related water research from 1993 -2011 (66 reports)	Starting point to look at membrane technology in WWBR
A META	79	1671/1/08: de Swardt BW, Barta B, A First-Order National Audit of Sewerage Reticulation Issues	2008		Big picture
A META	80	1605/1/07: van Zyl JE, and Geusteyn LC, Development of a National Water Consumption Archive: (Only available on CD)	2007		
A META	81	1213/1/05: Vosloo R, Bouman H, Survey of certain persistant organic pollutants in major South African waters (POPS)	2005	countrywide assessment of POPs in a selection of major water bodies, and would indicate geographical areas (such as industrial and or residential) where more concerted action, management or research needs to be focussed.	WWBR might take special care to develop knowledge or something to degrade these throughout the process stream
A META	82	TT 226/04: Conningarth Economists, Research impact assessment: Lessons to be learned from the cost-benefit analyses of selected WRC research projects.	2004	A sample, consisting of six research projects, was selected and evaluated by means of Cost-Benefit Analysis (CBA). These projects were the following: - ACRU Model Development - Hydrosalinity System Models - Surface Water Resources of South Africa - Biological Nutrient Removal - Dry Cooling in Power Generation - Combined Services Model	Provides insight from the overall gap analysis for a development plan for WRC-funding of WWBR research. ... the following policy and planning directives for future WRC research initiatives are proffered: - The CBA provides unequivocal evidence that the WRC research outputs have made a significant contribution to improving the economic welfare of South Africa - The growing importance of research projects dealing with water conservation and demand management is in line with the WRC's strategic focus and the government's development prerogatives - Agriculture remains the largest water user and, therefore, requires that a substantial amount of resources still be devoted to research activities that would promote more efficient use of water for irrigation purposes. However, the CBA results also show that research for other users is of great significance because of the potentially higher returns that can be expected on such outlays. - The CBA shows that research into new technologies and the

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					transfer thereof to the operational level provides handsome dividends. On the other hand, research projects which assist in the better planning of water projects in all their facets also produces more than reasonable returns. - The CBA results of the projects in question show that proportionally larger benefits can be obtained from research directed at reducing operational costs. This is despite the fact that, in some cases, such reductions go hand-in-hand with major capital expenditure.
A META	83	1185/1/04: Swartz CD, Ralo T, Guidelines for planning and design of small water treatment plants for rural communities, with specific emphasis on sustainability and community involvement and participation.	2004	The aims of this project included to create an understanding of the unit processes employed in small rural (drinking) water treatment plants, and to provide information on indigenous water treatment technologies.	Project included a workshop which can guide the WWBR data gathering workshop planning. Report has minimal relevance to WWBR.
A META	84	TT 115/99: DWAF, WRC, A framework for implementing non-point source management under the National Water Act	1999	The research set out to examine how best to implement the Water Act in terms of non-point water source management.	The legislature outlined in the Water Act will bind water production in WWBRs, and so this document may assist in assessing legal compliance in WWBRs.
A META	85	629/1/96: Palmer Development Group, Evaluation of solid waste practice in developing urban areas of South Africa: Executive Summary	1996	The focus of the report was to assess the factors which effect solid waste management, specifically in developing communities.	Limited applicability to WWBRs.
A META	86	561/1/94: Palmer Development Group, Water and sanitation in urban areas: Survey of on-site conditions	1994	Drinking water - Water supply, Sanitation - On site sanitation	Background information
A SOLIDS	87	1745/1/12: Still DA, Foxon KM, Understanding sludge accumulation in VIPs	2012	Disposal of dense pit sludge at wastewater treatment works has been found to quickly overload the works in addition to being counterproductive in a number of respects. The policy of the South African government stresses the value of human excreta as a resource although utilisation must be done within strict parameters due to the hazards of contamination. Most pits are filling in five to nine years. Pits generally fill at a rate of 40 litres per capita annum, with 60 litres per capita annum providing a safe margin for planning pit design and emptying programmes.	This gives an estimate input for a potential WWBR based on solid wastes.

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A SOLIDS	88	KV 248/10: Lutchamma-Dudoo C, Biologically Enhanced Primary Settlement:	2010	Investigation into using biological agents as settling agents to replace the more commonly used ferric chloride, to allow rural communities to become more self relient with regards wastewater treatment.	The technology could be applied in WWBRs, although its capabilities and limitations need to be further explored.
A SOLIDS	89	1524/1/07 Carden K, Armitage N, Winter K, Sichone O, Rivett U, Understanding the use and disposal of greywater in the non-sewered areas in South Africa.	2007	Situation analysis: Understanding the use and disposal of greywater in the non-sewered areas in South Africa.	More work required to contextualize (modular) wastewater biorefinery units in non-sewered areas – starting with a feasibility study, then with solid substrate bioprocess technologies
A SOLIDS	90	1550/1/07: Broadhurst JL, Hansen Y, Petrie JG, Waste Characterisation and Water-Related Impact Predictions for Solid Mineral Wastes: a new approach	2007	The need to improve the way in which solid mineral wastes are characterised is driven not only by the limitations in terms of current databases and methodolodies for the generation of such. There is also a requirement for a more systematic and rigorous approach which will ensure that the necessary data and information is integrated into the decision stages of a project life cycle in a time and cost effective manner. This project aimed to develop a generic and integrated methodology for predicting water-borne environmental impacts associated with solid mineral wastes.	Not directly relevant to WWBR, but the approach and methodology may be informative. The increased understanding afforded by this approach provides opportunities to influence and control behaviour, and eventually optimise waste management and minimise environmental imapcts across the entire life cycle of minerals operations.
A SOLIDS	91	1240/1/04: Marx CJ, Alexander WV, Johannes WG, Steinbach-Kane S, A technical and financial review of sludge treatment technologies	2004	The aim was to give a clear indication to metropolitan councils, municipalities and other sludge producers of the technologies available and applicable under local conditions, as well as an indication of the cost and economy of scale applicable to each process. The study includes an overview of current sludge management practices in South Africa, as well as an estimate of sludge quantities and qualities and a brief description of commonly used sludge treatment and disposal methods.	Applicable to sludges used/produced in WWBR, including the legal framework, using as a basis the Sludge Utilisation or Disposal Decision Flow Diagram (SUDDFD), as presented in the Addendum No 1 to the Permissible Utilisation and Disposal of Sewage Sludge (Edition 1), (Department of Agriculture et al 1997). The sludge treatment requirements and available technologies for each of the utilisation or disposal routes are listed in matrix form for easy reference and use. Also see if there are updated on this work.
A SOLIDS	92	1167/1/03: Schoeman JJ, Steyn A, Slabbert JL, Venter EA, Treatment of landfill leachate from hazardous and municipal solid waste	2003		
A SOLIDS	93	544/1/00: Norris GA, Sludge build-up in septic tanks, biological digesters and pit latrines in South Africa (ONLY PHOTO COPIES AVAILABLE)	2000		
A SOLIDS	94	TT 107/99: Ceronio AD, Van Vuuren LRJ, Warner APC: Guidelines for the design and	1999	Sludge drying / 'preprocessing'	Needs further work to consider drying beds as solid substrate bioreactors

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		operation of sewage sludge drying beds			
A SOLIDS	95	599/1/99: Pearson I, La Trobe B, Co-disposal and composting of septic tank and pit latrine sludges with municipal refuse	1999	Sludges cannot in general be simply composted on their own. It is necessary to ensure that pasteurization temperatures are achieved during the composting process to ensure that pathogenic organisms are eliminated, and weed seeds are made non-viable. To achieve such temperatures the following conditions are required: • A bulking agent to maintain pores and channels throughout the compost windrow for the continuous penetration of oxygen. • A method of promoting the flow of air through the windrow to support the active organisms responsible for the breakdown of the organic matter. • An insulating layer on the surface of the windrow to maintain internal temperatures and to trap malodours. In the tests carried out in this project, domestic refuse, garden refuse, and grass cuttings were used as bulking agents, and wood chips were used to cover and insulate the heaps or windrows. The project includes prelminary costing.	Large WWBR potential, specifically for best practice in SSF/biosolid bioprocesses. What has been done since the publication of this report? The report recommends further evaluation of "passive" aeration systems for compost windrows employing sewage sludge, which could be of use to WWBR as well.
A SOLIDS	96	391/1/96: Novella PH, Ross WR, Lord GE, Greenhalgh MA, Stow JG, Fawcett KS, The co-disposal of wastewater sludge with refuse in sanitary landfills	1996	Sanitary landfilling, whereby the waste is compacted and covered each day with a soil layer offers the most versatile method for the disposal of solid wastes in an economical and environmentally sound manner. Co-disposal (or joint disposal) in its widest sense, is understood to be the calculated and monitored interaction of wastewater sludge (or selected difficult industrial and commercial wastes) with municipal refuse in a properly controlled landfill site. ... difference perceptions of the values and dangers of the co-disposal practice have developed. The experimental runs established that excessive addition of sludge liquor caused the belly plate of the landfill compactor to sink too deep into the refuse-sludge mixture, thus retarding the manoeuvrability of the machine. The Safe Working Ratio of refuse to sludge liquor (by volume) for the winter and summer seasons was determined to be 6:1 and 4:1 respectively. The importance of moisture in solid-state anaerobic decomposition has been highlighted for optimising the physical, chemical and biological conditions for accelerated stabilisation of the landfilled waste. ... such an integrated waste management strategy would be advantageous in terms of improved pollution control.	Can this be interpreted for supplementary resources (e.g. lignocellulosic/organic biomass) for WWBR? Is this current practice, or is this report out of date?

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A?	97	1128/1/03: Naidoo V, Buckley CA, Survey and preliminary investigation into biodegradation of pesticide wastes	2003	The project outlines a comprehensive schedule of pesticide use and waste generation in Southern Africa as well as a comprehensive survey of existing technologies for the disposal of pesticides.	Limited applicability to WWBRs, although some pesticide treatment methodologies may be relevant if WWBRs are to treat pesticide wastes.
A2	98	TT 568/13: Ralivhesa K, van Averbeke W, Siebrits F, Production Guidelines for Small-Scale Broiler Enterprises	2013	Data or contacts of poultry wastewater?	
A2	99	1734/1/13: Brouckaert CJ, Mhlanga F, Arnold, Quantitative Assessment of Industrial effluents for discharge to sewer	2013	Quantitative approach to industrial effluents	
A3 HEALTH	100	TT 559/13: Herselman JE, Guideline for the Utilisation and Disposal of Water Treatment Residues	2013	Guidelines that describe the requirements for the disposal and/or use of water treatment residues.	WTRs can be used, and are products of WWBRs. The guidelines on their use and disposal could strongly influence WWBR set up and operation.
A3 HEALTH	101	TT 561/13: Masoabi D, Boyd LA, Thomas Coughlin T, Heath RGM, Endocrine-Disrupting Compounds - Sampling Guide. Volume II	2013		Analysis methods.
A4 ECON	102	KV 307/09: Naidoo D, Moola S, Place H, Discussion paper on the role of water and the water sector in the green economy within the context of the new growth path	2013	Literature review, and interview based research on the role of water in the green economy, and the economy in general (in as much as many sectors are heavily dependant on water).	This work contextualises the need for WWBRs, and the development of WWBRs can be placed in the green economic scenario.
A4 META	103	2075/1/13: Pegram G, Baleta H, Water in the Western Cape Economy	2013	This project investigates possible ways of assessing regional water resources in the Western Cape system (Berg and Breede-Overberg WMAs) from a political-economic and developmental perspective. Increasingly stressed water resources and the uncertainty of climate and development futures have highlighted the close interactions between water, energy and food security at a national level.	The project acknowledges that data throughout this project has been a challenge. Perhaps further work on WWBR can improve this case, and vice versa. An in-depth analysis of local level water in the economy implications is required. This is because initial presentations of this work have found the engagement with the private sector less compelling due to the scale of water and economy investigated (district level municipality or water management area). The same is expected to be the case for WWBR.
A4 META	104	1890/1/12: Duncker LC, CSIR, Establishment of a sanitation technology demonstration centre	2012	The concept, along with WADER – Water technologies demonstration programme (http://wader.org.za/), carries great potential to further applied studies and application towards the WWBR concept. See also http://www.csir.co.za/Built_environment/santechcentre/	Due to this project being in its infancy, little information was found regarding this.

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A5 ECON	105	TT 543/12: vd Merwe-Botha M, Quilling G, Drivers for wastewater technology selection: Assessment of the selection of wastewater treatment technologies by municipalities in relation to the management capability and legislative requirements	2012	A sobering observation of the constraints municipalities face when selecting treatment technologies, that highlight the challenge of wastewater biorefineries in this context.	More work required on how these factors are affected when an economic business case for bioproduction applies, e.g. would a privately managed plant be a better option.
A5 ECON	106	1805/1/12: Grové B, Frezghi M, Pott A, Lecler N, Development and testing of an integrated hydro-economic model	2012	Model linking hydrologic simulation with the economic optimisation to quantify possible impacts of changes in catchment water management scenarios.	Modelling could be used to quantify economics used in WWBRs, particularly in reference to water.
A5 META	107	2170/1/13: Siebrits R, Winter K, Identifying and Prioritising Water Research Questions for South Africa	2013	A new era in water research in South Africa began with the promulgation of the Water Research Act No. 34 of 1971. The Act led to the formation of the Water Research Commission (WRC) and the Water Research Fund with the purpose of initiating, managing and financing water research. This study commences with the identification of the prevailing paradigms that have influenced the history of water research in South Africa by analysing the publication output over the last four decades and in identifying research questions proposed by a range of researchers active in the water sector in South Africa.	A good overview of water related research In South Africa. This study needs to expand on this selection with regards to relevance to WWBR.
B		2144/1/14, Swanepoel C, Bouwman H, Pieters R, Bezuidenhout C, Presence, concentrations and potential implications of HIV-anti-retrovirals in selected water resources in South Africa		Develop extraction and analytical procedures for selected HIV-ARVs from water and fish.	The inflowing and outflowing concentrations over time on a range of different WWTPs with different efficiencies (based on Green Drop data) needs to be determined.
B		Technical note 3139: Myburgh PA; Lategan EL; Howell CL, Infrastructure for irrigation of grapevines with diluted winery wastewater in a field experiment	2015	Relatively simple infrastructure and procedure required to dilute the winery wastewater to COD levels ranging between 100 and 3 000 mg/ℓ in 15 m3 tanks	Scale up studies
B		1881/1/14: Myburgh PA and Howell CL, The impact of wastewater irrigation by wineries on soils, crop growth and product quality	2014	The possibility of re-cycling winery wastewater for vineyard irrigation was investigated in a field trial near Rawsonville in the Breede River Valley. Wastewater obtained from a co-operative winery was augmented to levels of 100 mg/L, 250 mg/L, 500 mg/L, 1000 mg/L,	The COD must be augmented to 3000 mg/L or less, preferably to less than 2000 mg/L to avoid unpleasant odours while irrigations are applied. Due to the possibility that direct contact with winery wastewater may cause off-odours in the wine, overhead sprinkler irrigation is not recommended if winery wastewater is

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				1500 mg/L, 2000 mg/L, 2500 mg/L and 3000 mg/L chemical oxygen demand (COD)	re-used for vineyard irrigation.
B	108	1942/1/13: Budhram S, Nyuswa M, Rajagopaul R, Thompson P,Operational and design considerations for high rate clarifiers in the South African water treatment industry	2013	high rate clarification technology was evaluated based on investigations conducted on a 500 m3/day demonstration model HR CSAV high rate clarifier	will assist water treatment designers and water treatment practitioners particularly in South Africa to make informed decisions on the appropriateness of high rate clarification processes under local conditions.
B	109	2005/1/12: Pocock G, Joubert J, Optimisation of Waste Stabilisation Ponds	2012	Waste Stabilisation Ponds using duck-weed	Waste stabilization ponds can be used for nitrogen removal and concentration in biomass from wastewaters. Duck-weed allows continual removal of biomass (as opposed to algae ponds), which could potentially be applicable to WWBRs.
B	110	1936/1/11: Burton SG, Welz PJ, Ramond J-B, Sheridan G, Kirby B, Schueller A, Rodriguez A, Pather-Elias S, Prins A, Cowan DA, Health for purpose in constructed wetlands	2011	Constructed wetlands to treat high COD winery wastewater	Molecular biology tools to assess the health of constructed wetlands, microbial community changes and the impacts of interventions (such as fertilizer addition). CWs are applicable to the polishing of wastewaters, potentially in WWBRs.
B	111	1658/1/11: Schoeman JJ, Sekgwela EI, Hallis D, South African clinoptilolite for the removal of NH3-N from secondary sewage effluent	2011	This project investigated the potential of reducing ammonia-nitrogen via inclusion of a selective ion-exchange system to the existing treatment train. Biological nitrification and algal ponds may not be suitable where low temperatures are encountered. Stripping and breakpoint chlorination are considered to be too expensive for the high ammonia-nitrogen concentration levels encountered in secondary effluent. Selective ion-exchange of ammonia-nitrogen using the natural zeolite, clinoptilolite, in the sodium form, which is not very sensitive to temperature fluctuations, and which is a locally occurring mineral, should be a suitable material for ammonia-nitrogen removal from secondary sewage effluent. Thus, the main aim of this investigation is to develop process design criteria and costs for the implementation of a South African clinoptilolite for ammonia-nitrogen removal from secondary effluents for pollution control.	Limited applicability in WWBRs, however, the concept of using local materials to improve the plant's operation could inform parallel processes in WWBRs.
B	112	1669/1/09: van der Merwe IW, Lourens A, Waygood C, Innovative approaches to brine handling	2009	Today, typical water recovery rates for different applications are: 40-45% for Sea water desalination, 70-85% for Industrial effluent and 85-90% for brackish water desalination. The major research effort in high recovery systems for in-land brackish water and industrial systems is in the region above 95% water recovery. The project investigated and identified innovative approaches to brine and sludge management; These innovative concepts were then compared with	Good guiding report on using brines in the WWBR context. Collected data about the present brine and sludge volumes in South Africa in an appropriate database. Volumes and sources of brine and sludge were determined through a survey of industry. For this survey, 268 companies were contacted, of which 185 positive responses were received. Despite the good response rate (69%), the development of a detailed database was

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				current best practises in order to identify those concepts which should be investigated further; For the promising concepts, a more detailed evaluation was performed in order to identify those with highest potential. Concepts evaluated include the 'WAIV' system, freeze desalination, and dewvaporation.	hampered by either a lack of data within some organizations, or an unwillingness to release detailed data due to commercial and other sensitivities... A total of just over 530 000 kl/d of effluent is discharged to inland systems, containing approximately 1060 t/d of salt (refer to the table in report). The report inclues a list of potential by-products from South African mine water.
B	113	1079/1/08: Loewenthal RE, Morgan B, Lahav O, Hearne G, Research on an Investigation into sulphur chemistry with specific application to biological sulphate-removal processes	2008	The principal aims to this contract were threefold: i. to investigate and model a sulphide chemistry in both the aqueous and gaseous phases, ii. to investigate and model the recovery of elemental sulphur through chemical oxidation of sulphide, and iii. to investigate and model the precipitation and recovery of metals.	The research presented in this report must be considered as a preliminary study into feasibility of applying biological – physical – chemical treatment processes to AMD waters.
B	114	TT 193/07: Rose PD, Hart OO, Dekker LG, Clark SJ, Integrated algal ponding systems and the treatment of domestic and industrial wastewaters: part 4: Report 7	2007	Initial studies in the production of high-value bio-products from halophilic micro-organisms in wastewater beneficiation using integrated Algal Ponding Systems.	The application of halotolerant microorganisms to WW treatment is extremely applicable to WWBRs, and this work should inform any WWBR work that considers saline compositions.
B	115	1544/1/07: Burton SG, Sheridan C, Law-Brown J, le Roes M, Cowan D, Rohr L, Mashapu N, Integrated research for use in constructed Wetlands for Treatment of Winery Wastewater	2007	Constructed wetlands for winery wastewater	Research applicable to the final polishing of wastewater treated in WWBR. More work in needed to characterize the applicability with a range of wastewater compositions.
B	116	971/1/07: OV Shipin OV, Meiring PGJ, Transforming the Petro Process for Biological Nutrient Removal	2007	Transforming an existing process to fulfill new functions	Can this process and/or methodology be adapted to bioproduction
B	117	763/1/07: Lew C, Biotechnological approach to the management of effluents from the Pulp and Paper Industry.	2007	Biological methods (white rot fungi and hemicellulytic enzymes) are used to treat effluent.	Application to WWBR. Lab scale - needs scale up
B	118	1539/1/06: Gaydon P, McNab N, Sahibdeen M, Pillay I, Mulder G, Thompson P, Evaluation of	2006	The simplest low technology units are anaerobic treatment systems such as septic tank and soil drains … Requirements for greater degrees of sophistication progressively bring in engineered pond and	Package plants most often fail in their ability to effectively nitrify ammonia and in disinfecting against bacteria, due to faults in design and operation, not due to the process technology per se. More work

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		Sewage Treatment Package Plants for Rural, Peri-Urban and Community use		wetland treatment systems, trickling filters, rotating biological contactors and mechanically aerated treatment systems.	required to evaluate the suitability of these package plants for modular use in wastewater biorefineries in communities. - link up with SEWPACKSA?
B	119	1361/1/06: Burton SG, Cowan DA, Garcin C, van Schalkwyk A, Werner C, A customised bioreactor for beneficiation and bioremediation of effluents containing high value organic chemicals.	2006	The development of the bioreactor was based on understanding of bioremediation of polyphenolic wastewaters, with the additional design and assembly of the components required for recovery of phenolic derivatives from treated effluents using hydrophobic membranes. In this project they developed technology to facilitate the extraction of hydrotyrosol from table olive wastewaters produced in the western Cape, and then to bioremediate the residual extracted wastewater.	A good example of producing valuable chemicals from wastewater - potential unit process in WWBR.
B	120	1364/1/06: Sigge GO, Britz TJ, McLachlan T,van Schalkwyk N, Treatment of apple and wine processing wastewaters using combined UASB technology and ozonation scenarios.	2006	Treatment of apple and wine processing wastewaters using either UASB or a combination of ozonation and UASB technologies. Report includes conclusions on cost efficiency of technologies.	This work is applicable to WWBRs, particularly if UASB technologies are going to form part of the process. It demonstrates UASB use with specific wastewaters, and this must be extended to include other wastewaters.
B	121	1338/1/05: Soteman SW, Ristow NE, Loewenthal RE; Wentzel MC, Ekama GA, Integrated mass balance models for chemical, physical and biological processes in wastewater treatment plants: Part One	2005	Integrated mass balance models for chemical, physical and biological processes in wastewater treatment plants: making wastewater biorefineries possible in principle.	More work required to adapt these mass balance models for wastewater biorefineries – product focused
B	122	1348/1/05: Heath RG, Coetser SE, Molwantwa J, Rose PD, Implementing the degrading packed bed reactor technology and verifying the longterm performance of passive treatment plants at Vryheid coronation	2005	The report details the long term full scale operation of a passive treatment method for acid rock drainage.	The research may be applicable in WWBRs, if the biorefinery is to treat acid rock drainage.
B	123	TT 195/04: Rose PD, Corbett CJ, Hart OO, Whittington-Jones KJ, Salinity, sanitation and sustainability: Report 9 (Rhodes BioSURE process: biodesalination of mine drainage wastewaters)	2004	biodesalination of mine drainage wastewaters	Relevance to WWBR of industrial (mine) wastewaters

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B	124	1084/1/04: Suruijal S, Tivchev G, Kasan HC; Bux F, Development of biological treatment technology for the remediation of edible oil effluent.	2004	The edible oil industry has been identified to be amongst the 75 industrial groupings in South Africa. In all, there are about 16 edible oil-processing plants, run by 10 separate groups. These industries refine and process approximately 300 000 tons of crude vegetable oil per year, which increases annually by about 3%. The objectives of the research included: • To investigate the source of effluent production during the different stages of refining • To chemically characterise the effluent. A preliminary costing analysis was also performed.	This report provides information on input streams to WWBR, and potential technologies to beneficiate them. The oil effluent was found to contain amounts of phytosterols, which could possibly be extracted, purified and sold as an animal feed supplement. An additional area identified is the need for a comprehensive analysis of the effluent. Settling problems as well as changes in microbial interspecies interactions have been noted in the pilot-scale activated sludge system.
B	125	1172/1/04: Rajagopaul R, Pillay VL, The evaluation and design of sludge dewatering and water filtration systems using tubular woven fabric technology	2004	Technology can be used in WWBR	DSP
B	126	1243/1/03: Van Hille RP, Antunes APM; Sanyahumbi D, Nightingale L, Duncan JR Development of integrated biosorption systems for the removal and/or recovery of heavy metals from mining and other industrial wastewaters and determination of the toxicity of metals to bioremediation processes	2003	Removal and recovery of heavy metal from mining and other industrial wastewaters. Pilot scale	Much of the methodology and research approach can be applied to the WWBR concept.
B	127	616/1/03: Duncan JR, Stoll A, Wilhelmi B, Zhao M, van Hille R, The use of algal and yeast biomass to accumulate toxic and valuable heavy metals from wastewater	2003	This project focussed on determining the efficiency and capacity of different types and forms of microbial biomass in removal of heavy metals from wastewaters generated by mining, electroplating, battery, tannery and other industries.	The application of heavy metal removal from wastewaters may be important in WWBRs, if WWs with a high heavy metal content are to be treated.
B	128	845/1/03: Antunes APM, Sanyahumbi D, Nightingale L, Payne R, Maclear A, Duncan JR, Development of bioreactor systems for the treatment of heavy metal containing effluents	2003	This report set out to evaluate the potential of algae and the water fern Azollu to accumulate heavy metals from effluents as well as exploit the exopolysaccharide production of a number of algae to improve metal removal efficiencies and from there optimise bioreactor design for metal removal on site.	The application of heavy metal removal from wastewaters may be important in WWBRs, if WWs with a high heavy metal content are to be treated. This technology would inform that unit process.
B	129	846/1/03: Brozel VS, Development of a continuous flow	2003	The aim of the project was to develop technology for the biodegradation of hydrophobic pollutants by emulsification using a	Investigate the use of rhamnolipid production in the WWBR context.

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		membrane bioreactor catalysing the solubilisation of hydrophobic pollutants by rhamnolipid-producing bacteria		membrane-supported biofilm producing the surfactant rhamnolopid produced by Pseudomonas aeruginosa.	
B	130	970/1/02: Hu Z, Sötemann SW, Vermande SM, Moodley R, Little C, Lakay MT, Wentzel MC, Ekama GA, External nitrification with the aid of fixed media trickling filters (TF) to increase the capacity of biological nutrient removal (BNR) suspended medium activated sludge (AS) systems	2002	External nitrification with the aid of fixed media trickling filters	An example of a bioreactor system likely to be suitable to the biorefinery. Needs further investigation
B	131	836/1/02: Coetzee PP, Meyer J, Evaluation and development of physical water treatment processes for the reduction of CaCO3 scale	2002	The focus was on the fundamental chemistry and physics of the processes involved in physical water treatment, the development of experimental and analytical methods to study the effects of physical fields on systems where scaling can occur, and the formulation of theoretical models to explain the mechanisms involved.	Limited application to WWBR, but included as an example/reminder to consider physical water treatment processes - both as an opportunity and a risk.
B	132	934/1/01: Van Heerden J, Ehlers MM, Korf C, Cloete TE, Active biomass fraction of MLSS and its role in biological phosphorus removal	2001	The report examines biological phosphorous removal in activated sludge WW treatment, modifiying a number of process variables to achieve maximum phosphorous removal.	Phosphorous removal will be an important part of WWBRs, and so this report can inform process considerations around that, however futher work in applying this technology to WWBRs is needed.
B	133	802/1/01: Dill S, Cloete TE, Coetser L, Zdyb L, Determination of the suitability of alternative carbon sources for sulphate reduction in the passive treatment of mine water	2001	to develop a quick test method for the assessment of potential carbon sources regarding their suitability for use in passive treatment systems for the use of sulphate reduction in small-scale anaerobic reactors.	the release of carbon from complex carbon sources over time to more fully understand the sustainability of carbon release from potential carbon sources.
B	134	822/1/00: Drysdale GD, Atkinson BW, Mudaly DD, Kasan HC, Bux F, Investigation of the microbial contribution to nutrient removal in an activated sludge wastewater treatment process	2000	Investigation of the microbial contribution to nutrient removal in an activated sludge by conducting a microbiological and plant parameter survey at different sites of WW treatment, and establishing the extent of correlation between microbial predominance and nutrient removal in different reactors.	The methodology used in this research is readily applicable to WWBR reactors, and would allow for tighter control of operations, and better uunderstanding of process parameters.

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B	135	933/1/00: Ntshudisane BM, Oosthuizen DJ, Ehlers TE, Cloete TE, Biolog for the determination of microbial species diversity and evenness in activated sludge systems	2000	Process analysis	Can this be adapted to be used in process control and analysis in WWBR
B	136	688/1/97: Bux F, Atkinson BW, Kasan HC, Laboratory and pilot-plant bioreactor development for remediation of metal-contaminated wastewater using activated sludge as biosorbent	1997	Remediation of metal-contaminated wastewater using activated sludge as biosorbent	Evaluate the impact of this work to metal-containing WWBR
B	137	427/1/95: Smollen M, Kafaar A, Development of electro-osmotic sludge dewatering technology	1995	Electro-osmotic sludge dewatering was found to be more effective than mechanical dewatering in the case of chemical gelatinous or biological fine particle sludges.	Work done was purely theoretical – no large scale electro-osmotic sludge dewatering plant existed at the time of the study. This work therefore needs to be validated before its aplicability to WWBRs can be assessed.
B	138	357/1/94: Bux F, Swalaha FM, Kasan HC, Microbiological transformation of metal contaminated effluents	1994	to develop cheaper, effective biosorbents to treat industrial wastes contaminated with metals.	Scale up needed
B	139	327/1/90: Nell JH, Van der Merwe M, Barnard RO, Evaluation of the active sewage pasteurisation (ASP) process for the treatment of sewage sludge	1990	Provides a method to detoxify sewage sludge such that it is suitable for unrestricted horticulture and agricultural use as a fertilizer.	More work required to determine if method is suitable for applications other than treating the sludge generated from dilute municipal sewage.
B	140	520/1/01: Pearson IA, Bhagwan J, Kariuki W, Banda W, Guidelines on appropriate technologies for water supply and sanitation in developing communities		Social aspects – community involved WWTW	Not really relevant
B AD		2105/1/14: Aoyi O; Apollo SO; Akach JWJP; Pete KY, Integrated photo-catalytic and anaerobic treatment of industrial wastewater for biogas production	2015	The treatment of high strength wastes such as molasses, textile, heavy metals and pharmaceutical waste water was investigated under different experimental conditions. An Integrated AD and Advanced oxygenation process (AOP) using South African zeolite was applied in the treatment of methylene blue dye in up-flow fixed bed bioreactor and UV photoreactor.	High cost of UV- rather use sunlight. Photodegradation of wastewater with high colour intensity difficult.
B AD	141	1538/1/09: Buckley AC Brouckaert CJ, A Feasibility Study	2009	Industrial wastewater, business model.	More work required to adapt this to include additional products in wastewater biorefinery context.

Category	Ref No	Authors & Title of Report	Year of publication	Value of research in context of wastewater biorefineries	Shortcoming of research in context of wastewater biorefineries / more work required
		in eThekwini Municipality on Anaerobic digestion for the Treatment of Toxic and High Strength Organic Wastes: A Study of the Business Case of Treating High Strength Industrial Wastes			
B AD	142	1216/1/05: Loewenthal RE, Ristow NE; Soteman SW; Wentzel MC; Ekama GA, Hydrolysis of primary sewage sludge under methanogenic, acidogenic and sulfate-reducing conditions,	2005	This report details the technical outcomes of investigatoins into the hydrolysis of primary sewage sludge, in order to produce accesible carbon for use in the BioSURE process which treats acid rock drainage.	The technology is applicable to WWBRs in the case where the WWBR treats ARD as well as sewage.
B AD	143	762/1/04: Sacks J, Buckley CA, Anaerobic digestion of high-strength or toxic organic effluents in available digester capacity.	2004	This project investigated the utilisation of available anaerobic digester capacity in KZN for the treatment of high-strength or toxic industrial effluents.	This work is applicable to WWBRs in as much as anaerobic digesters will likely form a key process within WWBRs, however this work covers a very specific area and question, and will need to be expanded upon to prove larger applicability.
B AD	144	455/1/01: Strydom JP, Mostert JF, Britz TJ, Anaerobic digestion of dairy factory effluents	2001	This research programme surveyed South African dairy industry to determine the present situation, requirements and need for effluent treatment; and investigated the use of anaerobic digestion of dairy wastewater.	Anaerobic digestion will likely form a key process in WWBRs, and so this report on ADs use for this specific application can inform that.
B AD	145	189/1/92: Division of Water Technology CSIR, Milnerton Municipality, University of Cape Town, Afrox Ltd, Evaluation and optimisation of dual digestion of sewage sludge. Executive Summary (and 189/2/92, 189/3/92, 189/4/92)	1992	Evaluation and optimisation of dual digestion of sewage sludge using an autothermal thermophilic aerobic reactor first stage and a mesophilic anaerobic digester second stage.	Analysis and modelling of the effect of industrial effluents discharged to municipal WWTWs. This work can be applied to WWBR in integrated municipal/industrial wastewaters.
B AD	146	87/1/84: Trim BC, Sludge stabilisation and disinfection by means of autothermal aerobic digestion using oxygen	1984		
B ALGAE	147	TT 390/09: Horan SJ, Horan MP, Mohale NG, Recovery and re-use of domestic wastewaters using	2009	Using algal biocatalysts – the entire Flamongo series of publications are informative.	Only sees algae as biomass for further use, needs more work to investigate commodity chemicals.

Category	Ref No	Authors & Title of Report	Year of publication	Value of research in context of wastewater biorefineries	Shortcoming of research in context of wastewater biorefineries / more work required
		integrated ponding systems: A key strategy in sustainable sanitation. Flamingo series no 13			
B BNR	148	1537/1/09: du Toit GJG, Parco V, Ramphao M, Wentzel MC, Lakay MT, Mafungwa H, Ekama GA, To investigate the performance and kinetics of biological nitrogen and phosphorus removal with ultrafiltration membranes for solid-liquid separation	2009	Aim 1: Evaluate biological nutrient removal (BNR) performance at typical membrane bioreactor (MBR) total suspended solids (TSS) concentrations (14-18 g/l). Aim 2: To compare the performance and kinetics of biological N and P removal under MBR conditions (high reactor TSS concentration (16 g/l)) with those in conventional BNR systems (low reactor TSS concentration (4 g/l)). Aim 4: Evaluate the impact of membrane solid liquid separation on the design of biological nutrient removal (BNR) activated sludge (AS) systems.	Adapt the findings to investigate the kinetics of bioproduction as well as nutrient removal in the wastewater context.
B BNR	149	1179/1/05: Cronje GL, Beeharry AO, Lakay MT, Wentzel MC, Ekama GA, Activity of heterotrophic and autotrophic biomass in BNR activated sludge.	2005	An investigation of the microorganism activity in biological nutrient removal, making wastewater biorefineries possible in principle.	More work required using this approach to determine activities of these organisms in a bioproduction context.
B BNR	150	692/1/02: Musvuto EV, Ubisi MF, Snyders M, Lakay MT, Wentzel MC, Treatment of wastewaters with high nutrient (N and P) but low organic (COD) contents.	2002	Treatment of wastewaters with high nutrient (N and P) but low organic (COD). This type of wastewater is often produced by primary forms of treatment (e.g. the supernatant of an AD), making this type of treatment especially relevant in terms of WWBR	The model developed needs to be experimentally tested over a wider range of water types, especially pH's, so as to be sure it applies in the context of a WWBR.
B BNR	151	137/1/86: Osborne DW, Lotter LH, Pitman AR, Nicholls HA, Enhancement of biological phosphate removal by altering process feed composition	1986	Biological phosphate removal	Useful in efficiently capturing phosphates in sewage. Could be applied in WWBR. Further work needs to be done on industrial wastewaters.
B BNR	152	TT 16/84: University of Cape Theory, design and operation of nutrient removal activated sludge processes	1984	The basis of biological nutrient removal, making wastewater biorefineries possible in principle	The model is limited in that it only considers nutrient removal and not recovery. The basis to maximize the maintenance coefficient of the microorganisms.
B DESAL	153	TT 266/06: du Plessis JA, Burger AJ, Swartz CD, Musee N, A desalination guide for South African municipal engineers.	2006	The purpose of this Guide is to: • provide a concise assessment of popular desalination technologies and related issues; • provide applicable guidance in the process of evaluating potential augmentation of municipal water supply through desalination, specifically within the context of available South African saline water sources. Such guidance is based on consideration of o saline water source quality and location,	Useful guide to inform WWBR considerations. Also helpful information in appendices. E.g. Appendix H: Membrane Technology companies (2006), Appendix I: Desalination plants in South Africa (owned by water supply authorities).

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				o desalination technology and peripheral process selection, o operating and maintenance aspects, o environmental and socio-economic implications, o capital and operating cost estimates.	
B MEMBRANE		TT 636/15: Turner KN , Naidoo K, Theron JG , Broodry J , Investigation into the cost and operation of Southern African desalination and water reuse plants, Volume I: Overview of Desalination and Water Reuse	2015	water reuse and desalination process into context and provide an understanding of current state-of-theart treatment processes and configurations, including how these relate to the technology used at the identified plants	Study done on coastal regions. Not inland
B MEMBRANE		2006/1/14: Baker PGL; Richards HL; Phelane L; Iwuoha EI, The Development of Nano-Composite Polysulphone Membranes with Reduced Fouling Properties for use in Wastewater Treatment	2014	The use of hydrogels as ultrafiltration-type membranes, have been proven to be excellent coatings for PSF membranes due to their amphiphilic nature, biocompatibility and excellent resistance to non-specific protein and other macromolecules adhesion. This area of research is very new and could hold the key to developing an anti-fouling membrane for use in wastewater purification.	Improve membranes but scale up necessary to evaluate the membrane performance in a small scale membrane reactor using simulated separation mixtures as well as real organic membrane reactor feed solutions. . One of the drawbacks of using metal nanoparticles in environmental applications relates to potential environmental health-related issues as a result of metal nanoparticles leaching into the environment.
B MEMBRANE	154	2010/1/12: Garcin CJ, Harrison STL, Pilot Scale Treatment of Table Olive Brines	2012	The membrane was able to satisfactorily separate the high Mw phenolic components from the waste stream resulting in clear brine stream that was then sent to the chromatography system; this was able to produce a purified brine stream for recycle, whilst retaining the antioxidants for recovery. An average of 360 g of antioxidant product was produced per 1 kL batch of wastewater processed. The process is only feasible if there are value-added products to be obtained from a waste stream; if wastewater treatment alone is considered, it is expensive due to the high cost of the speciality membranes and the chromatography resin used.	Good case study to be incorporated into a WWBR scenario.
B MEMBRANE	155	1371/1/07: Edwards W, Leukes WD, Bezuidenhout CC, Riedel K-HJ, Vladimir Linkov M, Jansen van Rensburg PJ, Neomagus HWJP, Burgess JE, Dual-Stage Ceramic Membrane Bioreactors for the Treatment of High-Strength Industrial Wastewaters	2007	Membrane bioreactors for treatment of high strength industrial wastewaters while generating stable adapted microbial consortium. Stripped Gas Liquor (SGL) industrial effluent (COD of ± 2000 mg.L-1) was used.	Application to WWBR
B MEMBRANE	156	1374/1/07: Pillay VL, Jacobs EP, Development of a combined activated carbon/microfiltration	2007	Textile effluent was treated with combined activated carbon/microfiltration	Application to WWBR. Lab scale - needs scale up

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		process for the treatment of industrial effluents			
B MEMBRANE	157	1372/1/06: Lewis A, Nathoo J, Prevention of calcium sulphate crystallisation in water desalination plants using slurry precipitation and recycle reverse osmosis (SPARRO)	2006		Preventative maintenance, or WWBR including desalination units (utilizing the brines)
B MEMBRANE	158	1384/1/04: Marah I, O'Donovan M, Martin R, Boberg D, Evaluation of microfiltration, ultrafiltration and nanofiltration for salt and chromium recovery from spent pickling and tanning effluent	2004	Salt and chromium recovery from spent pickling and tanning effluent	
B MEMBRANE	159	1035/1/01: Domrose SE, Sanderson RD, Jacobs EP, Burch G, Cleaning and pre-treatment techniques for ultrafiltration membranes fouled by pulp and paper effluent	2001	foulant characterisation and foulant removal from UF membranes used in industrial effluent (paper and pulp) treatment under laboratory-scale conditions and pilot scale	As elevation of the pH of the effluent feed to the UF plant reduced the rate and degree ofmembrane fouling considerably.
B MEMBRANE	160	847/1/98: Domrose SE, Finch DA, Sanderson RD, Development of transverse-flow capillary-membrane modules of the modular and block types for liquid separation and bioreactors	1998	Development of cost effective membrane cartridge modules of up to 10m2, multi-cartridge modules of up to 100m2, manifolding for capillary membrane modules and transverse flow capillary membrane module	Design optimisation
B MEMBRANE	161	548/1/97: Jacobs EP, Barnard JP, Investigation to upgrade secondary treated sewage effluent by means of ultrafiltration and nanofiltration for municipal and industrial use	1997	The objectives of the research were to determine to what extent medium-molecular-mass cut-off capillary ultrafiltration and tubular nanofiltration membranes, could be used to improve the quality of secondary treated sewage and water, over extended operating periods.	Membrane filtration is likely to be a useful technology for WWBRs, and the work presented in this report may inform operating and design decisions regaring membrane operating times.
B MEMBRANE	162	362/1/95: Malherbe GF, Morkel CE, Bezuidenhout D, Jacobs EP, Hurndall MJ, Sanderson RD, Industrial applications of membranes	1995	laboratory evaluation of various experimental membranes made at the Institute for Polymer Science (IPS) and made available in development quantities for use on real or simulated effluents, including evaluation at industrial sites. BRACKWATER TREATMENT, SASOL COOLING-WATER	Use in WWBR

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				BLOWDOWN, POTASSIUM BITARTRATE REMOVAL FROM WINE RESTS, SEAWATER PRETREATMENT FOR RO DESALINATION	
B MEMBRANE	163	242/1/90: Bailey AD, Dold PL, An exploratory investigation of crossflow microfiltration for solid/liquid separation in biological waste-water treatment	1990	exploratory investigation into the application of Crossflow Microfi1tration (CFMF) for solid/liquid separation in two biological wastewater treatment systems. The systems chosen were the Upflow Anaerobic Sludge Bed (UASB) reactor and the aerobic Activated Sludge systems.	operating conditions
B MEMBRANE	164	337/1/90: Strohwald NKH, Removal of algae from water by ultrafiltration	1990	(drinking water section – low concentrations?)	Feasible as DSP?
B NANO	165	KV 195/07: Schutte CF, Focke WW, Evaluation of Nanotechnology for application in water and wastewater treatment and related aspects in South Africa.	2007	Three general areas have been identified: (i) water treatment technology including development of improved membranes and development of activated filter media, (ii) development of real-time diagnostic tools for water quality assessment, (iii) development of membrane-based wastewater treatment technology. These may have application in WWBR.	Nanotechnology is a broad term, which is of limited usefulness. Some specific applications however may be useful, but these need to be evaluated on a case-by-case basis.
B SOLIDS	166	333/1/97: Whyte DC, Swartz CD, The removal of suspended solids from pulp and paper effluents by employing the combined sedimentation flotation process	1997	Suspended solids in the effluent of pulp and paper mills are comprised of both less dense particles (mainly fibres) and denser particles such as clay. this project investigated, at pilot scale, the use of a compact inclined plate settler integrated ahead of a flotation cell. The advantage of this configuration is the high rate of sedimentation coupled to the shorter solids retention time within the unit. The most significant conclusions of this study are that high percentages of removal for suspended solids can be obtained with the combined SEDIDAF process; the settling stage of the process contributes most to the overall removal of solids from the effluent; effective suspended solids removal can be obtained with settling in an inclined plate settler at surface loading rates as high as 10.9 m/h; improved suspended solids removal is obtained at lower flotation zone velocities in the DAF stage; the DAF stage does not only remove the organic fraction of the suspended solids but also inorganic particles; and, the settling stage does not only remove the inorganic fraction of the suspended solids, but also organic particles.	Investigate the application potential for WWBR. Has this been applied to industry since publication of this report?
B SSF	167	766/1/05: De Jesus AE, Heinze PH, Muller JR, Nortje GL, Utilisation of earthworms and associated systems for the	2005	A typical D-Grade abattoir (that slaughters up to 15 head of cattle per day) generates up to 1 ton of wet rumen contents and blood and up to 34.7 kl total wastewater per day. An important benefit of vermicomposting is that processing can take place in situ and that worthless or decomposing wastes need not be transported over long	Investigate the use of vermicompost or vermiculture for pre-treatment of biosolids before the Biosolids reactor, including further research aspects as highlighted in the report.. Investigate potential higher-value products from vermiculture. Lower value products include fertiliser, compost, potting soil, protein.

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		treatment of effluent from red meat abattoirs.		distances. The end products have value as fertilizer, compost, potting soil and as a protein source. The purpose of the current project was to "clean up" wastewater in addition to solid wastes. It differs from the standard vermicomposting process in that large volumes of wastewater pass through the system. The main problem that had to be solved was to ensure that the earthworms remained sufficiently active to convert the solid effluent to vermieompost under conditions where large volumes of liquid effluent passed through the system. A single container, adapted to ensure better filtration and harvesting of the vermicompost was designed and a laboratory-scale prototype built and evaluated. The earthworm ecosystem could be adapted to tolerate addition of blood provided it was not too concentrated (blood 0,7 % of the feed liquid). Provided the water could drain away within hours (about 3 hours in the present series of experiments), the earthworms were able to maintain a good speed of composting (10-15 cm per week) even when large volumes of water (similar to the amount of effluent from an abattoir) passed through the system. The system works well provided the layer of added solids does not exceed 2-3 cm per day; the liquid drains away fairly fast and aerobic conditions are maintained. The present process opens up the possibility to rid abattoir effluent of solids and to make the resultant liquid effluent more amenable to further treatment with existing systems. The effluent from the earthworm plant is not yet sufficiently clean to be released into the environment without further cleaning and polishing.	
B SSF	168	1129/1/04: Burton SG, Ryan DR, van Wyk L, Bioreactor systems using the white rot fungus Trametes for bioremediation of industrial wastewater	2004	Development of a practicable bioremediation process for using the enzymes of Trametes versicolor to degrade pollutants in specific industrial wastes, namely chlorinated aromatics and phenolics produced by the pulp-and-paper and petrochemical industry. Large scale, cost-effective applications of white-rot fungi to continuous treatment of liquid effluent has previously been hindered by the lack of suitable bioreactor systems. A hollow fibre membrane bioreactor and a trickle filter were investigated for suitability as supports for immobilised biofilms of T. versicolor and laccase production and pollutant degradation were successfully demonstrated in both reactor configurations. However, the need for a simple, cost effective, yet simple to upscale reactor system led to the investigation and development of an airlift loop reactor (ALR). Increased growth (10g/L dry mass) and enzyme production (12000U/L) as well as highly efficient effluent degradation (5% v/v/day) were achieved in the ALR	Investigate the value of this work in context of WWBR - does it optimise well as a unit process in the treatment strain? Take the system to larger scale and demonstrate its effectiveness at pilot scale.

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				in fermentations over two week periods. Immobilised biofilm reactors in the form of a Transverse Flow Membrane Bioreactor and a Trickle Bed Reactor were identified as suitable for growth, enzyme production and phenolic removal by T. versicolor.	
B SSF	169	331/1/01: Pretorius WA, Willie P, Oxygen transfer in filamentous biocultures	2001	In wastewater treatment, Chemical Oxygen Demand (COD) balances are used to determine the oxygen requirement of a particular biological wastewater treatment process. This same method, although very cumbersome, could be used to determine the oxygen transfer efficiency in a biological growth system. This was the method used in this study to determine the aeration efficiency under various experimental conditions.	Fairly fundamental work on aeration. May have WWBR application, specifically in biosolids reactor studies.
B SSF	170	535/1/98: van der Westhuizen TH, Pretorius WA, Use of filamentous fungi for the purification of industrial effluents	1998	This is a report on an investigation conducted to determine the potential of using the micro-screen process to convert industrial effluent COD into biomass that can be used for secondary purposes. The report describes the development of the process on one specific effluent, but the process is equally suitable for a large range of effluents in many of the organic industries world-wide. The effluent under discussion in this report is a typical low acetic acid containing effluent, but it also contains inhibiting substances that made conventional biological treatment difficult. Notes: Bacterial contamination above a certain degree influences the dewatering characteristics and product quality of the biomass. Two possibilities for commercial use of the biomass have been investigated, namely use of the dried biomass as protein source, and secondary batch fermentation of the harvested biomass to produce celluiase enzymes.	Liquid fungal culture application. The approach to challenges are useful for WWBR approaches, specifically since the process relies on dynamic selection principles to sustain the filamentous culture, any possible bacterial contamination had to be quantified.
B UASB	171	1248/1/06: Foxon KM; Buckley CA, Brouckaert CJ, Dama P, Mtembu DZ, Rodda N, Smith M, Pillay S, Arjun N, Lalbahadur T, Bux F, The evaluation of the anaerobic baffled reactor for sanitation in dense peri-urban settlements.(ABR)	2006	Technology that may be relevant for WWBR: The evaluation of the anaerobic baffled reactor for sanitation in dense peri-urban settlements.	Possible application to WWBR in dense peri-urban settlements, as a model of decentralised production.
B UASB	172	1364/1/06: Sigge GO, Britz TJ, McLachlan T, van Schalkwyk N,	2006	Lab scale experiments and scaled up to 600 L. Good report for WWBR.	

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		Treatment of apple and wine processing wastewaters using combined UASB technology and ozonation scenarios.			
B WETLAND	173	TT 438/09: Kotze DC, ASSESSING THE SUSTAINABILITY OF WETLAND USE	2009	A model developed to assist in assessing the ecological sustainability of wetland use, focusing on grazing of wetlands by livestock, cultivation of wetlands and harvesting of wetland plants for crafts and thatching.	Limited direct applicability to WWBRs, but could potentially be adapted to be applied to macrophyte operations.
B4 MEMBRANE	174	TT 556/12: Edwards W, Marshall Sheerene Sheldon MS, Zeelie PJ, De Jagers D, Dekker LG, Bezuidenhout CC, Water Reuse for Industrial Wastewater	2013	Performance of dual-stage Membrane BioReactor (MBR) for the treatment of textile and paper mill effluent, including economic viability assessment.	Technology applicable to WWBRs, already demonstrated on several wastewaters.
B4 MEMBRANE	175	2011/1/13: Tandlich R, Luyt C, Tyalana K, Moyo F, Application of emulsion liquid membranes in the extraction of rhodium from mining and metal refinery effluent	2013	This project set out to investigate the application of emulsion liquid membranes (ELMs) in recovering platinum group metals (PGMs) from the aqueous by-products of PGM refining. Extraction of Rh from aqueous matrices was tested and the results showed that the complete extraction of Rh was possible. This was achieved by the use of an optimised ELM. Carryover of the diluent components into the stripping phase and effluent was observed and further work is recommended to overcome this drawback.	A possible route to extract metals from wastewaters either as part of a WWBR process train or as a pre-treatment step to make the water more suitable for bioconversion.
B5 WETLAND		2104/1/14; Welz PJ, Ramond J-B, Cowan DA, Smith I, Palmer Z, Haldenwang R, Burton S, Le Roes-Hill M, Treatment of winery wastewater in unplanted constructed wetlands		Expanded on the knowledge generated from their previous WRC-funded project (K5/1936) to understand how constructed wetlands may be adapted for “real world applications”	By definition, constructed wetlands contain plants. This strict definition is debateable because many natural wetlands do not contain plants. Nevertheless, to avoid confusion, the systems used in the project are referred to as biological sand filters.
B6 NANO	176	1991/1/13: Pletschke B, Torto N, Frost C, Zeni Tshentu Z, Electrospun nanofibre-based strategies for removal and detection of water contaminants	2013	electrospun nanofibre-based devices for water purification as well as monitoring of water quality.	DSP for WWBR. Also for process analysis.
B6 NANO	177	1897/1/12: Leslie Petrik L, Ndungu P, Nanotechnology in water treatment	2012	removal of several inorganic and selected organic contaminants such as acid rock drainage (ARD) from various mines in the Gauteng and Mpumalanga regions, industrial brine effluents, dyes, and bacterial laden water	Technology that can be used in WWBR

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C	178	Biomimicry for Constructed Wetlands: Looking To Nature For Solutions On Water Treatment	2013	WRC project is ongoing. Biomimicry is a good design tool to facilitate systems thinking, and shares the holistic approach of wastewater biorefineries.	Data is lacking, this is not a robust engineering application. This may improve as this project progresses.
C	179	TT 565/13: Swartz CD, vd Merve-Botha M, Freese SD, Energy Efficiency in the South African Water Industry: A Compendium of Best Practices and Case Studies	2013	A stepping stone towards biorefineries using the cleaner production approach	Focused on energy reduction. More work required on on-site energy production and integrated unit operation, e.g. waste-heat recovery
C	180	KV 323/13: van Vuuren SJ, Loots I, van Dijk M, Barta B, Energy Generation using Low Head Technologies	2013	The results of investigation indicate clearly that there are significant potential for the development of low-head hydropower in ... wastewater treatment infrastructure. It is significant on the background of the potential reduction in electricity demand on the national grid presently supplying the conveyance, pumping and treatment of raw water and the treatment of large quantities of urban wastewater.	Co-generation may be of relevance to WWBR. It needs to be determined how the low head hydro technology will impact plant operation.
C	181	TT 546/12: Mvuma GG, Hooijman F, Brent AC, Oelofse SHH, Rogers DEC, Volume III: Development and assessment of technological interventions for cleaner production at the scale of the complex	2012	Key factors that influence the environmental sustainability of a large inland industrial complex: The Secunda Industrial Complex.	Assessment of cleaner production options and environmental assessment by LCA
C	182	TT 485/11: Barclay S, Trusler G, von Blottnitz H, Buckley CA, Kothuis B, Janisch C, Cleaner Production: A Guidance Document for the Mining Industry in South Africa	2011	Helping to implement cleaner production in mining industry	The use of cleaner production tools such as quick scan assessments, life cycle assessments, and cleaner production forums to encourage and motivate the mining industry to implement cleaner production in order to reduce their environmental impact and increase profitability
C	183	1553/1/11: Trusler G, Mzoboshe S, The introduction of cleaner production technologies in the South African mining industry: a summary report	2011	Cleaner production technologies describes a preventative environmental approach, aimed at increasing resource efficiency and reducing the generation of waste at source, rather than addressing and mitigating just the symptoms by technically treating an existing waste or pollution problem	Good guidelines for general approach in WWBR.
C	184	1898/1/11: Majozi T, Adekola O, Water Use Optimization in industry: A Mathematical Model for a Multipurpose Batch Plant	2011	It is desirable to minimize the production of pharmaceutical effluent at worst and eliminate it at best. This report presents a methodology to address the problem of wastewater minimization, over longer time horizons, including by extending the concept of water reuse to include a regeneration system. This study systematically presents	Can this model be applied to running WWBR efficiently? Probably of little relevance.

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				mathematical formulations and detailed case studies where these techniques have been applied with freshwater savings in excess of 25%.	
C	185	1542/1/08: Mbolekwa Z, Buckley CA The removal of reactive dyes from dye liquor for the reuse of salt, water and energy	2008	The aim of the project was toš establish the process parameters governing the recovery of water and chemicals for reuse from reactive dye baths using activated carbon.	Further research in this area should concentrate on evaluating different activated carbons and role of auxiliaries in activated carbon adsorption studies. This study proved that the activated carbon adsorption technique is the solution in reactive dyeing textile industries because of the possibility for re-use of water, salt and energy; thus enabling environmental improvements with savings in salt, energy and water.
C	186	1625/1/08: Majozi T, Gouws JF, Development of a complete process integration framework for wastewater minimisation in multipurpose batch plants.	2008	Development of a mathematical optimisation technique for wastewater minimisation, speicifcally in batch systems, that could be applied to industrial scales.	This technique could prove to be invaluable for WWBR reactor scheduling and optimisation.
C	187	1673/1/08: Mazema HK, Ally SH, Kamish W, Muhaydien A, A pilot study into available upstream cleaner production technologies for the petroleum refining industry to meet the requirements of the waste discharge charge system.	2008	Provides an assessment of the Cleaner Production technologies available to the petroleum refining industry, and the waste discharge charge system (WDCS) based on the available cleaner production initiatives.	NB for WWBR on site for petroleum industry
C	188	TT 283 & 4/07: Barclay S, Buckley C, Waste minimisation clubs in South Africa (Facilitation and Training Manual)	2007	Cleaner production initiative	
C	189	1368/1/07: Fraser D, Ndwandwe K, Basnal P,Isafiade A, Nyathi NS, Majozi T, Brouckaert CJ, Brouckaert BM, Water conservation through energy conservation.	2007	Reducing water usage through efficient energy, heat and water use.	Focused on heat exchanger networks for reduced energy consumption, and a similar method for water use reduction. More work required on on-site operation, e.g. waste-heat recovery.
C	190	1266/1/06: Grove B, Whole-farm model to optimise water use.	2006	Stochastic modelling of water usage on farms.	Package plants most often fail in their ability to effectively nitrify ammonia and in disinfecting against bacteria, due to faults in design and operation, not due to the process technology per se. More work required to evaluate the suitability of these package plants for

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					modular use in wastewater biorefineries in communities. - link up with SEWPACKSA?
C	191	TT 139/00: Barclay S, Buckley C, Waste minimisation guide for the textile industry: A step towards cleaner production. Vol I. (and TT 140/01 and TT 161/05)	2000	Minimising wastewater in textile industry	Development of flowsheets for minimising water and energy usage in the textile industry; Assessment and categorising of waste streams; Aim is to reduce environmental impact and comply with legislations
D		2131/1/15: Tesfamariam EH; Annandale JG; de Jager PC; Ogbazghi Z; Malobane ME; Mbetse CKA, Quantifying the fertilizer value of wastewater sludges for Agriculture	2015	To develop a user friendly sludge application rate advisor computer model that takes into account both the fertilizer value of sludge and crop nutrient requirements	Further analyses required i.t.o. effect of post wastewater treatment dewatering techniques on fertilizer value
D	192	KV 320/13: van Niekerk A, Schneider B, Implementation Plan for Direct and Indirect Water Re-use for Domestic Purposes-Sector Discussion Document	2013	focused specifically on the direct and indirect reuse of domestic treated wastewater as a proactive step to generate a sector discussion document for the progressive implementation of the Water Re-use Strategy. The project developed a plan to bridge the gap between the strategy and implementation of water re-use for domestic water use in consultation with the Department of Water Affairs.	Possible opportunities for WWBR ito Developing appropriate technologies and undertaking baseline studies to determine the status of indirect / direct domestic / potable water re-use
D	193	1724/1/12: Tesfamariam EH, Annandale JG, de Jager PC, Mbakwe I, van der Merwe P, Nobela L, van der Laan M, Sustainable Agricultural Use of Municipal Wastewater Sludge, 1724/2/12: The potential of sludge amended combustion coal ash residues	2012	An investigation of use of sludge (both municipal waste derived, and petro-chemical waste derived) for agriculture.	Recovery and re-use of N and P out of sludges for agriculture. More work required on the stages of processing that still considers the product (more than just soil additive) as originating from sludge.
D	194	TT 520/12: Fessehazion KM, Abraha AB, Everson CS, Truter WF, Annandale JG, Moodley M, Water use and nitrogen application for irrigation management of pasture production	2012	Water use and nitrogen application for irrigation management of pasture production	Water from WWBR could be used in pasture irrigation – this study informs that. However, limited applicability to WWBRs.
D	195	1937/1/11: Burton SG, Mupure CH, Horne KA, Jones S and Welz	2011	Beneficiation of Agri-Industry Effluents	Downstream processing of agri-wastes, for recovery of valuable products (phenols, antioxidants and sugars). A closer evaluation of

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		PJ, Beneficiation of Agri-Industry Effluents			the (economic) feasibility and market potential of concepts highlighted in this study.
D	196	1937/1/11: Burton SG, Mupure GH, Horne KA, Jones S, Welz PJ, Beneficiation of Agri-Industry Effluents	2011	The feasibility of wastewater beneficiation depends largely on the concentrations of valuable by-products present and the efficiency of the extraction processes that can be applied. The paper reviews apple and citrus wastewaters were analyzed.	Very early level research, possibly not considering economically viable, bulk commodity products that is expected to be more suitable for WWBR application.
D	197	TT 351/09: Herselman JE, Burger LW, Moodley P, Guidelines for the Utilisation and disposal of wastewater sludge, Volume 5, Requirements for thermal sludge management practices and for commercial products containing sludge.	2009	Report series aims to provide options and opportunities for WW sludge use innovation. Where wastewater sludge cannot be used as a resource, the guidelines also provide for its disposal in a responsible manner.	The guidelines will likely apply to the operation and products from WWBRs, however these guidelines will likely require further clarification and adjustment.
D	198	KV 187/07: Burton SG, Garcin CR, Aucamp JH, Beneficiation of wastewaters from the South African citrus industry- A feasibility study	2007	Examines two principal products (an oil and a carbohydrate) from citrus wastewaters, with preliminary technoeconomic evaluation.	Good applicability to WWBRs, giving a techno-economic assessment of a wastewater to products example.
D	199	1242/1/05: Petrik L, White R, M; Somerset V; Key D; Iwuoha E; Burgers C; Fey MV, Klink Utilization of fly ash for acid mine drainage remediation	2005	This report details the use of fly ash, from coal fired power, to treat acid rock drainage, and producing zeolite from the resultant product. This technique disposes of two hazardous materials, and produces a saleable product simultaneously.	This technique could prove useful in WWBRs if ARD is to be treated, and if fly ash is available. However, more work is required on the scale-up and techno-economic evaluation of the method.
D	200	1367/1/05: Christopher L, Bio-remediation and Bio-utilization of pulping and bleaching wastewaters.	2005	This technical paper demonstrates the reduction of toxic chemical use when using alternative bleaches, such as enzymtatic approaches. Furthermore, valuable products (such as the abovementioned enzymes) can be produced from the pulp wastewaters.	The application of the wastewater technology (cleaning pulp wastewater to produce enzymes) is applicable to WWBRs, while not the first part of the report.
D	201	1210/1/04: Snyman HG, van der Waals JH, Laboratory and field scale evaluation of agricultural use of sewage sludge.	2004	An investigation of use of sewage sludge in agricultural soil amendment, including composition and characteristics, and the potential for accumulation of heavy metals and pathogens.	potentially applicable to WWBRs, as sludge for agricultural soil amendment may be a valuable product. However, furth work on WWBR sludges in the same space are needed.
D	202	366/1/94: Loots PA, Oellermann RA, Pearce K, Pilot studies on phosphate crystallization in biological wastewater treatment systems	1994	Phosphate recovery using crystallization in biological wastewater treatment systems	Steady state was not achieved at the pilot scale and thus the study did not achieve the objective of full scale testing due to process instabilities. Potential to investigate work again if more detailed thermodynamic data relating to phosphate crystallization can be found.

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D AGRIC	203	TT 430/09: Stimie CM, de Lange M, Crosby CT, Erna Kruger E, Agricultural water use in homestead gardening systems	2009	To improve food security through homestead gardening, by developing and evaluating the appropriateness and acceptability of training material for water use management, training the trainers and training of household members in selected areas.	Some systematic thinking about disperse small scale processes could be applicable to regional WWBRs, however, mostly inapplicable.
E	204	2012/1/13: Randall D, Lewis A, Rodriguez-Pascual M, Nathoo J, Reddy T, Apsey G, Kapembwa M, Egan T, Chivavava J, Extended Investigations into Recovery of Water and Salts from Multi-component Hypersaline Brines using Eutectic Freeze Crystallization	2013	Recovery of water and salts from industrial (coal and platinum mining) wastewaters. This could be implemented in WWBR if a brine stream is part of the process.	This work needs to be extended to other complex brines, from other industrial sources. Application in WWBR to be shown.
E	205	2013/04/12; Dunn K; Rose P; Arthrospira (Spirulina) in tannery wastewaters. Part 1: The microbial ecology of tannery waste stabilisation ponds and the management of noxious odour emissions using microalgal capping Part 2: Evaluation of tannery wastewater as production media for the mass culture of Arthrospira biomass.	2013	(Spirulina) in tannery wastewaters. Part 1: The microbial ecology of tannery waste stabilisation ponds and the management of noxious odour emissions using microalgal capping Part 2: Evaluation of tannery wastewater as production media for the mass culture of Arthrospira biomass.	Possible application to WWBR, a case specific application
E	206	1543/1/10: Mapolie SF, Saptarshi, Darkwa J, Van Wyk JL,Industrial wastewater remediation via wet air oxidation using immobilised transition metal catalysts	2010	Using catalytic wet air oxidation for removing organic materials from industrial effluents. Using phenol as model chemical to be removed	Evaluation of suitable reactor systems for the catalytic processes.
E	207	1363/1/08: Binda M, Gounder P, Buckley CA, Barbara, Promotion of biodegradable chemicals in the textile industry	2008	Development of score system for tectile industry effluent. A pilot studt of implementing the score system at volunteer factories.	This methodology could be usefully applied to WWBRs, for influent and effluent analysis. However, more work is required to apply the methodology to other indutrial effluents.
E	208	1546/1/07: Petrik LF, Hendricks NR, Ellendt AAM, Burgers CL, Toxic element removal from water using zeolite adsorbents made from solid waste residues	2007	Preliminary study on the use of fly ash to neutralise acid rock drainage, and the subsequent production of zeolite adsorbant materials from the residue of this process for toxin removal from wastewaters.	Limited applicability to WWBRs, although toxin removal from WWs may be important in WWBRs.

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E	209	1377/1/05: Taljaard L, Venter A, Gorton D, An evaluation of different commercial microbial or microbially-derived products for the treatment of organic waste in pit latrines.	2005	Pit latrines operate on the principle of anaerobic decomposition. This process, however, is very slow, leading to organic waste build-up and subsequent system blockages. There are claims that the use of microbial or microbially-derived products for the treatment of organic waste in pit latrines controls odour and also reduces the bulk of the organic material. A total of 16 products were obtained. There were minimal changes in the pits treated with Product M and no changes in the control pits. The odour and the population of flies in the treated latrines (especially with Product B) disappeared after the first dosages, whereas bad odours and Hies persisted in the untreated latrines.	More work required to evaluate if these bioproducts can be produced from wastewater, as well as their efficacy in situ. Future work could include a biological study into the claimed mode of action of these biological products. The products should be evaluated on the basis of the amount and type of microorganisms and enzymes present, and compared to the information and claims on the specification sheets.
E	210	1072/1/05: Neomagus HWJP, Bio-polymeric heavy metal adsorbing materials for industrial wastewater treatment.	2005	The removal and recovery of heavy metals using the biosorbent chitosan is investigated. Since flakes do not have good adsorption characteristics and are difficult to use in large equipment, other configurations (beads, membranes and immobilised chitosan) were prepared for the experimental adsorption studies. The adsorption experiments were carried out at a laboratory scale. A gel type of material was formed, containing predominantly water (93-96%) and the balance chitosan (4 – 7%). For the chitosan beads, a novel adsorption model has been developed, which takes the acid base characteristics of the chitosan into account. The new model can therefore be used at any pH, for both adsorption and desorption. From this model, it could be concluded that the chitosan has the largest affinity to copper, followed by lead, nickel, zinc and cadmium.	Investigate the possibility of recovering chitosan from (fisheries?) wastes in a WWBR systems-thinking setup (even just a paper based study), explore where co-siting of metal recovery may be an option. Explore chitosan-related thinking in a dilute context: this study focused heavily on increasing concentration and throughput, which affects chitosan stability and costs. Market potential: "Since chitosan is only produced at a small scale, the prices on the world market are relatively high ($ 35/kg). Since the market for chitosan is rapidly growing (mainly for the application of fat absorber), price decreases are expected in the future."
E	211	1259/1/05: Petersen F, Aldrich C, Esau A; Qi BC, Biosorption of heavy metals from aqueous solutions.	2005	The project's objective was to investigate the feasibility of using biomaterials for the removal of heavy metals from aqueous effluents by first identifying a suitable biosorbent, characterizing the sorbent and evaluating its use on an industrial scale.	WWBRs will likely need to be able to treat wastewaters containing heavy metals, and so this technology could prove useful for their removal. However, further work on the limitations and applications of the technology is needed.
E	212	1170/1/04: Whiteley CG, Pletschke BI, Burgess JE, Tshivhunge AS, Ngesi N, Whittington-Jones K, Enongene G, van Jaarsveld F, Heron P, Rashamuse, Rose PD, Investigation into the enzymology of accelerated primary sewage sludge solubilisation and digestion in sulphate reducing systems.	2004	A study of a bioproduct (enzyme) to be used in sulphate reducing systems. This study has indicated that the enhanced mineralisation of complex particulate organic matter in sewage sludges relies primarily on enzymatic hydrolysis of the micromolecules. Furthermore it provides a view of the enzymology of the RSBR with respect to depth of the reactor and concomitant effect of levels of sulphide, sulphate and alkalinity/pH of the overall system.	More work required to evaluate if these enzymes can be produced as marketable and financially viable bioproducts from wastewater.

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E	213	723/1/04: Summers GF, Designed functionalized polymers by anionic macromolecular engineering for membrane development and fabrication.	2004		Bio application. Could be produced by WWBR (biologically or physic-chem)
E	214	1040/1/03: Graz CJM, Stilwell KM, McComb DG, A two-enzyme cleaning-in-place programme for South African dairies	2003	Production on enzymes (bioproduct) in a cleaning programme in SA dairies	To produce an economically viable biological cleaning system for dairy plants. To screen microbes isolated on-site in a dairy for their extracellular enzymes which can degrade milk constituents. Can this enzyme be used/produced in WWBR.
E	215	1083/1/02: Swalaha FM, Datadin S, Choonawala BB, Assessment and application of imported biomass for the bioremediation of heavy metal effluents	2002	Bioremediation	Assessing treatment options for metals contaminated waste streams from metal mining and metal processing facilities
E	216	932/1/02: Leukes W, Edwards W, Buchanan K, Bezuidenhoudt J, Jordaan J, Watcham C, Way-Jones N, Enzymatic defouling of ultrafiltration membranes: A defouling-on-demand strategy using immobilised enzymes	2002	Enzymatic cleaning has been offered as an alternative to chemical cleaning since enzymes are biodegradable and do not cause additional pollution problems. Enzymes are immobilised onto the ultrafiltration unit	Produced from WWBR. large-scale, low-cost production of the thermostable laccase should be developed for provision of sufficient enzyme for pilot-scale testing. Also, effective process design needs to be done to formulate this technology into a usable operating system.
E	217	623/1/96: Talbot MMB, Ascough SW, Rankin A, Bio-enhancement of a river system using a biological catalyst	1996	Bio-enhancement of a river system using a biological catalyst	If effective, can this biocatalyst be produced in WWBR?
E	218	531/1/96: Swart P, Maartens A, Engelbrecht J; Allie Z, Jacobs EP, The deveolpment of characteristics and cleaning techniques to classify foulants and remove them from ultra- and microfiltration membranes by biochemical means	1995	The deveolpment of characteristics and cleaning techniques to classify foulants and remove them from ultra- and microfiltration membranes by biochemical means. There are a number of commercial enzyme preparations available on the market that are used for cleaning purposes in the food industry These preparations are not specific and a broad spectrum of biological materials will be removed by them In membrane installations where fouling can be attributed to one or more main group(s) of biological molecules, the development of specialised enzyme systems, highly specific for particular fouling agents, will be the most cost and time effective method for the removal of foulants from the membrane surface.	Bio application. Could be produced by WWBR (biologically or physic-chem)
E	219	318/1/94: Cloete TE; Brözel VS; de Bruyn EE; Pietersen B,	1994	Recent studies have indicated that biofilm ecosystems respond to stress (i.e. biocides) in ways similar to macro-ecosystems. Generally,	Limited application to WWBR. Knowledge may be useful for optima process control in units.

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		Optimisation of biofouling control in industrial water systems		there is a decline in species diversity and a selection of more tolerant isolates. Mucoid mutants did not exhibit increased tolerance to bactericides, indicating that extracellular polysaccharide does not confer increased resistance to bacteria in biofilms. Attached cells were more resistant than free-living cells within 15 min following attachment. Cell age had a marked influence on resistance, where actively growing cells were most resistant and late stationary phase cells were least resistant.	
E	220	1165/1/06: Jacobs EP, Swart P, Bredenkamp MW, Allie Z, Govender S, Liebenberg L, van Kralingen L, Williams WT, Development of technology for the selective removal of bioactive pollutants by ligands, non-covalently immobilised on membranes.	2006	The development of a technique by which biologically active species, specifically endocrine disruptive chemicals, could be separated from water by way of selective ligands immobilised on membranes.	Technology applicable to WWBRs, especially if potable water is to be produced from wastewaters which may contain EDCs. However, this technology requires further development and scale-up.
F		1822/1/14, Ikumi DS, Harding TH, Vogts M, Lakay MT, Mafungwa H, Brouckaert CJ, Ekama GA, Mass balances modelling over wastewater treatment plants III	2015	To develop three phase (aqueous-gas-solid) steady state and dynamic mathematical models for the anaerobic and aerobic digestion of sludge; including waste activated sludge (WAS) produced by enhanced biological phosphorus removal (EBPR) plants, within a plant-wide setting.	No standardardisation in modelling software. Frustration between consultants and munciple users
F	221	TT 601/14: Environmentally Sustainable Beneficiation of Brewery Effluent: Algal ponding, Constructed Wetland, Hydroponic Vegetables and Aquaculture Clifford LW Jones, Peter J Britz, Rory Scheepers, Sean Power, Anneke Cilliers & Richard Laubscher Report to the Water Research Commission by Department of Ichthyology and Fisheries Science, Rhodes University WRC Report No TT 601/14	2014	This project aimed to develop a sequence of effluent treatment methods using existing technologies, such as algal ponding and constructed wetlands, to develop a unique, low cost, low-tech, environmentally sustainable industrial water treatment process. It also aimed to combine these technologies with the production of algae, vegetables and fish in such a way that the end result was not only treated industrial effluent, but also the production of recovered water available for reuse and/or used for producing valuable downstream products. The project's goal was to take industrial effluent and, using little more than the sun's energy and photosynthesis, turn it into clean water, valuable algae, fresh vegetables and fish (swordtail (Xiphophorus helleri) - classified as a nuisance pest though, so not suitable).	Has details of people who may be useful to guide WWBR further included in report (page xv). Is there a techno-eceonomic analysis done on this work? Would it be able to scale to other WWBR applications? From the report: In the initial baseline studies it was demonstrated that the high rate algal pond/wetland system was a viable alternative to an activated sludge system, with a substantially lower environmental impact and lower operating costs than the more conventional method of treating effluent. The geographic footprint (i.e. the space) required to operate a full-scale high rate algal pond or constructed wetland system would be substantial, so optimising its performance was identified as a priority. The research that followed was thus aimed at increasing the flow of effluent through the systems without compromising the efficiency of nutrient removal, thus determining the minimum size of the physical footprint required to treat a given volume of effluent. In autumn it was possible to reduce the hydraulic retention time of the HRAP from 18.6 d to 3.8 d, and in summer to 2.5 d. The drop in pH (9.0 to 8.5) and ammonia (6 to 2

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					mg/L) levelled off within 13 m of the linear wetland. Further work was required to (a) refine the required length of constructed wetland and (b) determine seasonal variation in the required hydraulic retention time in the constructed wetland.
F	222	TT 587-14: Verster B, Madonsela Z, Minnaar S, Cohen, Harrison STL, Introducing the wastewater biorefinery concept	2014	Introducing the biorefinery concept specifically – the precursor to this current project.	As an introduction, this study still lacks scope and depth of opportunities and risks (partially adressed in this current report)
F	223	1803/1/13: Blignaut J, de Wit M, Milton S, Esler K, le Maitre D, Mitchell S, Crookes D, A market for ecosystem goods and services following the restoration of natural capital: Volume 1: Main Report (and 1803/2/13)	2013	Integrated system dynamics model on the likely impact of restoration on the ecology, hydrology and economy of restoration sites	Ecosystem economics possibly applicable to WWBR, although products are not the focus of the model.
F	224	TT 399/09: Burton SG, Cohen B, Harrison S, Pather-Elias S, Stafford W, van Hille R, von Blottnitz H, Energy From Wastewater – A Feasibility Study (Essence Report)	2009	An overview of the chemical potential of wastewater, making wastewater biorefineries possible in principle	Only considers energy product, more work required for commodity chemicals, and Nitrogen and Phosphate containing product
F	225	1541/1/08: Mutambanengwe C, Oyekola O, Togo C, Whiteley CG, Production of Enzymes for Industrial Wastewater Treatment – Proof of Concept and application to the textile dye industry.	2008	Based on work on the BIOSURE process, this study undertook a thorough investigation to show that hydrogenase enzymes, also found within the biosulphidogenic reactor, could be used to bioremediate industrial waste effluent from the textile dye industry.	A good example of producing enzymes from wastewater via the BIOSURE process - possible case study of WWBR.
F	226	TT 235/04: Rouhani QA, Britz PJ, Contribution of aquaculture to rural livelihoods in South Africa: A baseline study	2004	Aquaculture is the beneficial and sustainable use of water as a medium in which to farm organisms, such as finfish, shellfish and aquatic plants, for example. The contribution of aquaculture to the livelihoods of rural communities was found to be negligible. “Small scale commercial” aquaculture projects were found to be more viable than “food security” projects. For "food security" projects, simple problems often resulted in project dysfunction or failure. Most projects had too many participants and the level of income per participant was very low. These "food security" type aquaculture projects were found to be unsustainable without ongoing technical support, and probably some structured “low interest” loans for set-up and input costs. The	Does WWBR count as aquaculture, and if so, which type? Community-public-private partnerships may be a suitable vehicle for promoting small-scale aquaculture projects. A public sector commitment on this scale requires clear policy objectives, sectoral plans and institutional coordination. The role of the public sector was analysed in this project in terms of emerging policy, the future of existing public sector aquaculture facilities, community public-private partnerships and interdepartmental coordination, and as such may prove useful to WWBR policy as well.

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				major weakness in all projects was a lack of training and experience in aquaculture and all projects required extension and technical support. Given the poor track record of the public sector in sustaining support to aquaculture projects, and the relative success of small-scale commercial projects, future policy should emphasise linkages to the existing private aquaculture sector by means of community-public-private partnerships.	
F	227	1081/1/04: Klusener CW, The development of a protein recovery technology at Sezela for the treatment of furfural plant azeotrope effluent with the simultaneous production of	2004	This document examines the use of filamentous fungi in the treatment of furfural effluent, producing a mycoprotein product for use in animal feed. The study focused on estimating a benchmark commercial value for the mycoprotein by evaluating it's use as an animal feed.	This study provides an excellent example of a process which could be incorporated in a WWBR, taking in an industrial effluent and simultaneously treating the WW and producing a salable product under non-sterile conditions.
F	228	1082/1/03: Christof LP, Further development of a biotechnological approach to the management of wastewaters from the pulp and paper industry	2003	Remediation of industrial wastewaters from the pulp and paper industry was investigated using biological methods such as pretreatment with enzymes, white-rot and mucoralean fungi. The wastewaters under study were derived from the extraction stage of the bleach plant as well as the spent sulphite liquor from the pulping stage of pulp production. Fermentation experiments for enzyme and gamma-linolenic production were carried out in shake flasks. Screening for best microbial sources was assessed according to levels of xylanase activity and single cell protein attained. The economical feasibility of the entire biobleaching technology using xylanases would be improved by utilising pulp mill wastewaters which at present are discarded as industrial waste.	Large WWBR potential. It has been demonstrated that implementation of the enzyme bleaching technology in the pulp and paper industry could improve the existing technology of pulp and paper manufacture in a cost-effective and environmentally friendly way. Trickling filters and RBC's were tested. The most efficient treatment system proved to be the rotating biological contactor where colour, bacterial growth inhibition levels, adsorbable organic halogen and chemical oxygen demand were decreased to a significant extent. Tall oil, which is a by-product derived from kraft mill spent liquor, could be utilised by selected fungi for production of high-value fatty acids such as gamma-linolenic acid.
F	229	939/1/03: Burton SG, Boshoff A, Foster I, Koteshwar K, Luke A, Mhlanga C, Nganwa P, Notshe T, Ryan D, Bioreactor systems for the conversion of organic compounds in industrial effluents to useful products.	2003	Focused on laccases, peroxidases and polyphenol peroxidases for the target groups of pollutants being phenolics, polyphenolics and related aromatic compounds. The research included investigations of enzyme production and biofilm growth as well as pollutant degradation. The enzymes in this study did not require cofactors such as NAD.	Significant WWBR potential. Work included fungal biofilms - Trametes versicolor and Neurospora crassa, which should be further investigated in WWBR application. Polyphenol oxidase (PPO) from mushrooms to synthesize cathechols should be investigated in the WWBR context.
F	230	TT 187/02: Rose PD, Salinity, Sanitation and Sustainability: A Study in Environmental Biotechnology and Integrated Wastewater Beneficiation in South Africa (Report 1) (and TT	2002	This report describes a twelve-year WRC investigation into an environmental biotechnology approach in the treatment of saline and sanitation wastewater, specifically focused on algal technologies.	The technologies used in this series of reports will be very applicable in WWBRs, and should inform research into any saline wastewaters.

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		188/02, TT 190/02, TT 191/02, TT 192/02, TT 196/02, TT 409/09)			
F	231	1054/1/01: Abbott G, Cultivation of high-value aquatic plants in restored urban wetlands for income generation in local communities ("new green" database)	2001	It is possible to cultivate economically valuable plants at high density in an altered urban wetland, and if growing conditions arc optimized then it should be possible for such a project to become a sustainable source of employment and wetland protection. Thus cultivation of economically valuable plants in wetlands can provide the economic incentives needed to ensure the continued preservation and rehabilitation of wetlands.	Relevance to the macrophyte bioreactor of a WWBR, and/or as it relates to Water sensitive urban design (WSUD). The Project Guidelines and Decision Support (Volume 1 of the HVAP project) should be used in determining the suitability of wetland sites for cultivation. Wetland culthation should ideally incorporate plants (in addition to the primary crop) which can fulfil multiple functions. However the environmental management requirements for wetlands will need lo be relaxed where possible to allow for cultivation of a number of useful plant species, some of which may not occur naturally in the wetland. A relaxation of the stringent standards of environmental protection imposed by the authorities will be required if altered wetlands are to become economically sustainable assets. Simple and low-cost solutions to irrigation, fertiliser and pesticide requirements should be used where possible.
F	232	182/1/89: Mitchell SA, The effective use of water by means of an algal aquaculture system	1989	The main aim of this project was to extend the technology of waste-water treatment by microalgae using organisms which would - (a) be cheap and easy to harvest so that the total suspended solids in effluent would conform to required standards; and (b) reclaim nitrogen from the waste stream in such a form that the biomass produced could be used as a supplement to stock feed. Systems employing a filamentous alga (Spirulina) and a grazing invertebrate (the fairy shrimp Streptocephalus macrourus) were compared. The long-term maintenance of a stable spirulina/zooplankton (Brachionus plicatili) polyculture is technically feasible with minimal agitation. It was found that sufficient agitation could be supplied with a power input of 10 kW/ha. It was also shown that it was to the Spirulina's advantange to be grown in polyculture with filter feeding invertebrates. These filter feeding invertebrates consumed the competing microalgae, and allowed the Spirulina to grow as a clean culture. Filter feeding invertebrates such as the brine shrimp Anemia and the water-flea Moina micrura were able to live successfully in Spirulina cultures while the Spirulina density was low, but rotifers such as Brachionus plicatilis and Hexarthra fennica were the only organisms able to live successfully in dense Spirulina cultures (Mitchell and Richmond, 1987).	Investigate this approach to 'DSP' in the WWBR, taking special note of the effect it would have on up- and downstream processes. Has any more up-to-date work been done in this field? While wastewater may be effectively treated by microalgae to remove dissolved solids, the algae must be removed from the effluent before the effluent will conform to the required effluent standards for total suspended solids. Harvesting the algae by flocculation, centrifugation or any other such method renders the process too expensive. This project investigated the possibility of using organisms that were large enough to be harvested easily to treat the wastewater. .

B SOUTH AFRICAN RESEARCH PUBLISHED IN JOURNALS

Table: B-1: Journal articles found on Scopus using keywords water, water treatment, South Africa, effluent and industrial

Category	Ref	Authors	Affiliation	Title of journal paper	Year of publication	Journal	Value of research in context of wastewater biorefineries	Shortcoming of research in context of WWBR / more work required
B	1	Simate, G.S.	School of Chemical and Metallurgical Engineering, University of the Witwatersrand	The treatment of brewery wastewater for reuse by integration of coagulation/flocculation and sedimentation with carbon nanotubes 'sandwiched' in a granular filter bed	2015	Journal of Industrial and Engineering Chemistry, 21, pp. 1277-1285.	This study deals with the integration of treatment systems and devices in order to reduce turbidity and chemical oxygen demand (COD) in brewery wastewater for re-use.	Lab scale study. Scale up work required.
A	2	Amdany, R., Chimuka, L., Cukrowska, E.	Molecular Sciences Institute, School of Chemistry, University of the Witwatersrand	Determination of naproxen, ibuprofen and triclosan in wastewater using the polar organic chemical integrative sampler (POCIS): A laboratory calibration and field application	2014	*Water SA*, 40 (3), pp. 407-414.	The study provides a method to determine the occurrence of two non-steroidal drugs and triclosan in wastewater using a polar organic chemical integrative sampler (POCIS)	Discrepancy in sample processing techniques
B	3	Welz, P.J [a], Palmer, Z.[ab], Isaacs, S.[a], Kirby, B.[b], le Roes-Hill, M. [a]	Biocatalysis and Technical Biology (BTB) Research Group, Cape Peninsula University of Technology	Analysis of substrate degradation, metabolite formation and microbial community responses in sand bioreactors treating winery wastewater: A comparative study	2014	Journal of Environmental Management, 145 (1), 147-156	The study yielded valuable insight that can be utilized in the design (configuration and operation) of full scale sand bioreactors.	

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B	4	Badejo, A.A., Ndambuki, J.M, Kupolati, W.K., Amuda, S.A.	Civil Engineering Department, Tshwane University of Technology	Performance of anaerobic digester-constructed wetlands system for brewery wastewater treatment	2014	Proceedings of the IASTED International Conference on Environment and Water Resource Management, AfricaEWRM 2014	The pilot plant study showed that Anaerobic Digester-constructed wetland (CW) combination has a high potential in brewery wastewater treatment.	
C	5	Mhlanga, F.T., Brouckaert, C.J.	Pollution Research Group, School of Chemical Engineering, University of KwaZulu-Natal	Characterisation of wastewater for modelling of wastewater treatment plants receiving industrial effluent	2013	*Water SA*, 39 (3), pp. 403-408.	The study provides a method to accurately characterise wastewater, focussing on the carbonaceous fraction. this information is instrumental in bioprocess modeling which aids in the design, modification and troubleshooting of wastewater treatment plants	This study focuses mainly on the carbonaceous fraction of wastewater, therefore further work needs to be carried out in order to characterise wastewater in terms of other important characteristics.
E	6	Nthumbi, R.M.[a], Catherine Ngila, J.[b], Moodley, B.[c], Kindness, A.[c], Petrik, L.[d]	[a]Kenyatta University, Kenya; [b]University of Johannesburg; [c]University of KwaZulu-Natal, School of Chemistry[d;] University of Western Cape	Application of chitosan/polyacrylamide nanofibres for removal of chromate and phosphate in water	2012	Physics and Chemistry of the Earth, 50-52, pp. 243-251.	The study focuses on the removal of phosphate and chromate which are prevalent in some industrial wastewaters. The work ultimately has applications making water contaminated with these anions safe for human consumption.	The work carried out was limited to lab scale experiments - thus further work will be required in order to determine if the methods developed can be applied on the scale required for a WWBR.
B	7	Simate, G.S.[a], Iyuke, S.E.[a], Ndlovu, S.[a], Heydenrych, M.[b]	[a] School of Chemical and Metallurgical Engineering, University of the Witwatersrand [b] Department of Chemical Engineering, University of Pretoria	The heterogeneous coagulation and flocculation of brewery wastewater using carbon nanotubes	2012	*Water Research*, 46 (4), pp. 1185-1197.	The ability of carbon nanotubes to act as a flocculant and/or coagulant is tested and compared to that of ferric chloride and it was found that traditional ferric chloride to be a more effective coagulant in all cases.	

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B	8	De Jager, D.[a] , Sheldon, M.S.[a] , Edwards, W.[b]	[a] Department of Chemical Engineering, Cape Peninsula University of Technology [b] Atl-Hydro, Fish Hoek, Cape Town, 8000, South Africa	Membrane bioreactor application within the treatment of high-strength textile effluent	2012	Water Science and Technology, 65 (5), pp. 907-914.	A dual-stage membrane bioreactor system with ultrafiltration modules was designed and used to successfully treat high-strength textile effluent to well below required discharge standards.	Such a reactor system should be tested at a larger scale using different types of effluents in order to determine of the same positive results can be obtained.
E	9	Opeolu, B.O.[a] , Bamgbose, O.[b] , Fatoki, O.S.[a]	[a] Department of Chemistry, Cape Peninsula University of Technology [b] Department of Environmental Management and Toxicology, University of Agriculture, Abeokuta, Nigeria	Zinc abatement from simulated and industrial wastewaters using sugarcane biomass	2011	*Water SA*, 37 (3), pp. 313-320.	This study assessed the potential of sugarcane biomass to remove zinc from standard solutions and industrial (paint and textile) wastewaters. Sugarcane biomass is therefore a potential alternative to expensive synthetic resins. Its biodegradability makes disposal environmentally friendly.	There is the need to further study the biomass in flow-through systems for industrial applicability.
B	10	Lin, J., Harichund, C.	School of Biochemistry, Genetics, and Microbiology, University of KwaZulu- Natal	Industrial effluent treatments using heavy-metal removing bacterial bioflocculants	2011	*Water SA*, 37 (2), pp. 265-270.	Bioflocculants were shown to successfully treat a heavy metal waste stream, removing several heavy metals effectively and simultaneously. The bioflocculant further removed almost all bacteria present and greatly reduced the turbidity of the wastewater.	The study suggests that that the use of bioflocculants may be effluent dependant. Therefore further study is recommended in order to determine the optimum conditions.

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B	11	Mack, C.L.[a], Wilhelmi, B.[a], Duncan, J.R.[a], Burgess, J.E.[a b]	[a] Department of Biochemistry Microbiology and Biotechnology, Rhodes University [b] Water Research Commission	Biosorptive recovery of platinum from platinum group metal refining wastewaters by immobilised Saccharomyces cerevisiae	2011	Water Science and Technology, 63 (1), pp. 149-155.	*Saccharomyces cerevisiae* has been found capable of sorbing numerous precious and base metals, and is a cheap and abundant source of biomass.	The sorption mechanism was found to be a chemical reaction, which made effective desorption impossible. When applied to PGM refinery wastewater, two key wastewater characteristics limited the success of the sorption process; high inorganic ion content and complex speciation of the platinum ions. The results proved the concept principle of platinum recovery by immobilised yeast biosorption and indicated that a more detailed understanding of the platinum speciation within the wastewater is required before biosorption can be applied.
B	12	Tabrizi, M.T.F., Glasser, D., Hildebrandt, D.	Centre of Material and Process Synthesis, School of Chemical and Metallurgical Engineering, University of the Witwatersrand	Wastewater treatment of reactive dyestuffs by ozonation in a semi-batch reactor	2011	Chemical Engineering Journal, 166 (2), pp. 662-668	The use of ozonation was shown to be effective in completely decolourising and partially oxidizing textile dyes.	More work is required to model the complex ozonation process. Such a model would be required in order to design an industrial decolouration plant.
B	13	Oboirien, B.O., Molokwane, P.E., Chirwa, E.M.N.	Department of Chemical Engineering, University of Pretoria	Bioremediation of organic pollutants in a radioactive wastewater	2009	Proceedings of the ICEM2007 - 11th International Conference on Environmental Remediation and Radioactive Waste Management, (PART B), pp. 873-876	[relevance - for radioactive waste]	

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C	14	Mhlanga, F.T.[a], Brouckaert, C.J.[a], Foxon, K.M.[a], Fennemore, C.[b], Mzulwini, D.[a], Buckley, C.A.[a]	[a] Pollution Research Group, School of Chemical Engineering, University of KwaZulu-Natal [b] eThekwini Water Services, 3 Prior Road, Durban 4041, South Africa	Simulation of a wastewater treatment plant receiving industrial effluents	2009	*Water SA*, 35 (4), pp. 447-454	A process model simulating the treatment of municipal wastewater, with a high proportion of industrial effluents was developed.	Perhaps such a process model could be adapted to a WWBR context, such that the entire process could be simulated to either aid in the design or operation of a WWBR.
B	15	Onyancha, D.[a], Mavura, W.[b], Ngila, J.C.[c], Ongoma, P.[b], Chacha, J.[d]	[a] Department of Chemistry, Nelson Mandela Metropolitan University [b] Department of Chemistry, Egerton University [c] School of Chemistry, University of KwaZulu Natal [d] Department of Chemistry, Jomo Kenyatta University of Agriculture and Technology	Studies of chromium removal from tannery wastewaters by algae biosorbents, Spirogyra condensata and Rhizoclonium hieroglyphicum	2008	Journal of Hazardous Materials, 158 (2-3), pp. 605-614	Algae biosorbents were used to effectively remove chromium from wastewater, which is of concern primarily to the tanning industry.	The scalability of the use of algae biosorbents to remove chromium from wastewaters requires further investigation.
B	16	Strong, P.J.[a b], Burgess, J.E.[a]	[a] Department of Biochemistry, Microbiology and Biotechnology, Rhodes University [b] CSIR Biosciences,	Fungal and enzymatic remediation of a wine lees and five wine-related distillery wastewaters	2008	*Bioresource Technology*, 99 (14), pp. 6134-6142	Wine distillery wastewaters were treated using fungi resulting in a reduction in COD, phenolic compounds and colour. The treatment of the wastewater with laccase reduced the presence of phenolics but increased the colour significantly.	The fungal treatment showed promise in treating wine distillery wastewater, the use of the same fungal treatment should be investigated to treat other types of wastewaters.

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D	17	Rava, E.[a d] , Schoeman, J.J.[a] , Allison, P.J.[b] , Dilsook, V.[c]	[a] University of Pretoria, Department of Chemical Engineering, Water Utilisation Division [b] Buckman Laboratories (Pty) Ltd (Hammarsdale) [c] Sappi Management Services - Technology Centre [d] Buckman Laboratories (Pty) Ltd (Bedfordview)	Management of hydrogen sulphide generation at a Kraft mill effluent plant	2008	*Water SA*, 34 (2), pp. 245-248	Sulphate reducing bacteria were successfully used to reduce the aqueous levels of H2S in Kraft mill wastewater, thereby reducing the odours emanating from the mill's effluent treatment plant.	The use of sulphate reducing bacteria could possibly employed to reduce odours from other effluents containing H2S.
D	18	Strong, P.J., Burgess, J.E.	Department of Biochemistry, Microbiology and Biotechnology, Rhodes University	Bioremediation of a wine distillery wastewater using white rot fungi and the subsequent production of laccase	2007	Water Science and Technology, 56 (2), pp. 179-186	*Trametes pubescens* MB 89 was shown to greatly improve the quality of wine distillery wastewater, which is known to be toxic to most biological treatment systems, while at the same time producing laccase.	The use of *Trametes pubescens* MB 89 to treat other types of wastewaters while simultaneously producing laccase should be furterh investigated.
E	19	Potgieter-Vermaak, S.S.[a] , Potgieter, J.H.[b] , Monama, P.[c] , Van Grieken, R.[a]	[a] Department of Chemistry, University of Antwerp [b] School of Chemical and Metallurgical Engineering, University of the Witwatersrand [c] Department of Chemistry, Tshwane University of Technology	Comparison of limestone, dolomite and fly ash as pre-treatment agents for acid mine drainage	2006	*Minerals Engineering*, 19 (5), pp. 454-462	The study reveals significant savings can be achieved by treating acid mine drainage when lime is replaced by either dolomite or fly ash.	The possibility of using fly ash and/or dolomite should be investigated when there is the need to raise the pH of wastewaters required to be treated.

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E	20	Jonker, A.[a c], Potgieter, H.[b d]	[a] Tshwane University of Technology [b] University of Witwatersrand [c] Department of Chemistry and Physics, Tshwane University of Technology [d] School of Process and Materials Engineering, University of the Witwatersrand	Physical properties of composites made from secondary cementitious materials with reference to their suitability for water filters	2005	Proceedings of the International Conference on Application of Codes, Design and Regulations, pp. 99-107	Selected waste (cementitious material) generated by the power generation, fertilizer and steel industries have shown promise in being used as a filter medium to treat industrial wastewater	The use of waste cementitious material to potentially remove various contaminants from industrial effluents should be further investigated to confirm their suitability in removing contaminants from industrial wastewater.
B	21	Lalbahadur, T.[a], Pillay, S.[b], Rodda, N.[b], Smith, M.[b], Buckley, C.[c], Holder, F.[a], Bux, F.[a], Foxon, K.[c]	[a] Centre for Water and Wastewater Technology, Durban Institute of Technology [b] School of Life and Environmental Sciences, Biochemical Research Group, University of Natal [c] School of Chemical Engineering, Pollution Research Group, University of Natal	Microbiological studies of an anaerobic baffled reactor: Microbial community characterisation and deactivation of health-related indicator bacteria	2005	Water Science and Technology, 51 (10), pp. 155-162	Moderate success in treating domestic wastewater was achieved using an anaerobic baffled reactor was achieved.	The reactor discharge was not below required contaminant levels and this was possibly due to hydraulic load limitations.
E	22	Esau, A.[a], Petersen, F.[b]	[a] Department of Chemical Engineering, Cape Technikon [b] Mintek, South Africa	Biosorption technologies for water treatment	2004	Waste Management and the Environment II, pp. 587-593	Biosorption of heavy metals such as Pb and Cu was shown to be effective (up to 100% removal) with fast kinetics, using eklonia maxima (brown seaweed). Using biomaterials is more cost effective than industrially used resins.	The work was conducted on a very small scale - thus the scalability of such biomaterials needs to be further investigated.

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E	23	Coopmans, E.J.A., Schwarz, H.P	Explochem Water Treatment (Pty) Ltd	Clarification as a pre-treatment to membrane systems	2004	*Desalination*, 165 (SUPPL.), pp. 177-182	Explochem Water Treatment has successfully design and built large scale water treatment plants that are effective in treating eutrophic algae laden water to drinking water standards.	
D	24	Boshoff, G.[a] , Duncan, J.[b] , Rose, P.D.[b]	[a] Environ. Engineering Research Centre, School of Civil Engineering, Queens University Belfast, Stranmillis Road, Belfast [b] Goldfields Biotechnology Laboratory, Dept. of Biochem. and Microbiology, Rhodes University	Tannery effluent as a carbon source for biological sulphate reduction	2004	*Water Research*, 38 (11), pp. 2651-2658	Sulphate removal of 60-80% was achieved using tannery effluent in pilot scale stirred tank reactor (STR), upflow anaerobic sludge blanket (UASB), and trench reactor (TR).	Although sulphate removal was achieved, COD removal rates decreased by 25%.
C	25	Gianadda, P., Brouckaert, C.J., Sayer, R., Buckley, C.A.	Pollution Research Group, School of Chemical Engineering, University of Natal	The application of pinch analysis to water, reagent and effluent management in a chlor-alkali facility	2002	Water Science and Technology, 46 (9), pp. 21-28	The concepts of water pinch analysis is introduced with the aim of reducing the amount of utility and process water used in the chlor-alkali process.	Water pinch analysis could potentially be used to maximise water treatment efficiency.
B	26	Mkhize, S.P., Bux, F	Ctr. for Water and Wastewater Res., Technikon Natal	Assessment of activated sludge to remediate edible-oil effluent	2001	South African Journal of Science, 97 (9-10), pp. 380-382	Anaerobic/aerobic sequencing batch reactor was used to remediate edible-oil effluent - greatly reducing COD as well as phosphates present.	Further investigation needs to be done to realise the full scale-up potential of this process when treating edible oil effluent. There is the possibility that this same reactor system could be used to treat other types of oil laden effluents.

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D	27	Greben, H.A., Maree, J.P., Singmin, Y., Mnqanqeni, S	Division of Water, Environment and Forestry Technology, CSIR	Biological sulphate removal from acid mine effluent using ethanol as carbon and energy source	2000	Water Science and Technology, 42 (3-4), pp. 339-344.	A biological sulphate removal process has been developed for the treatment of sulphate-rich industrial effluents, where sulphate is converted via sulphide to sulphur in an anaerobic single-stage reactor. Ethanol is used as carbon and energy source.	
B	28	Bell, J., Plumb, J.J., Buckley, C.A., Stuckey, D.C.	Sci., School of Chemical Engrg., Univ of Natal, Durban,	Treatment and decolorization of dyes in an anaerobic baffled reactor	2000	Journal of Environmental Engineering, 126 (11), pp. 1026-1032.	Decolorization of industrial wastewater from a food dye manufacturer in an anaerobic baffled reactor. Reduction in COD of 70% and color reduction of about 90% was achieved	Lab-scale study. Initially the tartrazine was not readily decolorized; however, decolorization improved with acclimation of the biomass
A	29	Pitman, A.R.[a] , Boyd, L.A.[b]	[a] Wastewater, Gtr. Johannesburg M., Braamfontein, [b] Health and Scientific Services, Gtr. Johannesburg Metropol. Council, Braamfontein,	Transforming local government wastewater departments — From adversary to industrial partner	1999	Water Science and Technology, 39 (10-11), pp. 39-45.	The need to remove nutrients from wastewater by biological means and dispose of sludge by-products in an efficient manner has prompted the Greater Johannesburg Metropolitan Council to adopt a new approach to the management of industrial discharges. Proposed rebate on the normal discharge tariff will encourage the discharge of industrial effluents having a high readily biodegradable concentration (which would assist the BNR process).	Those effluents having high concentrations of heavy metals (which would degrade the reuse value of sludge by-products) would be discouraged by means of an additional penalty above the normal discharge tariff.

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D	30	Zhao, M., Duncan, J.R., Van Hille, R.P.	Dept. of Biochem. and Microbiology, Rhodes University	Removal and recovery of zinc from solution and electroplating effluent using *Azolla filiculoides*	1999	*Water Research*, 33 (6), pp . 1516-1522	A method to recover zinc from electroplating effluent using Azolla filiculoides was investigated using batch columns. The mechanical stability and flow permeability of Azolla filiculoides. Complete desorption of the bound zinc was also achieved.	Azolla filiculoides could be potentially used to recover zinc from other effluents, such as ARD containing zinc.
B	31	Edwards, W.[a] , Bownes, R.[a] , Leukes, W.D.[a] , Jacobs, E.P.[b] , Sanderson, R.[b] , Rose, P.D.[a] , Burton, S.G.[a]	[a] Goldfields Biotechnology Centre, Dept. Biochem. Microbiol., Rhodes U. [b] Institute for Polymer Science, Stellenbosch University	A capillary membrane bioreactor using immobilized polyphenol oxidase for the removal of phenols from industrial effluents	1999	Enzyme and Microbial Technology, 24 (3-4), pp. 209-217.	A capillary membrane bioreactor has been developed and tested for the removal of phenolic compounds from synthetic and industrial effluents. Almost complete removal of the colored quinones and associated polymers from the permeate was observed.	
D	32	Atkinson, B.W., Bux, F., Kasan, H.C.	Ctr. for Water and Wastewater Res., Department of Biotechnology, Technikon Natal	Considerations for application of biosorption technology to remediate metal-contaminated industrial effluents	1998	*Water SA*, 24 (2), pp. 129-135.	A pilot-plant feasibility study, using waste activated sludge to bioremediate a metal plating effluent, showed that the currently used method of chemical precipitation is more cost-effective. This paper describes the factors that must be considered when selecting bioremediation as a cleanup technology for inorganics.	

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D	33	Atkinson, B.W., Bux, F., Kasan, H.C.	Department of Biotechnology, Technikon Natal	Bioremediation of metal-contaminated industrial effluents using waste sludges	1996	*Water Science and Technology*, 34 (9 pt 5), pp. 9-15	Activated sludge was used to biosorb metal contaminated effluent on a laboratory scale using up-flow column bioreactors. The average adsorptive capacity of the biomadd was 80%, eithing the first 15 minutes.	This same activated sludge process could be potentially used to recover metals from other metal contaminated effluents, such as ARD. However, scale up issues would potentially have to be dealt with.
B	34	Schoeman, J.J., Steyn, A., Scurr, P.J.	Watertek, CSIR	Treatment using reverse osmosis of an effluent from stainless steel manufacture	1996	*Water Research*, 30 (9), pp . 1979-1984	The work showed that it is possible to treat neutralized spent acid effluent (seepage) effectively using reverse osmosis for effluent volume reduction, water recovery and pollution control.	Cost remains a significant factor when considering any type of RO technology. RO is an option for very difficult to treat effluents and could possibly used as a final polishing step.
A	35	Haarhoff, J.[a] , Van Der Merwe, B.[b]	[a] Department of Civil Engineering, Rand Afrikaans University [b] City Engineer's Department, Windhoek, Namibia	Twenty-five years of wastewater reclamation in Windhoek, Namibia	1996	*Water Science and Technology*, 33 (10-11), pp. 25-35	A comprehensive review of the wastewater water reclamation plant in Windhoek. The paper details the systems used and the results obtained by the plant over the past 25 years and the plans to expand its capacity from 4800 to 21000 m^3/day.	The review can provide good insight of a working water reclamation plant that has been successful and operating on large scale.
C	36	Maree, J.P., Du Plessis, P	Division of Water Technology, CSIR	Neutralization of acid mine water with calcium carbonate	1994	*Water Science and Technology*, 29 (9), pp. 285-296	Calcium carbonate is investigated as an alternative to lime for the neutralization of acidic effluent with varying degrees of success. Calcium carbonate is an attractive alternative given its low cost, simple doing system required and low solubility at pH less than 7.	

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B	37	Howgrave-Graham, A.R., Isherwood, H.A., Wallis, F.M.	Dept. Microbiology Plant Pathology, University of Natal,	Evaluation of two upflow anaerobic digesters purifying industrial wastewaters high in organic matter	1994	Water Science and Technology, 29 (9), pp. 225-229	Two full-scale anaerobic digesters, one a clarigester purifying a maize processing wastewater and the other with an upflow anaerobic sludge blanket (UASB) configuration treating brewery effluent contained well setting, granular sludges efficient in pollutant removal.	
C	38	Kilani, J.S.	Univ of Durban-Westville, Durban	Compatibility study of the effects of dairy and brewery effluents on the treatability of domestic sewage	1993	*Water SA*, 19 (3), pp. 247-252.	The results indicate that the dairy and the brewery wastes have no adverse effect on the treatability of the domestic sewage. Furthermore, the effluents from the 5 ponds have BOD/COD ratios within ranges that are generally accepted as indicating a high degree of biodegradability. They would therefore not be expected to have any adverse effect on the efficiency of secondary biological treatment processes.	

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B	39	Jackson-Moss, C.A., Maree, J.P., Wotton, S.C.	Division of Water Technology, CSIR	Treatment of bleach plant effluent with the biological granular activated carbon process	1992	Water Science and Technology, 26 (1-2), pp. 427-434.	Bleach plant effluent from the pulp and paper industry was treated by means of the anaerobic biological granular activated carbon process. It was found that over 50% of the COD and colour could be successfully removed from this effluent. The adsorptive capacity of the activated carbon was extended as a result of microbial activity inside the anaerobic reactor. The results of this investigation suggest that the anaerobic biological granular activated carbon process could be used to alleviate the pollution problems experienced by the pulp and paper industry.	

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B	40	Cowan, J.A.C., MacTavish, F., Brouckaert, C.J., Jacobs, E.P.	Steffen, Robertson and Kirsten Inc, Johannesburg, South Africa	Membrane treatment strategies for red meat abattoir effluents	1992	*Water Science and Technology*, 25 (10) , pp. 137-148.	These treatment techniques have now been lifted from the research phase into commercial application on small scale (25m3/d) using full size modules. The South African Abattoir Corporation, as the major representative of the industry in South Africa, has undertaken to assess the value of membrane treatment processes as a part of a number of effluent treatment strategies. Ultrafiltration will consistently remove 90% COD, 85% phosphate from the effluent, and provide a relatively non-fouling feed for reverse osmosis which produces a high quality reusable water for abattoir use.	
B	41	Buckley, C.A.	Pollution Research Group, Department of Chemical Engineering, University of Natal,	Membrane technology for the treatment of dyehouse effluents	1992	*Water Science and Technology*, 25 (10) , pp. 203-209	In this paper, dye chemistry is summarized and ten of the most commonly used dye types are identified. For color removal purposes the dyes are grouped into three classes. Four membrane processes are described which have been used in South Africa for the treatment of dyehouse effluents.	
B	42	Strohwald, N.K.H., Ross, W.R.	Membratek (Pty) Ltd, Noorder Paarl, South Africa	Application of the ADUFR process to brewery effluent on a laboratory scale	1992	*Water Science and Technology*, 25 (10) , pp. 95-105	An anaerobic digestion – ultrafiltration (ADUFR) unit successfully treated brewery effluent on a lab scale, reducing COD by up to 99%, with no membrane fowling.	

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E	43	Meyer, V., Carlsson, F.H.H., Oellermann, R.A.	Division of Water Technology, CSIR, PO Box 395, Pretoria	Decolourization of textile effluent using a low cost natural adsorbent material	1992	*Water Science and Technology*, 26 (5-6), pp. 1205-1211.	colour from textile-plant effluents, tests were run using several low cost natural adsorbent materials including vermiculite, sawdust, barbecue charcoal, maize stalks, sand, rice husks and peatmoss. With the exception of vermiculite, more than 50% of the colour was removed from the wastewater, with barbecue charcoal and rice husks showing the best adsorptive qualities (67% and 65% respectively). Under simulated industrial conditions on a laboratory scale a fixed-bed reactor was used to investigate the adsorption capacity of barbecue charcoal with respect to colour removal. An average of 28% of colour was removed at a hydraulic retention time (HRT) of 1.6 h over a period of 25 days. The effect of pH on the adsorptive capacity with respect to colour removal and represents a relatively cheap adsorbent material compared to conventionally used granular activated carbon.	
B	44	Maree, J.P., Hulse, G., Dods, D., Schutte, C.E.	Division of Water Technology, CSIR	Pilot plant studies on biological sulphate removal from industrial effluent	1991	*Water Science and Technology*, 23 (7-9), pp. 1293-1300	A biological sulphate removal process was developed for the treatment of sulphur rich industrial effluents.	

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B	45	Maree, J.P., Strydom, W.F.	National Institute for Water Research, CSIR	Biological sulphate removal from industrial effluent in an upflow packed bed reactor	1987	*Water Research*, 21 (2), pp . 141-146	The removal of sulphate (by conversion to sulphide then elemental sulphur) by two bacteria has been shown to symbiotically occur in a upflow packed bed reactor.	
	Using the keyword search terms wastewater biorefinery and SA as affiliate							
F	46	Singh, B., Guldh, A., Singh, P., Rawat, I., Bux, F., Singh, A.	Centre for Environmental Sciences, Central University of Jharkhand, Ranchi, India and Institute for Water and Wastewater Technology, Durban University of Technology	Sustainable production of biofuels from microalgae using a biorefinary approach	2015	*Applied Environmental Biotechnology: Present Scenario and Future Trends, 115-128*	*The value added product derived from biorefinery basket includes pigments, nutraceuticals, and bioactive compounds. The use of industrial refusals for biomass production includes wastewater as nutrient medium and utilization of flue gases (CO2) as the carbon source for culture of microalgae. These processes have the potential to reduce fresh water footprint and carbon footprint.*	

Category	Ref	Authors	Affiliation	Title of journal paper	Year of publicatio n	Journal	Value of research in context of wastewater biorefineries	Shortcoming of research in context of WWBR / more work required
F	47	Rawat, I., Bhola, V., Kumar, R.R., Bux, F	Institute for Water and Wastewater Technology, Durban University of Technology	Improving the feasibility of producing biofuels from microalgae using wastewater	2013	Environmental Technology (United Kingdom), 34 (13-14), pp. 1765-1775.	The use of a biorefinery approach sees the production costs reduced greatly due to utilization of waste streams for cultivation and the generation of several potential energy sources and value-added products while offering environmental protection. The use of wastewater as a production media, coupled with CO 2 sequestration from flue gas greatly reduces the microalgal cultivation costs. Conversion of residual biomass and by-products, such as glycerol, for fuel production using an integrated approach potentially holds the key to near future commercial implementation of biofuels production.	
F	48	Rawat, I., Ranjith Kumar, R., Mutanda, T., Bux, F.	Institute for Water and Wastewater Technology, Durban University of Technology	Dual role of microalgae: Phycoremediation of domestic wastewater and biomass production for sustainable biofuels production	2011	Applied Energy, 88 (10), pp. 3411-3424	This paper discusses current knowledge regarding wastewater treatment using HRAPs and microalgal biomass production techniques using wastewater streams. The paper discusses biodiesel production via transesterification of the lipids and other biofuels such as biomethane and bioethanol which are described using the biorefinery approach.	

C ANALYSIS OF SOUTH AFRICAN WASTEWATER STREAMS FOR BIOREFINERY FEEDSTOCK

There were a number of issues encountered in compiling data which would explicate the current status of South African wastewaters. These are explained in Chapter 4. A key difficulty is the variability within the reporting, not least how the concentrations of the components in the wastewater are determined and then given. In order to create a set of data where comparisons can be made, it was decided to attempt a standardisation of units for all quantities presented here.

C.1 Conversion Calculations for Concentration of C, N and P

The most challenging conversions were for carbon, nitrogen and phosphorus content of the wastewaters. In terms of initial analysis of the WWBR potential of a feedstock it was decided that the data needed are concentrations of C, N and P (Sections 7.3.1 and 8.1.1). However, this is seldom how these are reported and the desired form was calculated from reported forms as follows.

C.1.1 Concentration of carbon

It is assumed that all carbon is present as organic carbon. In waste waters the organic carbon is reported in three ways.

- TOC: Total Organic Carbon
- This is the concentration of carbon in the wastewater and is the measure used here.
- COD: Chemical Oxygen Demand
- The amount of oxygen needed for complete oxidation of organics per volume of wastewater.
- BOD: Biological Oxygen Demand
- The amount of oxygen needed for decomposition of organic compounds by microorganisms.
- The BOD is often reported with a subscript which relates to the number of days the test was run for, usually 5 or 7. Alternatively the test can be run until the decomposition is complete.

The relationship between these measures is represented in Figure: C-1.

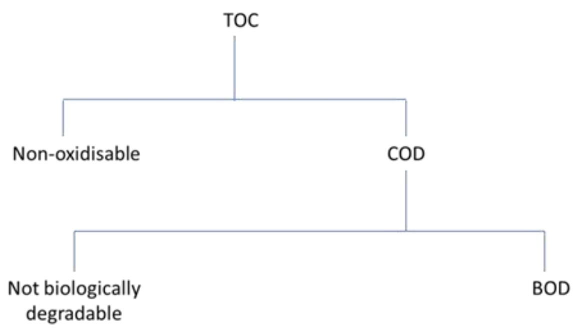


Figure: C-1: Relationship between measures of carbon concentration in organic wastewaters (adapted from Davies, 2005)

COD and BOD are the more frequently reported measures of organics in wastewaters because one or both of these is usually part of the regulated water quality for an effluent. This is a direct measure of how "polluting" the organic compounds in the wastewater are, reflecting the complexity of the compounds.

Theoretical ratio between COD and TOC

There is a theoretical COD (assuming full oxidation) which can be easily calculated for single simple organic compounds. The COD to TOC ratio is easily derived from this. For example:

Acetate

$CH_3COOH + 2\ O_2 \Rightarrow 2CO_2 + 2H_2O$

full oxidation uses 2 mol O_2 for 1 mol CH_3COOH

COD of CH_3COOH = 2*(2*16) = 64 g/mol CH_3COOH

equivalent to 2 C (atomic mass 12) = 2*12 = 24

COD/C = 64/24 = 2.6667 g/g

20 mg/ℓ COD of acetate is equivalent to 1/2.6667 x 20 mg/ℓ C = 7.49 mg/ℓ C

Methane

$CH_4 + 2O_2 \Rightarrow CO_2 + 2H_2O$

full oxidation uses 2 mol O_2 for 1 mol CH_4

COD of CH_4 = 2*(2*16) = 64 g/mol CH_4

equivalent to 1 C (atomic mass 12) = 12

COD/C = 64/12 = 5.3333 g/g

20 mg/ℓ COD of methane is equivalent to 1/5.3333 x 20 mg/ℓ C = 3.75 mg/ℓ C

This ratio applies to any organic compound containing no oxygen atoms, which supplies an upper value for this ratio.

Formic Acid

$CHOOH + ½\ O_2 \Rightarrow CO_2 + H_2O$

full oxidation uses ½ mol O_2 for 1 mol CHOOH

COD of CHOOH = ½*16 = 8 g/mol CHOOH

equivalent to 1 C = 12

COD/C = 8/12 = 0.6667 g/g

20 mg/ℓ COD of acetate is equivalent to 1/0.6667 x 20 mg/ℓ C = 30 mg/ℓ C

This value forms a minimum for this ratio.

Table: C-1: Some theoretical ratios of COD to TOC

				ratio calculation	COD/C
Acetate:	$CH_3COOH + 2\ O_2$	$\Rightarrow 2CO_2 + 2H_2O$	1 mole acetate is 64 gCOD	64/(2*12)	2.667
Propionate:	$CH_3(CH_2)COOH + 3½\ O_2$	$\Rightarrow 3CO_2 + 3H_2O$	1 mole propionate is 112 gCOD	112/(3*12)	3.111
Butyrate:	$CH_3(CH_2)_2COOH + 5\ O_2$	$\Rightarrow 4CO_2 + 4H_2O$	1 mole butyrate is 160 gCOD	160/(4*12)	3.333
Valerate:	$CH_3(CH_2)_3COOH + 6½\ O_2$	$\Rightarrow 5CO_2 + 5H_2O$	1 mole valerate is 208 gCOD	208/(5*12)	3.466

Empirical ratio between COD and TOC

However in streams containing mixed complex organic compounds the ratios between COD, BOD and TOC are empirical and vary significantly depending on the type of organics present in the specific wastewater stream. Henze, et al. (2008) tabulate typical ratios for various measures and components of municipal wastewater, including those in Table: C-2.

Table: C-2: Typical empirical ratios between COD and other measures for municipal wastewater (Henze, et al., 2008)

Ratio	High	Medium	Low
COD/BOD	2.5 – 3.5	2.0 – 2.5	1.5 – 2.0
COD/VSS	1.6 – 2.0	1.4 – 1.6	1.2 – 1.4
COD/TOC	3.0 – 3.5	2.5 - 3	2.0 -2.5

The relationship of COD to TOC for settled influent and for effluent in municipal wastewater was investigated by Dubber and Gray (2010). They report a strong linear relationship with a slope of 3.0 which corresponds with the upper mid-rage value given in Table: C-2.

The ratio of COD/TOC for industrial wastewaters is variable. However, the value of 3 is the midpoint between the highest possible COD/TOC of 5.333 and the lowest possible value of 0.667. It is thus likely to be a close approximation for all excepting the most specialised of the industrial wastewaters.

For the purposes of the data contained in the table in this report, a conversion factor of COD/TOC of 3.0 has been used where measured TOC is not available.

C.1.2 Concentration of nitrogen

Nitrogen present in wastewater can be reported in three different ways.

- TKN: Total Kjeldahl Nitrogen
- This is the sum of organic nitrogen, ammonia (NH_3), and ammonium (NH_4^+) in the sample.
- Organic nitrogen consists of protein, urea and nucleic acids.
- Nitrates: NO_3^-
- Nitrites: NO_2^-

Total Kjeldahl Nitrogen

This value is already a direct nitrogen concentration

Nitrates

NO_3^- molecular mass 14 + (3*16) = 62

N atomic mass 14

N/NO_3^- = 14/62 = 0.2258

Nitrites

NO_2^- molecular mass 14 + (2*16) = 46

N atomic mass 14

N/ NO_2^- = 14/46 = 0.3043

Ammonium

Occasionally NH_4^+ is reported instead of TKN

NH_4^+ molecular mass 14 + (4*1) = 18

N atomic mass 14

N/ NH_4^+ = 14/18 = 0.7778

Total nitrogen (TN)

TN = TKN + (NO_3^-)-N + (NO_2^-)-N

C.1.3 Concentration of phosphorus

The measure of phosphorus is usually given as phosphate (PO_4^{3-}) concentration.

PO_4^{3-} molecular mass 31 + (4*16) = 95

P atomic mass 31

P/ PO_4^{3-} = 31/95 = 0.3263

C.2 General Data for Industrial Wastewaters

C.2.1 Summary data used in this report for industrial wastewaters

The COD, NO^{3-} or NO^{2-} or NH^{4+} or TKN or TN and $PO4^{3-}$ data was used from various references and is subsequently referenced in Table: C-3. This table should be read together with the table in Section 4.1.3.

Table: C-3: Composition of selected South African wastewaters

Industry Sector	COD (mg/l)	NO^{3-} or NO^{2-} or NH^{4+} or TKN or TN (mg/l)	PO_4^{3-} or TP (mg/l)	TSS (mg/l)	pH	Reference
Municipal	500-1200	30-100 (TN)	6-25	250-600	7-8	(Henze, et al., 2008)
Abattoir (poultry)	1300-7500	100-250 (TKN)	100-250 (TP)	200 -1200	7.0-7.2	(Molapo, 2009)
Abattoir (red meat)	2380-8942	0.71-24 (TKN)	nl	189-3330	5.7-8.4	(DWA SA, 2001)
Brewing	3000 (a)	25-80 (TN) (b)	10-50 (TP) (b)	200-1000 (b)	5.5 (a)	b (Burton, et al., 2009) c (Brito, et al., 2007)
Canning	700-6500	nl	nl	nl	4.4-11.7	(Binnie and Partners, 1987)
Cleaning and cosmetics	2134-8477	5 (nitrate) & 36 (ammonia)	55	nl	8-9	(Cloete, et al., 2010)
Dairy	10000-20000	400 (TN)	40 (TP)	4500	8.2	(Du Preez, 2010)
Distillery (Alcoholic beverages)	3100-120000	100-64000 (TN)	240-65700 (TP)	2400-5000	3-5.4	(Melamane, et al., 2007)
Dyeing and colouring	217-1992	nl	nl	nl	10-12	(Cloete, et al., 2010)
Edible oil (#)	16000-250000 (c)	16.1 -45.9 (c)	550-4400 (d)	715-29330 (c)	1.8-10.5 (e)	(c) (Roux-Van der Merwe, et al., 2005) (d) (Surujlal, et al., 2004) (e) (Steffen, Robertson & Kirsten Inc, 1989d)
Fishery	1600-10000	0.7-69.7 (NH3)	nl	200-10000	6.4-10	(Chowdhury, et al., 2010) (Quiroz, et al., 2013)
Laundry	330-1390	0-3	21-35 (nitrate)	nl	9	(Cloete, et al., 2010)
Petroleum	7896	13.5 (ammonia) 2.23 (nitrate) 40.6 (TKN)	nl	nl	4.2 – 9.1	(Gasim, et al., 2012)
Pulp and Paper	700-1200	8.7 (ammonia) & 1.52 (nitrate)	4	6000	6-8	(Cloete, et al., 2010)
Soft drinks	87-725000	nl	nl	10-19000 (TDS)	2.8-12.2	(Pollution Research Group, 2015)
Sugar	1500 - 2000	deficient	deficient			(Mooij, et al., 2015)
Textiles	537-9553	<1	1-39	950-4850 TDS (f)	5-12	(Cloete, et al., 2010) (f) (Steffen, Robertson and Kirsten Inc, 1993)
Winery	800-12800 (g)	110 (h)	52		4.0-5.7 (i)	(g) (Welz, et al., 2015) (h) (Cai, et al., 2013) (i) (Brito, et al., 2007)
TKN Total Kjeldahl Nitrogen TN Total nitrogen TP Total phosphorous						

Industry Sector	COD (mg/l)	NO^{3-} or NO^{2-} or NH^{4+} or TKN or TN (mg/l)	PO_4^{3-} or TP (mg/l)	TSS (mg/l)	pH	Reference
TSS Total supended solids nl not listed						

C.2.2 Additional general data for industrial wastewaters

Data compiled from Cloete et al. (2010) is shown in Table: C-4 and from Burton et al. (2009) in Table C.5.

Table: C-4: Industrial water use and effluent release (adapted from Table 5.1 WRC Report Number 1547/1/10 (Cloete, et al., 2010))

Source	Annual H_2O consumption (Mm^3)	Annual effluent production (Mm^3)	COD (mg/l)	N (mg/l)	P (mg/l)	pH	EC (mS/m)
Cement	4.6543	0.1827	nl	nl	nl	nl	nl
Chemical	0.7419	0.1369	217	0	0	9.0-11.0	193-1500
Cleaning	0.746	0.3143	4850-8477	0-5	55	8	43.75
Dye and colouring	0.8955	0.645	217-1992	nl	nl	10.0-12.0	347-1234
Ferrous metal	133.78	1.5639	nl	nl	nl	2.92-9.83	nl
Plastics	0.0033	0	nl	nl	nl	nl	nl
Paint: powder	0.0203	0.0005	161-1093	0-3 nitrite/nitrate 1-4 ammonia (N)	0-40	8-9	45-195
Paint: oil & water			1823-4205	1-2 nitrite/nitrate 1-13 ammonia (N)	2-27	6-8	36-149
Petroleum	136.26	23.617	31-49	2.0-5.0	nl	nl	63-1364
Pulp and Paper: paper recycling	44.063	39.488	14225	1.52 nitrite/nitrate	4	8	144
Pulp and Paper: carton recycling & manufacturing			3667	3 nitrite/nitrate	6		105
Tannery: cattle	0.1707	0.0135	2108	1 nitrite/nitrate 31 ammonia (N)	8	7	350
Tannery: sheep & game			560	0 nitrite/nitrate 2 ammonia (N)	2		935
Textiles	5.0511	3.1146	537-1623	0-<1	1-36	6/8/2014	95-228
Washery/Laundry	0.234	0.2186	330-1390	0-3	21-35	9	99-512

Table: C-5: Examples of South African wastewaters containing fermentable substrates (adapted from Burton et al, (2009) Table 11)

Wastewater	COD (g/L)	Volume (ML per year)	Load (Mg/year)
Sewage	0.8 – 1.2 Ave = 0.86	2 766 400	2 379 104
Dairy*	1.5 – 9.2 Ave = 5.3	6 346	33 637
Red meat and poultry abattoirs	11 – 21 Ave = 16	11 000 – 31 000	336 000
Olive production	55 – 201 Ave = 100	89	8 900
Fruit processing	5 - 15 Ave = 10	14 000	140 000
Grain and grape distilleries	25 – 45 Ave = 30	Grain: 63 Grape: 342	12 150
Sugar cane molasses from distilleries	35	3 500 – 4 000	131 250
Winery	6	1 000	6 000
Brewery	3	28 000	23 533
Textile industry	0.1 – 2.5 Ave = 1	25 000	25 000
Pulp and paper	0.7	80 000	56 000
Petrochemical waste	0.2- 0.9 Ave = 0.7	crude: 1 140 synthetic: 3 048 re-refinery: 2 - 11	2 939

* Only the formal dairy is considered here. Other animal husbandry sectors (cattle for beef, pigs and chickens are not shown) here

Some general data on agro-food industrial wastewater (Rajagopal, et al., 2013) has been given in Table: C-6for reference.

Table: C-6: Characteristics of typical agro-food industrial wastewater adapted from Rajagopal, et al. (2013)

Industry	TS (mg/ L)	TP (mg/ L)	TN (mg/ L)	BOD (mg/ L)	COD (mg/ L)
Food processinga	-	3	50	600–4,000	1,000–8,000
Palm oil mill	40	-	750	25	50
Sugar-beet processing	6100	2.7	10	-	6 600
Dairy	1,100–1,600	-	-	800–1,000	1,400–2,500
Corn milling	650	125	174	3 000	4 850
Potato chips	5 000	100	250	5 000	6 000
Baker's yeast	600	3	275	-	6 100
Winery	150–200	40–60	310–410	-	18,000–21,000
Dairy	250–2,750	-	10–90	650–6,250	400–15,200
Cheese dairy	1,600–3,900	60–100	400–700	-	23,000–4,0000
Olive mill	75 500	-	460	-	130 100
Cassava starch	830	90	525	6 300	10 500
Notes: a contains flour, soybean, tomato, pepper and salt. TS: total solids; TN: total nitrogen; TP: total phosphorus; BOD: biochemical oxygen demand; COD: chemical oxygen demand					

C.3 Municipal wastewater (Section 4.2)

Municipal WWTW have been well characterised in terms of capacity by the GreenDrop initiative of the Department of Water and Sanitation. Data from their report (DWS SA, 2014) is extracted into Table: C-7. The composition of typical raw municipal wastewater with the normal contribution of industrial wastewaters is given ((Henze, et al., 2008)) in Table: C-8. Local data taken from Verster et al. (2013), who collected site specific wastewater data from the Athlone and Mitchell's Plain WWTWs in Cape Town, are given Table: C-9.

Table: C-7: Size distribution of 824 WWTW from 152 municipalities (adapted from GreenDrop report (DWS SA, 2014))

	Micro size < 0.5 ML/day	Small size 0.5 – 2 ML/day	Medium size 2 – 10 ML/day	Large size 10 – 25 ML/day	Macro size > 25 ML/day	Undetermined	Total
No of municipal WWTPs	168	269	232	65	62	28 (43)	824
Total design capacity (ML/day)	37.55	256.88	1019.73	939.90	4178.30		6432.36
Total daily inflows (MlLday)	9.39	85.43	485.65	496.05	3923.06		4999.58
% plants	20.4	32.6	28.2	7.9	7.5	3.40	100.0

Table: C-8: Composition of typical raw municipal wastewater (adapted from Henze et al. (2008))

Parameter (in mg/L)	High	Medium	Low
COD,total	1200	750	500
COD soluble	480	300	200
COD suspended	720	450	300
BOD	560	350	230
VFA (as acetate)	80	30	10
N total	100	60	30
Ammonia-N	75	34	20
P total	25	15	6
Ortho-P	15	10	4
TSS	600	400	250
VSS	480	320	200

Table: C-9: Athlone and Mitchell's Plain municipal WWTW composition data (adapted from Verster et al. (2013))

	Athlone WWTW raw wastewater	Mitchells Plain WWTW raw wastewater
	Mean ± SD	Mean ± SD
COD (mg/L)	880±526	1465±560
TKN (mg/L)	56±13	92±45
NH3 (mg/L)	32±7.6	
Total P (mg/L)	9.2±2.4	19±12
Ortho P (mg/L)	5.5±1.7	
SS (mg/L)	351 ±149	750±360
VSS (mg/L)	304 ± 108	
pH	7.25±0.28	
Conductivity (mS/m)	140±23	
Cl (mg/L)	211±42	
Alkalinity (mg/L)	275±42	
(Athlone data 1997 – 2010, Mitchells Plain data 2008)		

C.4 Data for Specific Industrial Wastewaters

The source data used to calculate the values presented in Section 4.3 are tabulated here.

C.4.1 Pulp and Paper industry (Section Error! Reference source not found.)

Table: C-10: Annual combined wastewater data (prior to any on-site treatment) for the South African pulp and paper industry sector (adapted from Burton et al. (2009))

Mill	Wastewater (ML)	Est wastewater (ML)	COD (mg/L)	pH	Temperature (°C)
Mondi					
Merebank	10264	10085	470-1659	–	–
Richards Bay	21361	21300	1399	8.24	44.38
Felixton	1933	2000	22842	–	–

Mill	Wastewater (ML)	Est wastewater (ML)	COD (mg/L)	pH	Temperature (°C)
Piet Retief	566	1750	6021	–	–
Springs	1046	1008	1940	–	–
Sappi					
Saiccor	33320	32582	615-3073	–	–
Stanger	6248	3760	319-1175	–	–
Enstra	7586	6227	578-1929	–	–
Adamas	506	462	848-3221	–	–
Ngodwana	10413	13996	1219-4607	–	–
Tugela	15470	6387	358-1305	–	–
Cape Kraft	428	408	595-4167	–	–
Nampak					
Bellville	655	576	733-2443	–	–
Kliprivier	506	432	711-2372	–	–
Riverview	208	180	721-2404	–	–
Rosslyn	298	320	671-4698	–	–
Kimberly-Clark					
Enstra	803	864	897-2989	–	–
New Era					
Gayatri	–	360	625-4375	–	–
Other	–	1210	789-3116	–	–
Total	**111611**	**103907**			
average	**6565**	**5469**			
stdev	**9322**	**8660**			
The "Est wastewater" column contains the values calculated using the mills' pulp and paper production figures and the various specific wastewater flows figures gained from the literature					

C.4.2 Petroleum industry (Section 4.3.2)

The capacity and potential wastewater produced for each of the six South African petroleum refineries is tabulated in Table: C-11. The minimum and maximum effluent likely to be produced at each capacity has been calculated for values given in Burton et al. (2009), and the average taken.

The conversion from barrels (bbl) per day to metric tonnes is also shown, with 1 bbl equivalent to 0.1183432 metric tonne. For the coal to liquid (CTL) process 120 000 metric tonnes of coal per day is converted into 150 000 barrels of oil per day in the Sasol Secunda plant (Schutze, n.d.). The conversion of natural gas to barrels of oil equivalent is: 1000 m^3 natural gas equivalent to 6.29 barrels of oil equivalent (BOE) (Selena Oil and Gas, n.d.).

Table: C-11: Production capacity of the South African petroleum industry (adapted from (SAPIA, 2014)

							Effluent calculated (m^3/day) from values in Burton et al. (2009)				Average effluent produced	
	Capacity (BBl/day)		Capacity (m3/day)		Capacity (tonnes/day)		at 0.1m^3 WW/ton crude		at 5m^3 WW/ton crude		ML/day effluent	
	2007	2014	2007	2014	2007	2014	2007	2014	2007	2014	2007	2014
Sapref	180000	180000	28618	28618	21294	21294	2129	2129	106470	106470	54.30	54.30
Enref	125000	120000	19873	19078	14788	14196	1479	1420	73938	70980	37.71	36.20
Chevref	100000	100000	15899	15899	11830	11830	1183	1183	59150	59150	30.17	30.17

Natref	108000	108000	17171	17171	12776	12776	1278	1278	63882	63882	32.58	32.58
Sasol	150000	150000	23848	23848	17745	17745	1775	1775	88725	88725	45.25	45.25
PetroSa	45000	45000	7154	7154	5324	5324	532	532	26618	26618	13.57	13.57
Total	**708000**	**703000**	**112563**	**111768**	**83756**	**83165**	**8376**	**8316**	**418782**	**415825**	**213.58**	**212.07**

C.4.3 Poultry abattoirs industry (Section 4.3.3)

From the Molapo (2009) study, the composition of poultry abattoir wastewater is given in Table: C-12, while the slaughtering capacity of these plants is given in Table: C-13. The estimated wastewater generated from the number of plants with their C, N and P content has been calculated and is summarized in Table: C-14.

Table: C-12: Poultry abattoir wastewater content loads adapted from Molapo (2009)

Parameter	Load
pH	7.0 – 7.2
BOD (mg/L)	700 – 4 000
COD (mg/L)	1 300 – 7 500
TSS (mg/L)	200 – 1 200
TKN (mg/L)	100 – 250
TP (mg/L)	100 – 250
fat, oil & grease (mg/L)	100 – 1 000

Table: C-13: Slaughtering capacity of poultry-abattoir plants (Molapo, 2009)

Units slaughtered daily	Frequency (n=26)	Occurrence (%)
800 – 20 000	14	53.9
20 001 – 40 000	3	11.6
40 0001 – 60 000	1	3.8
60 001 – 80 000	1	3.8
80 001 – 100 000	1	3.8
More than 100 001	6	23.1

Table: C-14: Estimated wastewater generated and respective C, N and P content from the number of poultry-abattoir plants presented in Molapo (2009)

Units Slaughtered per year	Estimated Wastewater (ML)	C Content (tonnes per year)	N content (tonnes per year)	P content (tonnes per year)	Fats, grease and oils (tonnes per year)
800 – 20 000	1.58	2.1 – 11.8	0.16 – 0.39	0.16 – 0.39	0.16 – 1.6
20 001 – 40 000	2.08	2.7 – 15.6	0.21 – 0.52	0.21 – 0.52	0.21 – 2.1
40 001 – 60 000	0.66	0.9 – 5.0	0.07 – 0.17	0.07 – 0.17	0.07 – 0.66
60 001 – 80 000	0.78	1.0 – 5.9	0.08 – 0.20	0.08 – 0.20	0.08 – 0.78
80 001 – 100 000	0.50	1.9 – 11.1	0.15 – 0.37	0.15 – 0.37	0.15 – 1.5
> 100 000	20.1	26.1 – 150.6	2.0 – 5.0	2.0 – 5.0	2.0 - 20
TOTAL	25.7				

C.4.4 Red meat abattoirs industry (Section 4.3.3)

The waste per slaughter unit according to Neethling (2014) is 818 L of effluent and 31 kg of solid waste. A slaughter unit is based on weight and carcass weight equivalents (cwe) are to 1 cow, bull or ox; 2 calves; 1 horse; 6 sheep or goats; 4 porkers; 2 baconers or 1 sausage pig.

From the webpage (RMRD SA, n.d.) between 2.4 and 2.6 million cattle and approximately 6.3 million sheep and goats were slaughtered in South Africa annually over the last number of years. From a DAFF report (DAFF SA, 2012)1 approximately 2.4 million pigs were slaughtered during 2011. During 2008 the production of mutton reached the peak of 163 million kg (0.163 million tonne), followed by downward trend is due to changing lifestyle of majority of consumers and in 2010 mutton production reached about 130 million kg (0.13 million tonne) (DAFF SA, 2011). The number of cows slaughtered in 2009 was estimated at 113 2000 (Index Mundi, 2010).

Using these figures an approximate cwe of 4.5 million per annum for red meat slaughter can be derived.

C.4.5 Dairy industry (Section 4.3.3)

In the annual report of the Milk Producers Organisation (MPO, 2016) milk production from 2013 to the beginning of 2016 is shown. The seasonality of milk is clearly visible with the lowest yields from April to July and highest from September to November (Figure: C-1). Information regarding water usage and effluent production for the primary dairy industry is enumerated in Table: C-15

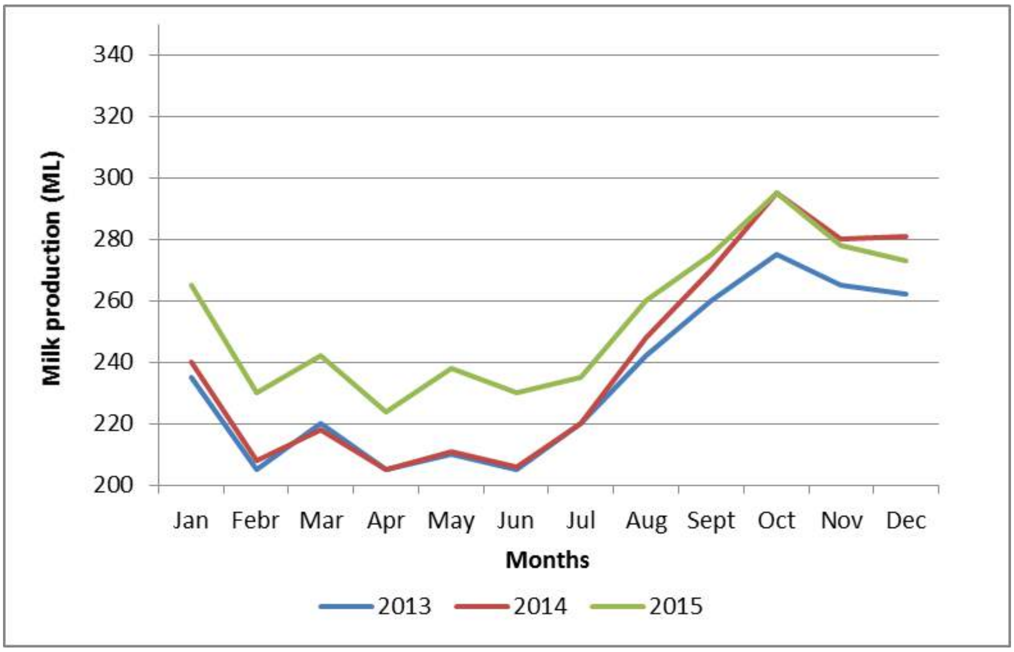


Figure: C-1: Milk production trends in South Africa for the period 2013 to 2015 adapted from “Dairy Market Trends 2016” (MPO, 2016)

Table: C-15: Information regarding the water usage and effluent production from five different milking parlours in the Free State and the Western Cape (Du Preez, 2010)

Milk Parlour	Area	No cows	Total milk production (L/day)	Water source	Total water use (A) (L/day)	Average wastewater generated per cow (L/ cow/day)	Average wastewater generated per cow during CIP (L/ cow/day)	Avg wastewater generated per litre milk produced (L/L-milk produced)
MP1	Free state	410	9500	Borehole	5953	15	4.88	0.63
MP2	Free state	250	7500	Borehole, natural spring and dam	4109	19	5.45	0.55
MP3	Western Cape	1100	34000	Borehole	36421	33	6.04	1.07
MP4	Western Cape	400	13000	Borehole	14025	35	6.00	1.08
MP5	Western Cape	650	24000	Municipal and borehole	35665	51	6.43	1.49

(A)These values where obtained from Du Preez (2010) Table 2-3 and consists of (1)Wastewater generated during milking process (udder cleaning, boot washing, etc), (2) Wastewater generated during washing of milking equipment (CIP washing of milking equipment and milk storage tanks), (3) (C) Wastewater generated during floor flushing (floor or gathering area and milking parlour)

Table: C-16: Dairy wastewater content loads taken from The Water Wiki (IWA, 2009)

Parameter	Load
TSS (mg/l)	100 – 1000
BOD (kg/ton of milkl)	0.8 – 2.5
COD (kg/ton of milk)	1.5 times that of BOD
TN (mg/l)	6% of BOD
TP (mg/l)	10 - 100

Dairy effluents contain dissolved sugars, protein and fats. The waste load equivalents of specific milk constituents are: 1 kg of milk fat = 3 kg COD; 1kg of lactose = 1.13 g COD; and 1 kg protein = 1.36 kg COD (IWA, 2009).

Secondary Dairy Industry

Studies report different pollutant load parameters. Strydom et al. (1997) reported a chemical oxygen demand (COD) of 5 340 mg/L (16 020 mg-C/L) from cheese manufacture; 4 656 mg/L (13968 mg C/L) from milk and 1 908mg/L (5724 mg C/L) from milk powder or butterfat products. NatSurv 4 (Steffan, Robertson and Kirsten Inc, 1989a) reported COD for whole milk of 210 000 mg/L (630 000 mg C/L), skimmed milk of 100 000 mg/L (300 000 mg C/L), butter milk of 110 000 mg/L (330 000 mg C/L) and whey of 75 000 mg/L (225 mg C/L). Therefore, pollutant loads for grey water produced from different products varies with the type of product. If an average COD value is taken from all the data listed above from Strydom et al. (1997) of 3 968 mg/L (11 904 mg-C/L) with a effluent volume of 4 500 ML/year then the amount of C that can be recovered is 53 568 tonne carbon per year (Table: C-17).

The World Dairy Summit (2012) reported 131 primary producers which have units processing their own milk, as well as 163 entities which purchase raw milk for processing.

Table: C-17: Volume, concentration and complexity data for the South African secondary dairy industry

effluent volume in South Africa	**total estimated effluent volume in South Africa**	*ML/year*	4 500 (estimate from 1986)
	Days of operation	*days*	365

	total estimated effluent volume in South Africa	*ML/day*	12.3
cross-reference	to worksheet with primary data and calculations.		
distribution: number of plants	**TOTAL**		**294**
	micro	*<0.5 ML/day*	
	small	*0.5-2 ML/day*	
	medium	*2-10 ML/day*	
	large	*10-25 ML/day*	
	macro	*>25 ML/day*	
concentration	**estimated average carbon content**	*mg/L*	11 904
	estimated average nitrogen content	*mg/L*	nl
	estimated average phosphorus content	*mg/L*	nl
	pH		2.2 – 11.8
	conductivity	*mS/m*	nl
complexities	**solids component**		nl
	toxic compounds		nl
	metals		nl
	complex organics		nl
	other valuable components		nl

C.4.6 Soft drinks industry (Section 4.3.4)

Data from the NatSurv 3 (Pollution Research Group, 2015) report gives the following information from the responses to a survey sent to 67 soft drink companies in Table: C-18.

Table: C-18: Summary of the survey findings for the South African soft drink industry, adapted from NatSurv 3 (Pollution Research Group, 2015)

Sector	Parameter	No. of companies	Range	Overall average
Carbonated soft drinks	Production (ML/year)	9	40 to >500	240
	Water usage (Ml/year)	9	60 to >500	300
	Specific water intake (SWI) (L/L)	9	1.2 to 2.5	1.6
	Wastewater (ML/year)	8	20 to 260	113
	Specific effluent volume (SEV) (L/L)	8	0.2 to 1.4	0.6
	pH (1)	7	2.8 to 12.2	-
	COD (mg/L) (1)	7	87 to 725000	-
	TDS (mg/L) (1)	2	10 to 3500	-
	SS (mg/L) (1)	2	53 to 130	-
Bottled water	Production (ML/year)	2	1 to 100	42
	Water usage (Ml/year)	1	2 to 5	=
	Specific water intake (SWI) (L/L)	1	1.2 to 1.5	1.2
	Wastewater (ML/year)	1	0.2 to 0.4	-
	Specific effluent volume (SEV) (L/L)	1	0.12	0.12
	pH	0	-	-
	COD (mg/L)	0	-	-
	TDS (mg/L)	0	-	-
	SS (mg/L)	0	-	-

Fruit juice	Production (ML/year)	5	1 to 60	17.5
	Water usage (Ml/year)	3	0 to 120	40
	Specific water intake (SWI) (L/L)	4	1.0 to 4.5	2.2
	Wastewater (ML/year)	3	0 to 400	130
	Specific effluent volume (SEV) (L/L)	4	0.1 to 3.8	1.7
	pH (1)	2	6.1 to 11	-
	COD (mg/L) (1)	2	175 to 18 000	-
	TDS (mg/L) (1)	2	600 to 19 000	-
	SS (mg/L) (1)	2	55 to 800	-
(1) These figures were taken from municipal figures given in Table 6.2 (Pollution Research Group, 2015)				

C.4.7 Alcoholic beverage industry (Section 4.3.4)

The alcholic beverage market data for the 12 month period from July to June 2010/2011 according to a report by Holtzkampf (2012) the volume and market share data is summarized in Table: C-19.

Table: C-19: Volume of alcoholic beverages and market share for the period 2010/2011 (Holtzkampf, 2012)

Beverage	Examples	Volume (000 L)	Market share value %
Spirits	brandy, vodka, whiskey, gin, cane	14 419 932	21.9
Wine	sparkling wine, red, white and rose wines	6 259 808	9.5
Fortified wine	sherry	1 163 817	1.8
Ready to drink (RTD)	Savanna, Hunters, Smirnoff spin, Klippies and Coke, cocktails	8 206 200	12.5
Beer		35 831 400	54.4
Total		65 881 157	100.0

Breweries

The composition of brewery wastewater content loads are given in Table C-19 to C-23 from various reference sources.

Table: C-20: Brewery wastewater content loads (IWA, 2009)

Parameter	Load
TSS (mg/l)	10 – 60
BOD (mg/l)	1000 - 1500
COD (mg/l)	1800 – 3000
TN (mg/l)	30 - 100
TP (mg/l)	10 – 30
pH	Average of 7
Temperature	30 ºC

Table: C-21: Typical specific pollution loads from breweries in South Africa, adapted from NatSurv 1 (Binnie and Partners, 1987)

Brewery	Average beer produced/month (ML)	Average water intake/month (ML)	Average effluent produced (ML)	Specific water intake (L/L)	Specific effluent volume (L/L)	COD (mg/L)	SS (mg/L)	TDS (mg/L)
A	17.1	102.5	70.5	6.0	4.1	20 000	4 000	5 600
B	9.0	79.1	40.0	8.8	4.4	20 000	2 900	9 900
C	18.2	129.0	93.0	7.1	5.1	10 400	2 900	8 300
D	14.0	77.0	nl	5.5	nl	nl	nl	nl
E	2.0	13.7	nl	6.8	nl	nl	nl	nl
F	16.0	100.80	43.5	6.3	2.7	700	nl	nl
G	8.3	61.700	51.7	7.4	6.2	9 400	nl	nl
H	5.2	34.7	25.3	6.7	4.9	10 700	1 600	nl
Total	89.8	598.5	324.0	6.66	3.6			

Table: C-22: Typical breakdown of the specific pollution loads within a brewery adapted from NatSurv 1 (Binnie and Partners, 1987)

Area	Specific effluent volume (L/L)	COD (mg/L)	SS (mg/L)	TDS (mg/L)
Brewhouse	0.5	3 700	700	500
Cellars	1.15	3 100	2 100	500
Packaging	1.5	3 500	negligible	200
Utilities	1.35	100	100	6 700
Totals	4.5	10 400	2 900	7 900

Table: C-23: The typical volume of beer produced, effluent generated and COD of brewery wastewater, adapted from Burton et al. (2009)

Brewery	Beer (ML)	Wastewater (ML)	COD (mg/L)	effluent/beer (L/L)
Alrode	688.5	2 203	3	3.20
Rosslyn	597.2	1 911	3	3.20
Prospecton	439.6	1 407	3	3.20
Newlands	373.3	1 194	3	3.20
Ibhayi	199.1	637	3	3.20
Chamdor	182.5	584	3	3.20
Polokwane	124.4	398	3	3.20
Total	2 604.6	8 334	3	3.20

Table: C-24: Characteristics of brewery trade wastewater, adapted from Cloete et al. (2010)

Characteristics	Amount
Water to beer ratio	4-10 hL water/ hL beer
Wastewater to beer ratio	1.3 -2 hL/hL lower than the water to beer ratio
BOD	0.6-1.8 kg BOD/ hL beer
SS	0.2 -0.4 kg SS/hL beer
COD/BOD	1.5 -1.7
Nitrogen	30-100 g/m^3 wastewater
Phospherous	30-100 g/m^3 wastewater
Heavy metal concentration	Very low

1 hL is 100 L. This measure of voluem unit is used throughout the beverage industry

Wineries

Production in the wine industry is summarised in the SA Wine industry Statistics (SAWIS, 2016) report and the production figures are shown in Table C-24 with the corresponding estimated effluent production using an SEV of 1 to 4 L-effluent/L-wine (Welz, et al., 2015).

Table C-24 Production and effluent figures for the south African wine industry (SAWIS report, 2016)

Table: C-25: Production and effluent figures for the south African wine industry (SAWIS, 2016) with calculated possible effluent figures using SEV range from (Welz, et al., 2015)

				Effluent produced (1-4 L/L-wine)	
Production (in ML/year)	**2013**	**2014**	**2015**	**2015 (1L/L)**	**2015 (4L/L)**
Wine	915.5	958.8	968.4	968.4	3 874
Wine for brandy	42	53.6	41.8	41.8	167
Distilling wine	140.7	133.6	112.9	112.9	452
Grape juice concentrated and grape juice	58.7	35.1	30.9	30.9	124
TOTAL	**1 156.9**	**1 181.1**	**1 154**	**1 154**	**4 616**

In the SAWIS (2016) report the following definitions were given:

"**Wine** includes all the products below.

- **Natural wine** is non-fortified and non-sparkling wine, including perlé wine which is wine carbonated to the extent that the pressure in the container in which it is sold is between 75 and 300 kPA. It also includes any grape juice or must and grape juice or must concentrate used in the sweetening of such natural wine.
- **Fortified wine** is non-sparkling wine which has been fortified with wine spirit. It includes the volume of wine spirit used in the fortification process.
- **Sparkling wine** is wine carbonated (either by fermentation or by impregnation with carbon dioxide) to the extent that the pressure in the container in which it is sold is more than 300 kPa. It includes any grape juice or must and grape juice or must concentrate used in the sweetening of such sparkling wine.

Wine for brandy is wine specially prepared for double distillation in a pot still and then, as distillate, matured for a peroid of at least three years in oak casks with a capacity of not more than 340 litres.

Distilling wine is wine specially prepared for distillation to spirits intended for use in brandy or other spirits, for fortification of wine or for industrial purposes.

Grape juice concentrate and grape juice refers to unfermented, undiluted or concentrated juice from grapes destined for use in non-alcholic products such as fruit juices."

The wine industry is quite difuse with many small producer and only a few large throughput entities. The breakdown for 2014 (WOSA, 2015; Froud, 2016) is given as follows: primary wine producers (farmers) 3 314 and cellars crushing grapes (wineries) 559, of which 49 are producer cooperatives and 25 are producing wholesalers.

Table: C-26: Volume, concentration and complexity data for the wine industry

effluent volume in South Africa	**total estimated effluent volume in South Africa**	*ML/year*	2 421
	Days of operation	*days*	365
	total estimated effluent volume in South Africa	*ML/day*	6.63
cross-reference	to worksheet with primary data and calculations.		
	TOTAL		**4 000**

distribution: number of plants	**micro**	*<0.5 ML/day*	
	small	*0.5-2 ML/day*	
	medium	*2-10 ML/day*	
	large	*10-25 ML/day*	
	macro	*>25 ML/day*	
concentration	**estimated average carbon content (range)**	*mg/L*	20 400
	estimated average nitrogen content (range)	*mg/L*	110
	estimated average phosphorus content	*mg/L*	52
	pH		
	conductivity	*mS/m*	
complexities	**solids component**		stems, skins
	toxic compounds		
	metals		
	complex organics		
	other valuable components		

Distilleries

The composition of various distillery wastewaters according to Melamane et (2007) is shown in Table C-26. The last three columns are more relevant for fuel ethanol distillation which would be counted as one of the "Other Organic-Based Industries".

Table: C-27: Chemical characteristics of distillery wastewaters, adapted from Melamane, et al. (2007)

	Type of wastewater				
Parameter	**Distillery wastewater**	**WDW ***	**Vinasse**	**Raw spent wash**	**Molasses wastewater**
pH	3.0 -4.1	3.53-5.4	4.4	4.2	5.2
Alkalinity (meq/L)	0	30.8-62.4	-	2	6000
EC	346	-	-	2530	-
Phenol (mg/L)	-	29-474	477	-	450
VFA (g/L)	1.6	1.01-6	-	-	8.5
CODt (g/L)	100-120	3.1-40	-	37.5	80.5
CODs (g/L)	-	7.6-16	97.5	-	-
BOD5 (mg/L)	30	0.21-8.0	42.23	-	-
TOC (mg/L)	-	2.5-6.0	36.28	-	-
VS (g/L)	50	7.340-25.4	-	-	79
VSS (g/L)	2.8	1.2-2.8	-	-	2.5
TS (g/L)	51.5-100	11.4-32	3.9	2.82	109
TSS (g/L)	-	2.4-5.0	-	-	-
MS (g/L)	-	6.6	-	-	30
MSS (g/L)	-	900	100	-	1100
TN (g/L)	-	0.1-64	-	202	1.8
NH4+ (mg/L)	-	140	-	125-400	-
NO3- (mg/L)	4900	-	-	-	-
TP (g/L)	-	0.24-65.7	-	0.24	-
PO_4^{3-} (mg/L)	-	130-350	-	139	-

	Type of wastewater				
Parameter	Distillery wastewater	WDW *	Vinasse	Raw spent wash	Molasses wastewater
* Wine distillery wastewater					

C.4.8 Edible oil industry (Section 4.3.4)

NatSurv 6 (Steffen, Robertson & Kirsten Inc, 1989d) reported total quantity of edible oil produced in 1989 was 250 000 t/a (0.25 Mt/year) and was expected to increase by 3% per annum using data from 11 oil plants. Using the compound interest equation (Equation 1) the total quantity of edible oil was estimated to be 0.56 Mt/year in 2016. The water used and wastewater generated for 2016 was calculated from the 1989 data using linear extrapolation and the values are shown in Table: C-28.

$$FV = PV \times (1+r)^n \qquad \text{Equation 1}$$

where FV is the future value, PV the present value, r is the percentage as a decimal and n is the number of years

Table: C-28: Data from 1989 and estimated values for 2016 for edible oil industry in South Africa

	1989 (NatSurv 6)	2016 (Calculated)
Oil produced (MT/year)	0.25	0.56
Water consumption (ML/year)	1 700	3 776
Water effluent (Ml/year)	612.5	1 361

Table: C-29: Results of the chemical analyses of an oil containing effluent over a period of 12 months, adapted from (Roux-Van der Merwe, et al., 2005)

Test performed	Sample 1	Sample 2	Sample 3
COD (mg/L)	251 630	35 000	15 280
Oil and grease (mg/L)	200 560	28 146	12 224
Conductivity (mS/m)	88.2	268	148
Suspended solids (mg/L)	29 330	15 410	715
Na (mg/L)	nl	1 000	410
TKN (mg/L)	16.1	45.9	40.7

Surujlal et al. (2004) studied the biological treatment technology for the remediation of edible oil effluent from one factory that produced 96 t/day of refinery effluent. The following parameters were recorded for the effluent (Table C-29).

Table: C-30: Effluent parameters collected, adapted from Surujlal et al. (2004)

Parameter (mg/L except pH)	June		July		August		September		October	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
pH	4.6-5.9	5.6	8.8-10.6	9.5	5.7-7.0	6.5	7.1-8.1	7.7	7.6-9.9	8.6
COD	7590-7380	7630	7550-8710	8160	1025-1270	1115	7250-7590	7400	11700-11810	11763
PO43- (-P)	500-590	550	910-1140	1020	1640-1680	1660	4320-4510	4400	2110-2180	2140
TKN (-N)	6.08-7.96	6.93	3.21-6.26	4.78	6.54-7.19	6.82	6.98-8.67	7.65	4.36-5.81	4.98
NH4+(-N)	0.98-1.51	1.25	0.41-0.76	0.6	1.39-2.62	2.0	1.09-1.21	1.15	2.09-3.6	2.69

SO42-	4980-5910	5550	5280-5830	5600	3410-3530	3470	5690-5980	5800	1170-1400	1260
FOG	249-266	256	103-121	111	325-352	340	581-631	628	297-319	308
TSS	239-281	265	379-388	383	98-134	112	256-274	265	309-340	322
Alkalinity	487-542	520	265-492	480	1670-1760	1720	616-649	630	742-778	766

C.4.9 Canning industry (Section 4.3.4)

The WRC report of Binnie and Partners (1987) used data from 1979 and 1980 on canning of certain fruit and vegetables; given in Table: C-31 are the water intake, effluent volumes, COD. No measurements were done for nitrogen and phosphorous content in these effluents. Information on fruit waste streams of South Africa is available in Khan et al. (2015) and the relevant data are summarised in Table: C-31.

Table: C-31: Data for canning of certain fruits and vegetables, adapted from Bennie and Partners (1987)

	Raw material processed (tonnes/yr)	Water intake (ML/y)	Estimated effluent volume (ML/yr)	Target specific effluent volume (ML/t processed)	wastewater/ water intake (ML/ML)	COD (mg/L)	SS (mg/L
Canning of apples	15 670	104	62.68	0.004	0.603	6 500	400
Canning of apricots	28 104	154	115	0.0041	0.747	4 500	400
Canned beans in tomato sauce	6 220	124	100	0.0161	0.806	1 628	154
Canning and bottling of beetroot	2 330	18.5	11.6	0.0050	0.627	1 943	213
Canning of corn	7 895	73	24	0.0030	0.329	1 500	250
Canning of green beans	2 300	17	14.95	0.0065	0.879	927	180
Canning of guavas	9 509	60.7	45.5	0.0048	0.750	700	195
Canning of peaches	132 361	910	700	0.0053	0.769	700	195
Total	**204 389**	**1 461.2**	**1 073.73**				

Table: C-32: Fruit waste streams of South Africa, adapted from Khan et al. (2015)

	Olives	Citrus	Grapes	Apples
Raw material	2000 tonnes olives processed for olive oil; 1500 tonnes processed for table olive production (2012/2013 season)	441899 tonnes citrus (ornages, lemons, limes, grapefruit and naartjies) processed (2011/2012 season)	1649 tonnes processed for preserves and canning; 151628 tonnes are dried; 1413533 tonnes pressed for wine spirits and juice production (2011/2012 season)	244469 tones processed for juice, jams, preserves; 1110 tonnes dried
Wastewater volume	80-120L/ 100 kg olives processed (0.8 – 1.2 L/kg)	1.5 ML/tonne citrus fruit produced	1000 ML/ year	5-10%
composition	Low pH (4.5) 2.6% total sugars	15% soluble solids, 30% pulp 100-2000 COD mg/L Nitrogen and phosphorous concentration low	Variable. Composition in g per 100g: 1.8-3.7% soluble sugars,1.8-3.8% protein, 0.3-1.0% lipids, 19.5-40.8% cell wall polysaccharides and lignin (dietary fibre)	Low pH of 3.3; Total solids of 115-135 g/L; Total nitrogen of 2.2-2.9 g/L; Total carbon 44.3-51.9 g/L;

				Total carbohydrates 56.2 – 66 g/L; Total protein 28.8-33.8 g/L; Lipids and other micronutrients 5.1-5.9 g/L
Complexity	Phenolic compounds, rich in antioxidants	Terpenes, rich in antioxidants	Phenolic compounds, antioxidants, pigments	antioxidants

C.4.10 Confectionary industry (Section Error! Reference source not found.)

In a study by Ersahin et al. (2011) confectionery wastewater was characterised as summarised in Table: C-33.

Table: C-33: Characterization of the wastewater discharged from the confectionery industry, adapted from Ersahin et al. (2011)

		Reference			
Parameter	**Unit**	(El-Gohary, et al., 1999)	(Orhon, et al., 1995)	(Diwani, et al., 2000)	(Ozturk & Altinbas, 2008)
COD	mg/L	4 475	2 840-6 220	5 000	19 900
$COD_{soluble}$	mg/L	-	2 500-5 400	-	-
BOD	mg/L	2 200	1 840-4 910	3 200	-
TKN	mg/L	100	33-55	-	-
TP	mg/L	172	8,6-65	-	-
TSS	mg/L	649	260-440	177	1 050
VSS	mg/L	490	-	-	-
pH	-	-	4-5,1	6	-
Oil and grease	mg/L	367	-	-	-

C.4.11 Textiles industry (Section 4.3.5)

From the NatSurv 13 report (Steffen, Robertson and Kirsten Inc, 1993) 15 textile mills were assessed for the annual production, and wastewater generated as well as the composition of the streams. In 1990 the water intake was reported to be approximately 30 x 10^6 m^3/a (30 000 ML/year or 0.03 million Ml/year) for the textile industry with effluent produced about 70 to 80% of intake which equates to 22 500 ML/year (0.0225 million ML/year). The SWI was found to vary from 95 to 400 L/kg of material processed (Steffen, Robertson and Kirsten Inc, 1993).

The study done by Cloete, et al. (2010) indicated the annual water consumption was 5.0511 Mm^3/year (5051.1 ML/year) while the effluent generated was 3.1146 Mm^3/year (3114.6 ML/year) which is about 62% of the water consumption. The COD values ranged from 537 to 9553 mg/L (1 611 – 28 659 mg-C/L), the nitrate concentrations were less than 1 mg/L (0.226 mg-N/L) but the phosphate concentration ranged from 1 to 39 mg/L (0.326 - 12.8 mg-P/L). The pH of the effluent varied between 5 and 12 with the majority of the textile effluents had a pH of above eight.

In general the effluents form the textile industry have high salinity due to the salts ($NaNO_3$, NaCl and Na_2SO_4) that are added to the dye baths to improve the fixation of the dyes on the fabrics (Imran, et al., 2015), which results in the wastewaters having a high electrical conductivity. There is a wide range of pH values due to the nature of the salts and dyes that are used in the process (Imran, et al., 2015). In some cases there are high metal concentrations (Cd, Cr, Co, Cu, Hg, Ni, Mg, Fe and Mn), colour and relatively high COD (Imran, et al., 2015; Steffen, Robertson and Kirsten Inc, 1993). They also contain auxiliaries such as detergents, bleaching agents, softeners and finishing chemicals (Barclay & Buckley, 2004).

The study by Barclay and Buckley (2004) on the treatment of textile and industrial effluent focussed on ways to treat textile effluent either on-site or at the WWTW with the focus on colour removal.

Table: C-34: Volume, concentration and complexity data for the textile industry

effluent volume in South Africa	**total estimated effluent volume in South Africa**	*ML/year*	30 000
	Days of operation	*days*	365
	total estimated effluent volume in South Africa	*ML/day*	82.2
cross-reference	to worksheet with primary data and calculations.		
distribution: number of plants	**TOTAL**		
	micro	*<0.5 ML/day*	
	small	*0.5-2 ML/day*	
	medium	*2-10 ML/day*	
	large	*10-25 ML/day*	
	macro	*>25 ML/day*	
concentration	**estimated average carbon content**	*mg/L*	151 350
	estimated average nitrogen content	*mg/L*	0.113
	estimated average phosphorus content	*mg/L*	6.53
	pH		5-12
	conductivity	*mS/m*	30-1517
complexities	**solids component**		
	toxic compounds		Azo dyes
	metals		Cd, Cr, Co, Cu, Hg, Ni, Mg, Fe and Mn
	complex organics		
	other valuable components		salts

C.4.12 Cleaning products manufacture (Section 4.3.5)

Cloete et al. (2010) reported that in Cape Town and Tshwane the cosmetics manufacturing, cleaning and toilet preparation industries have effluents totalling 0.3143 Mm^3/year (314.3 ML/year) with COD values ranging from 2 134 to 8 477 mg/L (6 402 – 25 431 mg-C/L), 3 to 36 mg/L ammonia (2.33-28 mg-N/L) and 5 mg/L nitrate (1.13 mg-P/L) as well as 55 mg/L (17.9 mg-P/L) phosphate. The pH value of the effluent varied between 8 and 9. The conductivity ranged from 43.75 to 412 mS/m.

Table: C-35: Volume, concentration and complexity data for the cleaning products manufacture industry

effluent volume in South Africa	**total estimated effluent volume in South Africa**	*ML/year*	314.3
	Days of operation	*days*	365
	total estimated effluent volume in South Africa	*ML/day*	0.86
cross-reference	to worksheet with primary data and calculations.		
	TOTAL		
distribution: number of plants	**micro**	*<0.5 ML/day*	
	small	*0.5-2 ML/day*	
	medium	*2-10 ML/day*	
	large	*10-25 ML/day*	
	macro	*>25 ML/day*	
concentration	**estimated average carbon content**	*mg/L*	15917
	estimated average nitrogen content	*mg/L*	8.15
	estimated average phosphorus content	*mg/L*	17.9
	pH		8-9
	conductivity	*mS/m*	43.75-412
complexities	**solids component**		
	toxic compounds		
	metals		
	complex organics		
	other valuable components		

D PRELIMINARY EXPERIMENTAL EVALUATION OF SELECTED BACTERIAL BIOPRODUCT

In Chapter 5 poly- γ- glutamic acid was identified as a potential bioproduct for the bacterial bioreactor step in a WWBR. On further refinement, PGA was selected as the product of primary interest going forward. This harmonises with the suggestion in Verster, et al. (2014) where it is similarly identified as suitable for recovery from wastewater.

Here the growth kinetics of *Bacillus* sp grown on synthetic waste to produce PGA is investigated. The relationships between product formation, growth and operating temperatures are studied. The temperature parameter was changed from the base case optimum condition (37 °C) to a lower temperature (30 °C) to compare cell growth and product formation owing to the desire to run these reactor systems without the need for heating or cooling.

D.1 Materials and Methods for Poly- γ- Glutamic Acid Studies

D.1.1 Inoculum

A glycerol stock culture of *Bacillus subtilis* DmB55, isolated by Madonsela (2013) was previously stored at -60°C, was cultured on Trypto-soy (TS) (30g/ℓ) agar streak plates for 12 hours. A wire loop of a colony of each isolate was transferred to 50 mℓ Trypto-soy medium in a 250 mℓ Erlenmeyer flask and cultivated at 37°C with orbital shaking at 200 rpm for 12 hours. An aliquot of this culture was transferred to a 1 litre Erlenmeyer flask containing 250 mℓ Modified Medium E (MME) (Madonsela, 2013) to achieve an optical density at 600 nm of 0.1 (Madonsela, 2013). The pre-inoculum culture was cultivated for 24 hours at 200 rpm and 37°C and sampled every 3 hours for analysis. An aliquot of this culture was transferred to the 1 litre Erlenmeyer inoculum flask containing fresh MME to achieve an optical density of 0.1. This seed culture was used to inoculate the bioreactor at 37°C with an agitation of 200 rpm. Figure: D-1 illustrates the timeline used to prepare seed culture.

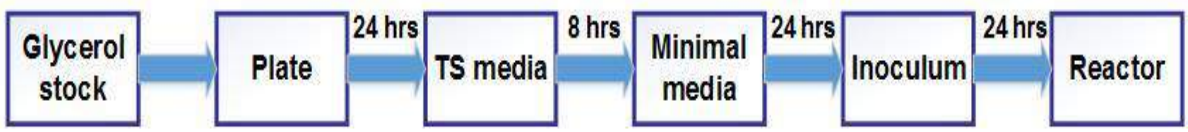


Figure: D-1: Inoculum train for bacterial cultures

D.1.2 Medium composition

The Modified medium E (MME) composition was 20 g/ℓ glucose, 1 g/ℓ glycerol, 12 g/ℓ citric acid, 3.48 g/ℓ ammonium chloride, 2.99 g/ℓ di-potassium hydrogen phosphate. The pH was adjusted to 6.5 using 5M NaOH before autoclaving.

D.1.3 Bioreactor conditions

The experiments were performed in triplicate in six parallel 300 mℓ mini bioreactors with the Sixfors® bioreactor system (Figure: D-2). A working volume of 250 mℓ and MME medium were used. The inoculum was added to achieve an optical density of 0.1 (A_{600}). The reactor conditions were set at an initial aeration rate of 1 vvm (0.25 ℓ/min), 37°C, pH 6.5 and an initial agitation rate of 200 rpm (Madonsela, 2013). Dissolved oxygen, pH and temperature were logged using the Iris ® software. A sample volume of 2 mℓ was taken every three hours for biomass, substrate and product analysis. The base case experiment was performed at 37°C and the temperature study at 30°C, both in triplicate.

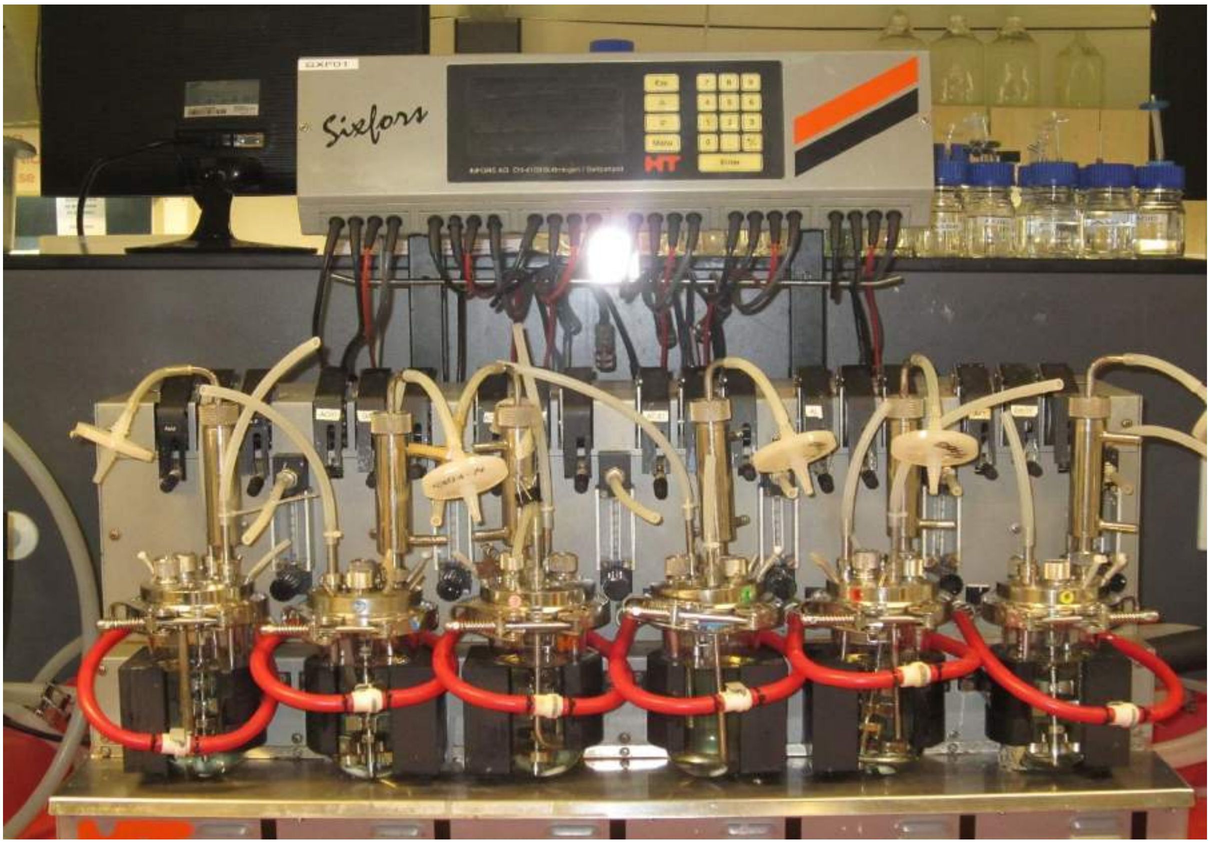


Figure: D-2: Sixfors® bioreactor system

D.1.4 Analyses

Cell dry weight (CDW)

CDW was measured from the 2 mℓ samples that were taken at discrete sampling times. These were pipetted into pre-dried and pre-weighed microfuge tubes and centrifuged at 15 000 rpm for 10 minutes. The supernatant was decanted and stored for further analysis. The pellet was resuspended, washed with phosphate buffer, and centrifuged. The wash buffer was discarded and the cell pellet and centrifuge tube was dried overnight in an 80°C oven (CeBER, 2014).

Cell concentration by Absorbance

The turbidity of the suspension was read using a Helios Alpha spectrophotometer at a wavelength of 600 nm. A correlation curve of cell concentration (CDW) in g/ℓ to A_{600} was constructed (Figure: D-3). There is a direct correlation between CDW and OD with approximately OD = 1.41 CDW – 1.32. This was used to determine the CDW at different stages of growth from optical density measurements. In all cases, appropriate dilutions in water were made to ensure that absorbance readings lay below 1.0 unit. The plot was constructed by multiplying the dilution factor by the OD reading.

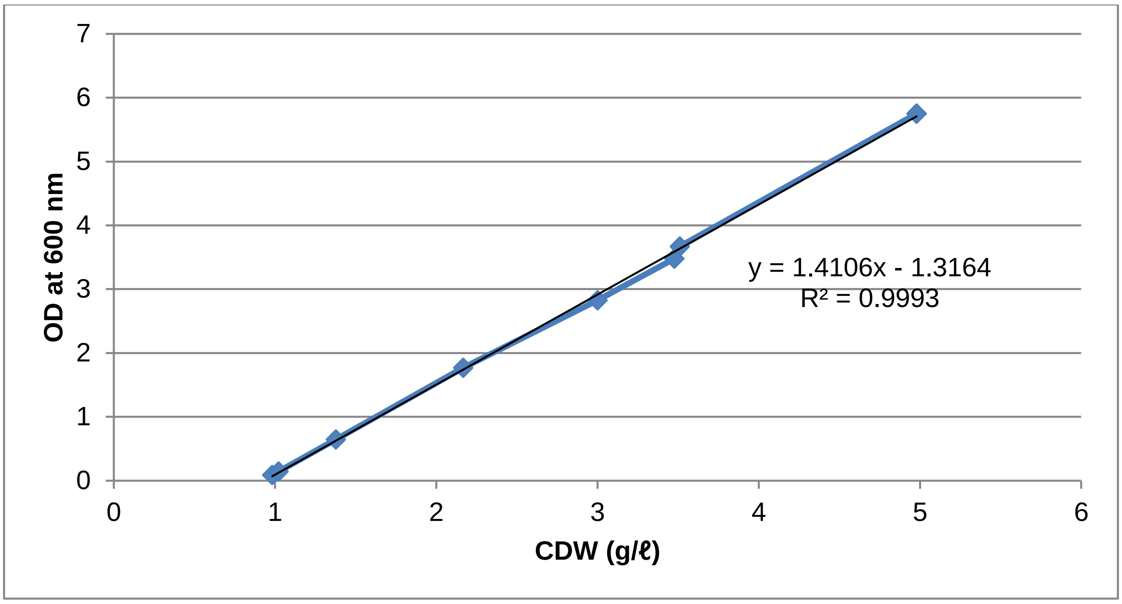


Figure: D-3: Correlation curve between CDW and OD for the base case and temperature study

Substrate analysis (HPLC)

The supernatant recovered from CDW measurements was used for HPLC analysis of the substrate concentrations (glucose, glycerol and citric acid). A Biorad Aminex ® HPX-87H organic acids column on a Waters 717 plus high performance liquid chromatography system with a refractive index detector Agilent 1100 was used. The mobile phase was 0.005M sulphuric acid (acidified water). The pump flow rate was set at 0.5 mℓ/min and the column temperature at 60 °C. Standards of glucose, citric acid and glycerol were prepared with concentrations ranging from 0.0 to 1.0 g/ℓ to plot the respective standard curves (CeBER, 2014).

γ -PGA extraction and spectrophotometric quantification

γ-PGA was determined according to the method by Zeng et al. (2012). The extraction steps are illustrated in Figure: D-4 and the amount of γ-PGA was measured spectrophotometrically at an absorbance of 197 nm on a Thermo Helios δ UV-Vis spectrophotometer.

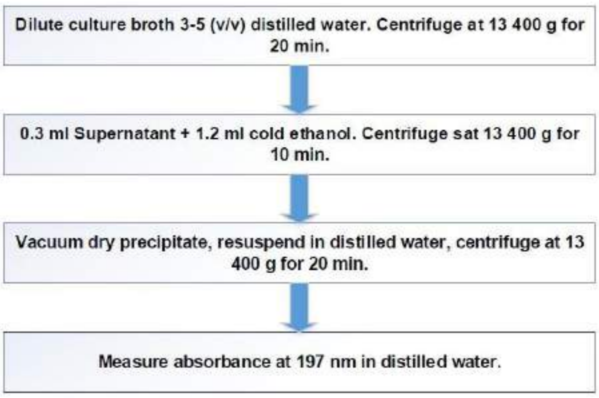


Figure: D-4: Procedure used to determine the amount of PGA produced (Image redrawn from Zeng et al. (2012))

Quantification of γ-PGA

The folding structure of γ-PGA is dependent on the concentration of the polymer as well as the pH of the solution. At low concentrations of PGA and at a pH value lower than 7, the molecule adopts an αhelical shape. At higher concentrations and pH values, the polymer conformation adopts a β-form. (Candela & Fouet, 2006). These changes affect the physical properties which, in turn, affect the UV absorption spectrum as well as the Fourier transform infrared spectrum (Zeng, et al., 2012). UV scans were completed using the Thermo Helios UV spectrophotometer. A stock solution of 1 g/ℓ γ-PGA (Xarealin, China) was prepared in the solvents tabulated in Table: D-1. All stock solutions (besides the water solution) were stored at 37 °C to ensure that the polymer was completely dissolved. The stock solution was diluted to the range 0.02 to 0.2 g/ℓ at 37 °C. These samples were syringe filtered and scanned at a UV range of 190 to 390 nm (Zeng, et al., 2012). All of these scans were repeated in duplicate to ensure reproducibility. The results of the optimisation study are presented in Section D.2.

Table: D-1: Solvents assessed as solvents for PGA UV scans

Solvent	**pH**
Deionised water	6.5
Formic acid	4
Formic acid	2
HCl	4
HCl	2

Selection of the suitable solvent.

γ-PGA standard in formic acid

This set of scans in formic acid at pH 2.0, a PGA show peak at 250 nm with an increase in peak height with concentration as shown in Figure: D-5. The maximum absorbance obtained from these scans was approximately 0.0859 at 0.2 g/ℓ PGA. The absorbance range of the standards in formic acid (pH 2) are considerably lower than with the other solvents.

The scans using formic acid as a solvent at pH 4 (Figure: D-6) indicate that the peaks do not align and thus it would be problematic to obtain exact values when the peak shifts slightly. The maximum peak obtained was at a wavelength of 198 nm for the more concentrated sample at 0.2 g/ℓ and 194 nm for 0.02 g/ℓ.

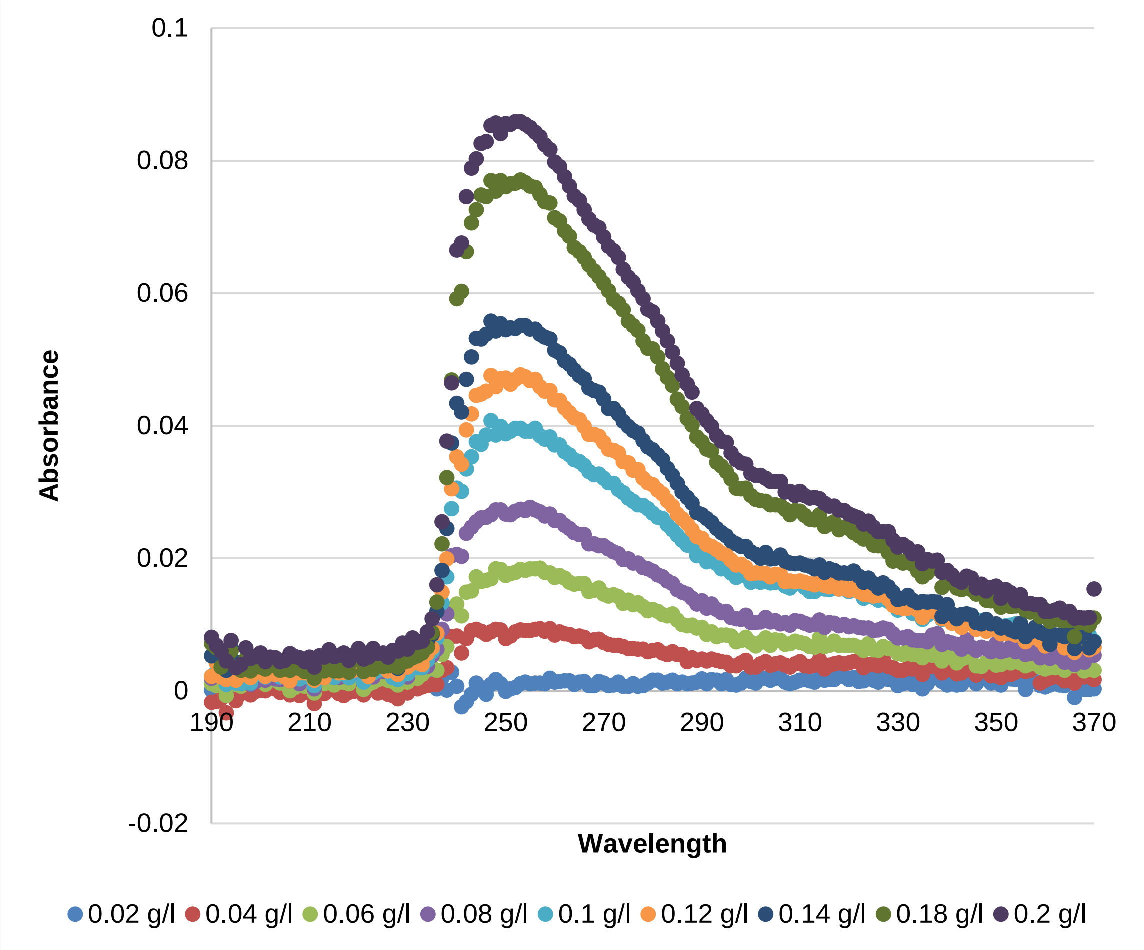


Figure: D-5: Scans of a range of PGA concentrations from 0.02 g/ℓ to 0.2 g/ℓ in formic acid pH 2

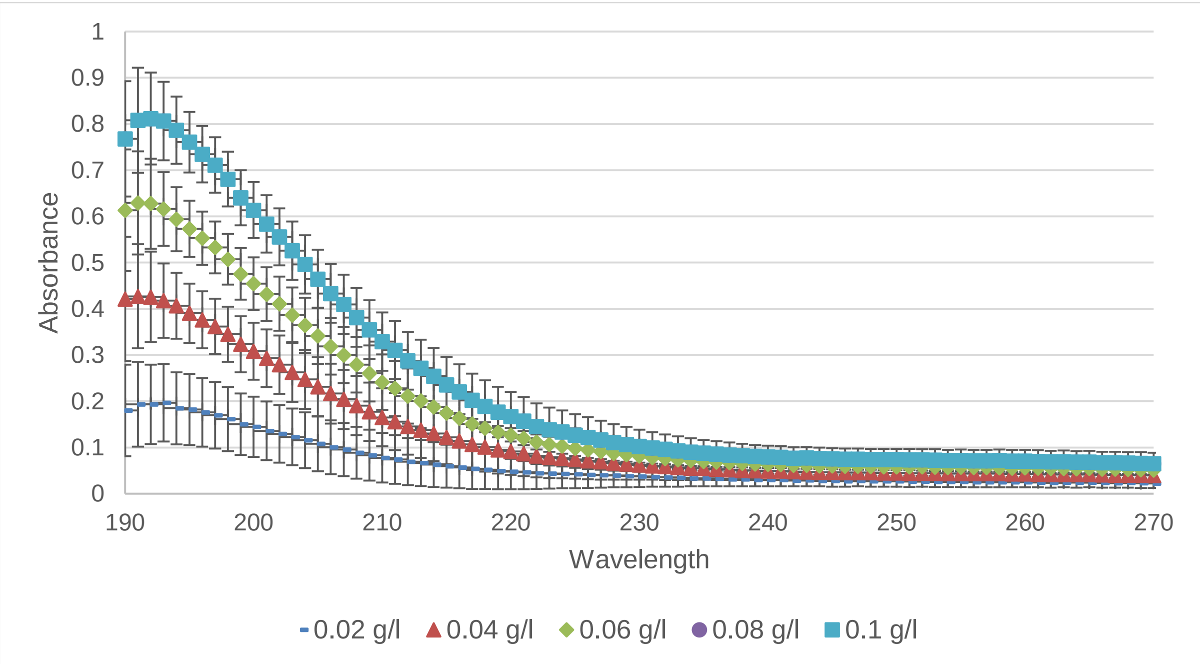


Figure: D-6: UV scans of a range of PGA concentrations 0.02 g/ℓ to 0.12 g/ℓ in formic acid (pH 4) *γ-PGA standard in HCl*

The scans using HCl at pH 4 as solvent clearly indicate that this solvent is not suitable since the peaks shift to the right with an increase in polymer concentration (Figure: D-7). Reproducibility is questionable with the size of the error bars.

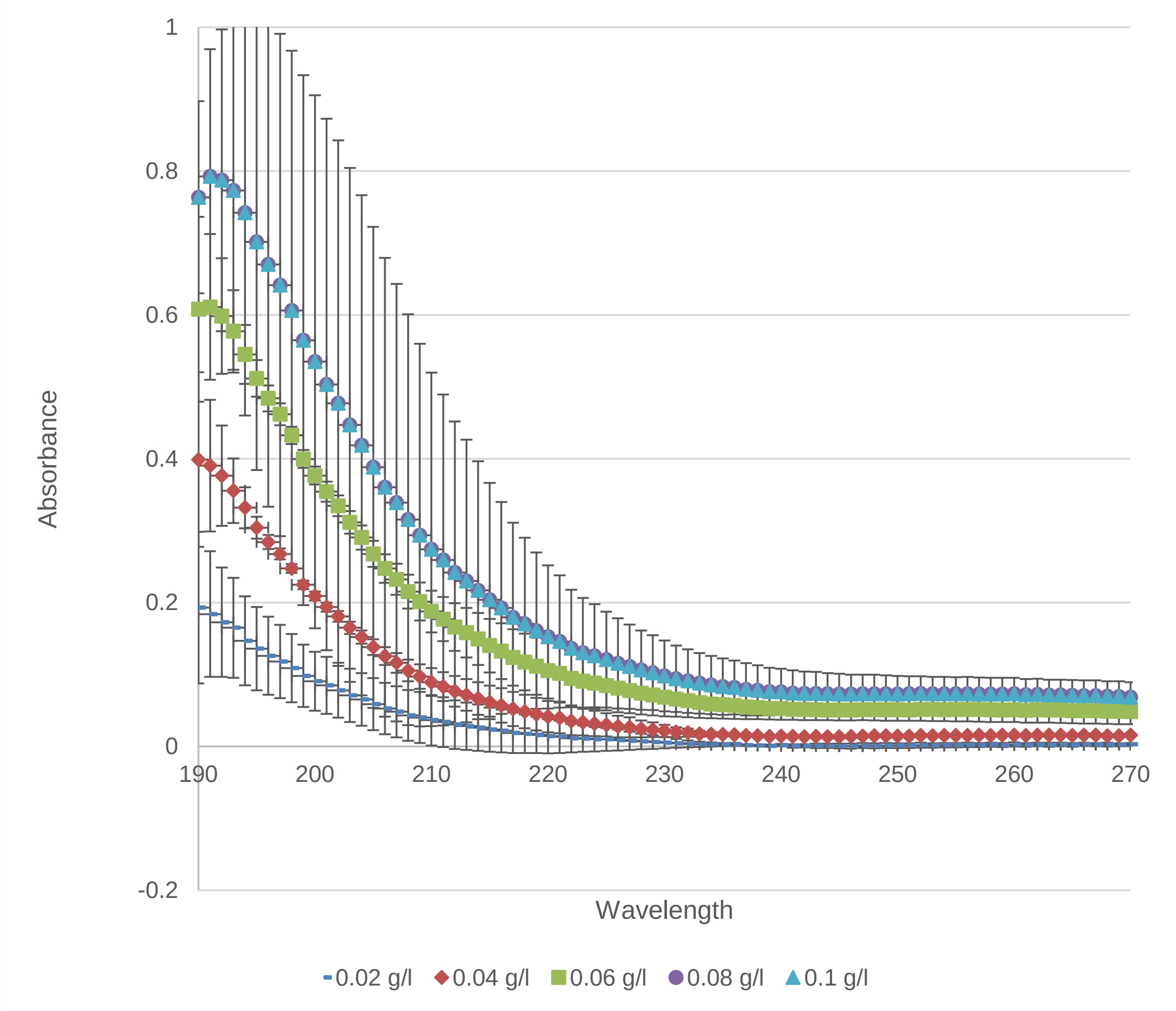


Figure: D-7: UV scans of a range of PGA concentrations from 0.02 g/ℓ to 0.12 g/ℓ in hydrochloric acid (pH 4)

γ-PGA standard in deionised water (pH 6.5).

The scans in Figure: D-8 indicate that the peak absorbance of PGA in deionised water is at 197 nm. The peaks are evenly spaced and the wavelength for maximum absorbance does not deviate with concentrations ranging from 0.02 g/ℓ to 0.08 g/ℓ.

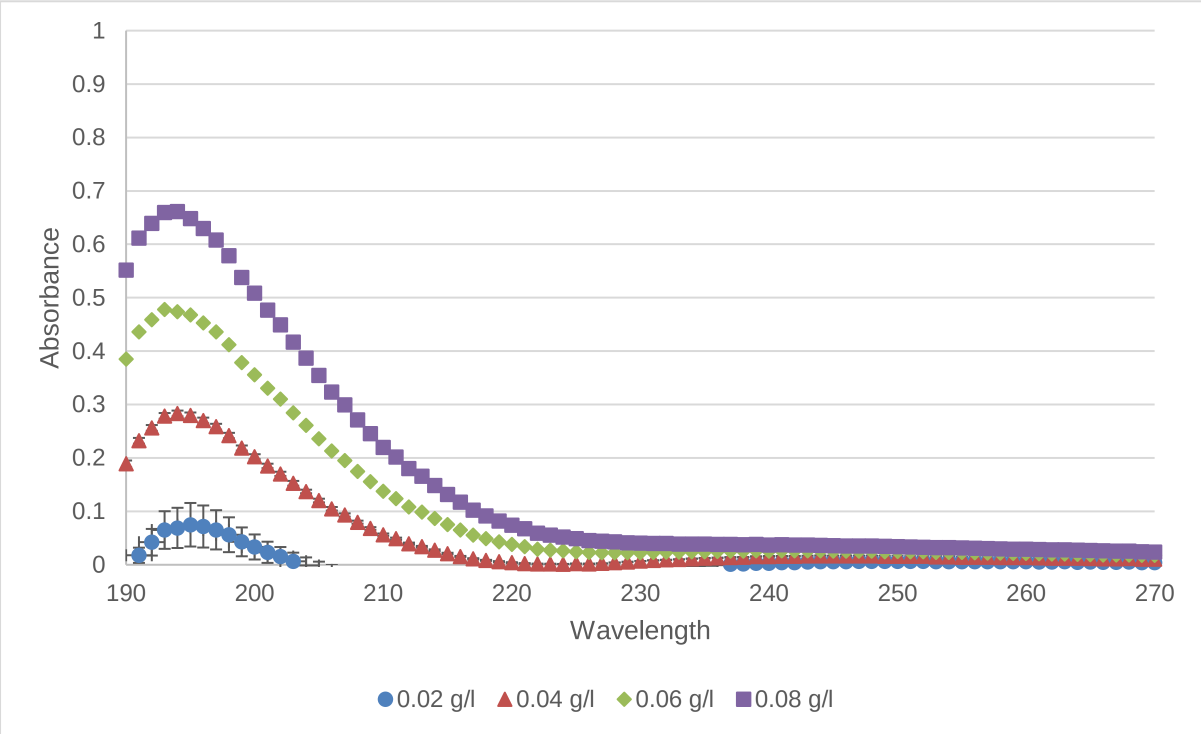


Figure: D-8: UV scans of a range of PGA concentrations from 0.02 g/ℓ to 0.08 g/ℓ in deionised water (pH 6.5)

Deionised water was thus selected as the suitable solvent for γ-PGA quantification at a wavelength of 197 nm. The polymer dissolves easily in water at room temperature. In addition, this is the cheapest option. The peaks are aligned and there is even spacing between them indicating a linear dependence of absorbance on concentration across these dilutions. Extracted samples require dilution to the range 0.02 - 0.08 g/ℓ to obtain accurate results. The standard curve in Figure: D-9 was used to determine γ-PGA concentration in the samples after extracting the crude polymer using the protocol outlined in Section BBBBBBBBBBBBBBBBBBBBBBBBBBBBBB.43.7228096.

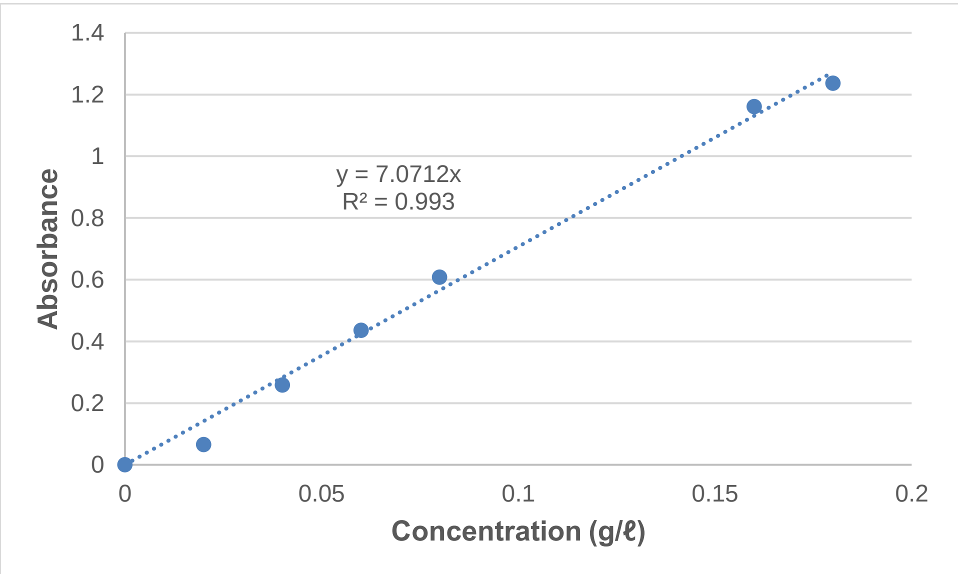


Figure: D-9: Standard curve for γ-PGA analysis

D.2 Temperature Study Using Isolate 1 (*Bacillus subtilis*)

D.2.1 Growth study

Figure: D-10 provides a summary of the results of the base case experiment (37 °C) and the temperature study at 30 °C. For the base case, sampling took place at three hour intervals when the culture began to enter exponential phase at 9 hours. These experiments were repeated in triplicate. The pH decreased from pH 6.9 at the start of the exponential phase (9 h) to pH 6.0 at the mid-exponential phase (24 h), thereafter it increased to pH 6.2 for the remainder of stationary phase (48 h). The CDW increased from 1 g/ℓ at 9 hours to 3.5 g/ℓ at 24 h to a final CDW of 5 g/ℓ at the end of the experiment. The optical density (OD) shows a similar trend with the highest reading of 5.75 at 48 hours.

On studying the effect of reducing temperature to 30 °C the pH started lower at pH 6.3 compared to pH 7 at 37 °C, but was also controlled at pH 6.4 by addition of 5M NaOH or HCl. The CDW (30 °C) followed the same trend as CDW (37 °C) but at a slower rate and only reached a maximum of 4.4 g/ℓ compared to the base case experiment (5.0 g/ℓ) at 48 hours. The OD similarly follows the base case trend, but more slowly and reaching only 4.9 compared to 5.75 at 48 hours, which is a decrease to 85% at the lower temperature.

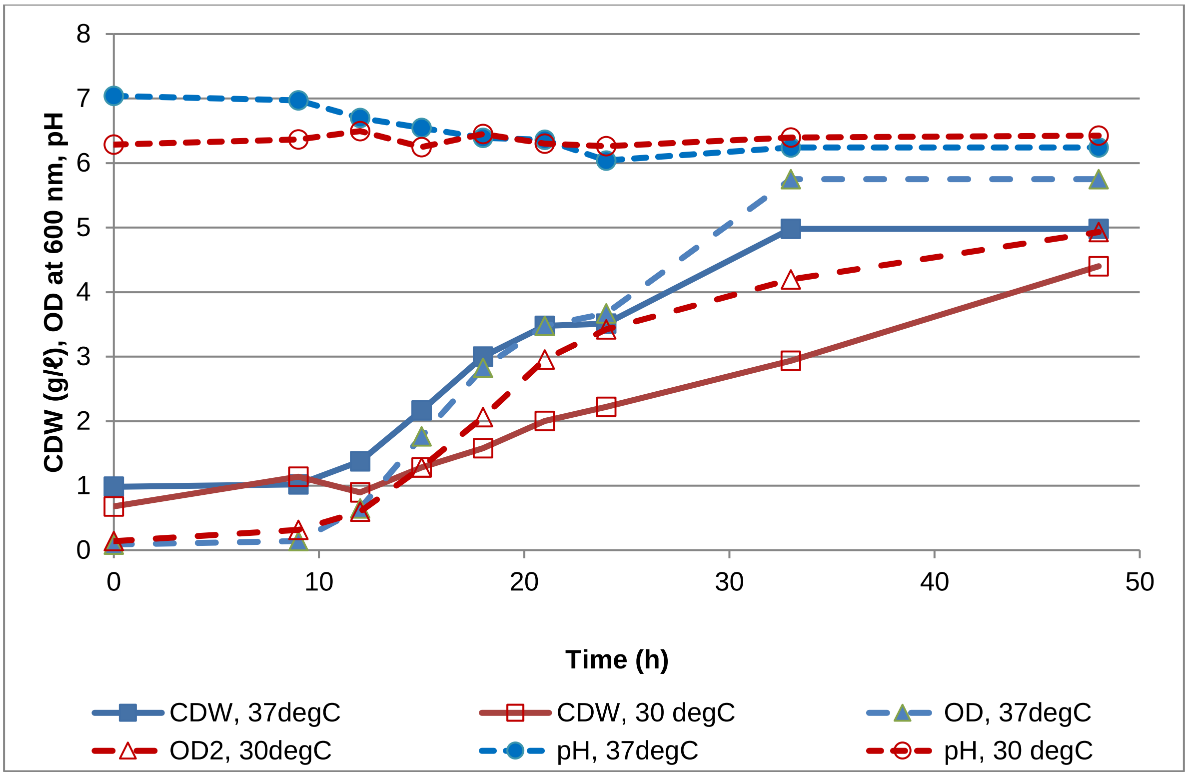


Figure: D-10: Growth curves for *Bacillus* sp at 37 °C and 30 °C

D.2.2 PGA productivity study

The volumetric PGA concentration and culture OD are shown in Figure: D-11 for the 37 °C and 30 °C cultures. Although an increase in temperature was beneficial to cell growth, it was not beneficial for PGA production. A maximum PGA concentration of 3.4 g/ℓ was obtained at 37 °C compared to 6 g/ℓ at 30°C. Therefore, an increase of a factor of 1.8 in PGA concentration was achieved by a 7 °C decrease in temperature.

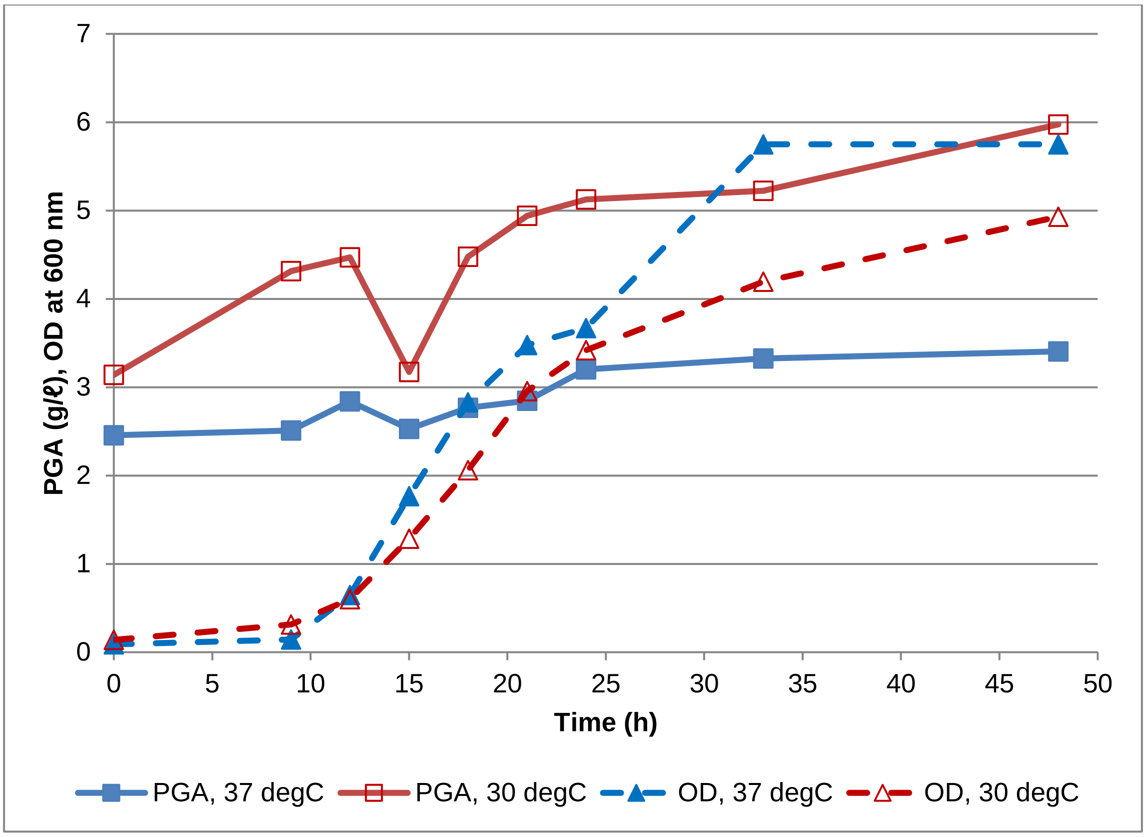


Figure: D-11: PGA production and cell growth during 37 °C and 30 °C experiments

The biomass and product yield coefficients at 37 °C and 30 °C are summarized in Table: D-2. The yield coefficients were calculated on an overall basis. The μ_{max} at 37 °C was calculated between 12 and 18 hours; for 30 °C it was calculated between 12 and 21 hours representing the exponential phase. The μ_{max} values were calculated to be 0.3 h^{-1} at 37 °C and 0.18 h^{-1} at 30 °C. Madonsela (2013) obtained a similar μ_{max} of 0.287 hr^{-1} for the base case at 37 °C.

The maximum concentration of γ-PGA produced was 4.97 g/ℓ for the base case (37 °C) and 5.97 g/ℓ for the temperature study (30 °C), compared to maximum biomass concentrations of 4.98 g/ℓ at 37 °C and 4.40 g/ℓ at 30 °C. A comparison between the $Y_{p/s}$ values of 0.25 g/g for the base case and 0.63 g/g at 30°C shows that γ-PGA production favours the lower temperature. The cell growth rate was higher at the higher temperature; however the specific γ-PGA production rate was higher at the lower temperature. This could be due to a stress response to the lowered temperature.

Table: D-2: Summary of yield coefficients, biokinetic constants and maximum concentrations achieved

Factor	37 °C	30 °C
$Y_{x/s}$	0.16 g/g	0.36 g/g
$Y_{p/s}$	0.25 g/g	0.633 g/g
μ_{max}	0.3 h^{-1}	0.18 h^{-1}
Max A_{600}	5.75	4.93
Max biomass conc	4.98 g/ℓ	4.40 g/ℓ

D.2.3 Recommendations for further studies

The production of γ-PGA was affected by temperature. A decrease in temperature from 37 to 30 °C increased the amount of γ-PGA produced by a factor of 1.8 to a final concentration of 6 g/ℓ. Higher temperatures were more favourable for growth with a maximum cell concentration of 5 g/ℓ and a maximum specific growth rate of 0.3 h^{-1} achieved.

It is recommended that experiments should be performed at room temperature to further the temperature study. It is also recommended that a fed batch configuration should be explored with the aim of extending the time period for biomass and PGA production, allowing an increased volumetric output of the desired polymer. These studies combined will then be valuable in modelling the reactor system using the derived biokinetics for this micro-organism to determine the expected temperature range with scale and climate, resulting from metabolic energy generation and heat loss from the bioreactor, in the absence of heating and cooling.

D.3 Material and Method for growth curve base case Using Isolate 1 *(Bacillus subtilis)*

As a precursor to the bioreactor work in the MBBR and AGS reactors, a preliminary base case reactor run in 5 litre New Brunswick CSTRs was commissioned, in duplicate. The objectives of this study was to obtain a good understanding of the organism, and the growth and substrate utilisation and production of γ-PGA under ideal conditions. The results of this reactor run were used as a starting point to optimise the experiment further and taking it into fed batch experiments in due course.

D.3.1 Inoculum

A glycerol stock culture of *Bacillus subtilis* DmB55, isolated by Madonsela (2013) was cultured in 10 mℓ Trytone Soy Broth (30g/ℓ) in a 125 mℓ Erlenmeyer flask for 12 hours and then streaked onto Tryptone Soy plates for 8 hours. A wire loop of a colony of each isolate was transferred to 50 mℓ Tryptone Soy Broth in a 250 mℓ Erlenmeyer flask and cultivated at 37°C with orbital shaking at 200 rpm for 12 hours. An aliquot of this culture was transferred to a 1 litre Erlenmeyer flask containing 250 mℓ Modified Medium E (MME) (Madonsela, 2013) to achieve an optical density at 600 nm of 0.1 (Madonsela, 2013). The pre-inoculum culture was cultivated for 24 hours at 200 rpm and 37°C and sampled every 3 hours for analysis. An aliquot of this culture was transferred to a 2 litre Erlenmeyer inoculum flask containing fresh MME to achieve an optical density of 0.1. This seed culture was used to inoculate the 5 litre bioreactor at 37°C with an agitation of 200 rpm. Figure: D-1 in section D.1.1 illustrates the inoculation train used.

D.3.2 Medium Composition

The half strength Modified medium E (MME) composition was 10 g/ℓ glucose, 0.5 g/ℓ glycerol, 6 g/ℓ citric acid, 1.74 g/ℓ ammonium chloride, 1.495 g/ℓ di-potassium hydrogen phosphate, 0.25 g/ℓ magnesium sulphate, 0.052 g/ℓ manganese sulphate, 0.02 g/ℓ ferric chloride and 0.075 g/ℓ calcium chloride. The pH was adjusted to 6.5 using NaOH pellets before autoclaving.

D.3.3 Bioreactor conditions

The experiments were performed in duplicate in 7 litre New Brunswick CSTRs. A working volume of 5 litres and half strength MME medium were used. The inoculum was added to achieve an optical density of 0.1 (A_{600}). The reactor conditions were set at an initial aeration rate of 1 vvm (0.25 ℓ/min), 37°C, pH 6.5 and an initial agitation rate of 200 rpm. (Madonsela, 2013). Dissolved oxygen, pH and temperature were monitored. The agitation rate was set as a function of the dissolved oxygen and adjusted as necessary to main a DO at 30%. A sample volume of 30 mℓ was taken every three hours for biomass, substrate and product analysis. Due to foaming, some challenges were faced and this resulted in the agitation rate and aeration rate being continuously monitored and adjusted, along with Antifoam 204 addition to prevent foam build up in the reactor headspace.

D.3.4 Analyses

The methods used to find CDW and OD were the same protocol followed in D.1.4.

Substrate analysis (HPLC)

The supernatant recovered from the CDW measurements was used for HPLC analysis of the substrate concentrations (glucose, glycerol and citric acid). A Biorad Aminex ® HPX-87H organic acids column on a Thermo 2 high performance liquid chromatography system with a refractive index detector by Finnigan Surveyor Plus was used. The mobile phase was 0.005M sulphuric acid (acidified water). The pump flow rate was set at 0.3 mℓ/min and the column temperature at 65 °C. Standards of glucose, citric acid and glycerol were prepared with concentrations ranging from 0.0 to 1.0 g/ℓ to plot the respective standard curves.

Optimisation of γ-PGA extraction and spectrophotometric quantification

Following on from the work in section BBBBBBBBBBBBBBBBBBBBBBBBBBBBBB.43.7228096, γ - PGA extraction and spectrophotometric quantification, further optimisation of this method was required due to the fluctuation in the pH values of the deionised water. Since this method is highly pH sensitive (Zeng, et al., 2012), it was increasingly difficult to produce an accurate standard curve that was standardised and gave accurate repeatability of sample analysis.

The use of a sodium phosphate buffer at pH7 was proposed. The buffer was made up and the pH checked. A stock solution of 1 g/ℓ was made up in the buffer and triplicate serial dilutions made from 0.04 to 0.2 g/ℓ. The standards were then filtered using a 0.22 µm syringe filter and scanned in the UV range from 190 nm to 250 nm. Figure: D-12 shows the full scan of the standard dilutions from a range of 190 to 250 nm, with a maximum peak absorption at 204 nm (indicated by the dashed vertical line).

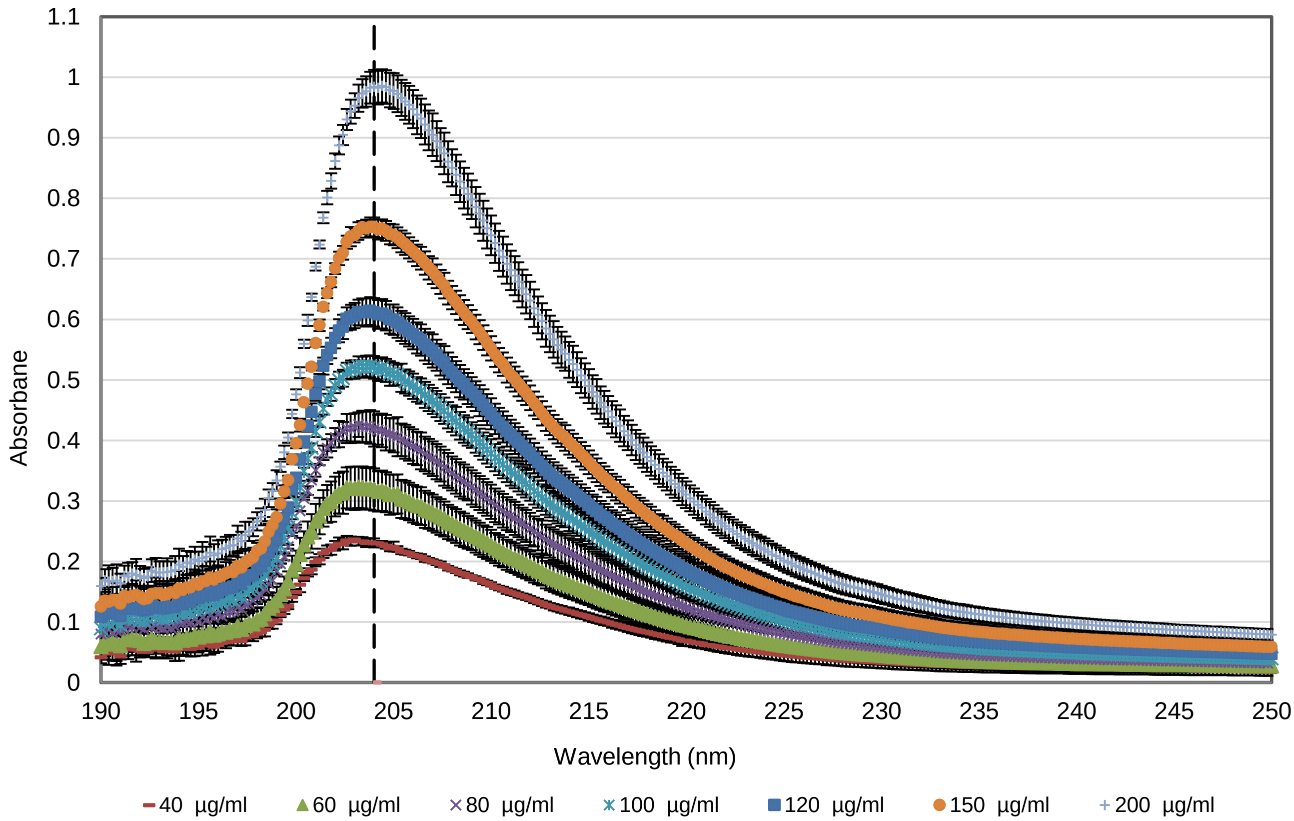


Figure: D-12: Standard PGA UV Scans in phosphate buffer at varying concentrations. The vertical dashed line indicates the maximum absorbance at 204 nm

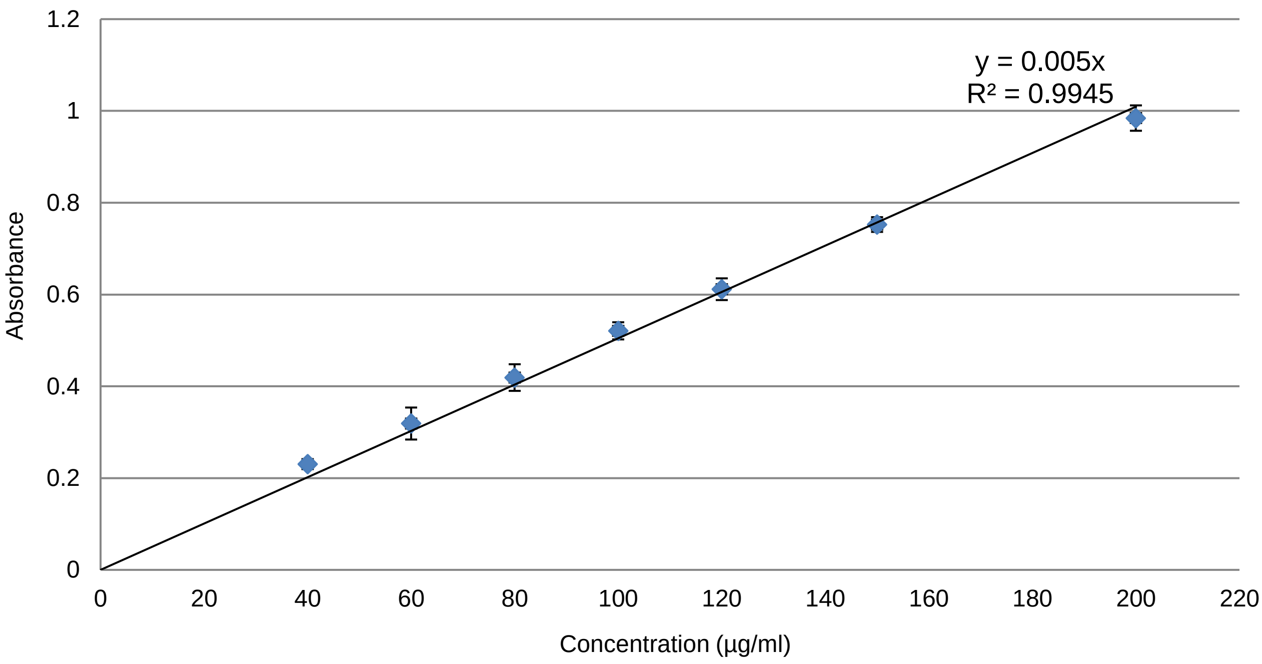


Figure: D-13: Standard curve of PGA scans at a maximum absorbance of 204 nm

The absorbance of each standard dilution at 204 nm was then plotted to yield a standard curve in Figure: D-13. The use of a pH 7 buffer was validated by scanning samples form the batch fermentation to ensure a maximum absorbance at 204 nm. The figure below illustrates the scans from three samples of the first batch reactor and the 0.2 g/l of γ-PGA standard. The peak of absorbance is at 204 nm.

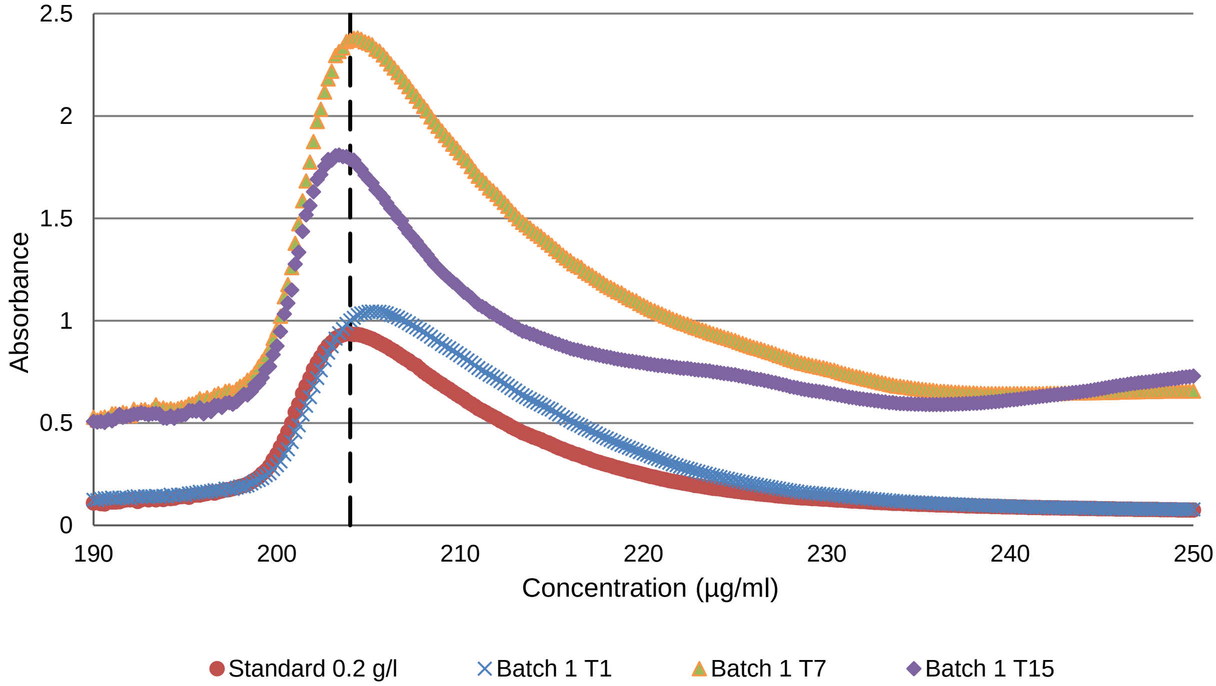


Figure: D-14: Graphical comparison of the UV scans of samples in batch reactor 1 and a standard of PGA of 0.2 g/ℓ, showing the maximum absorption at 204 nm

D.4 Base Case results

Two batch fermentations were set up at a 5 litre working volume in New Brunswick CSTRs, using half strength MME. They were operated at 37 °C and 200 rpm. Sampling took place at three hour intervals. The culture began to enter exponential phase at 8 hours for the batch reactors. The pH was externally controlled with 5M NaOH and 5M H_2SO_4. The growth data is summarised in Figure: D-15 and Figure: D-16.

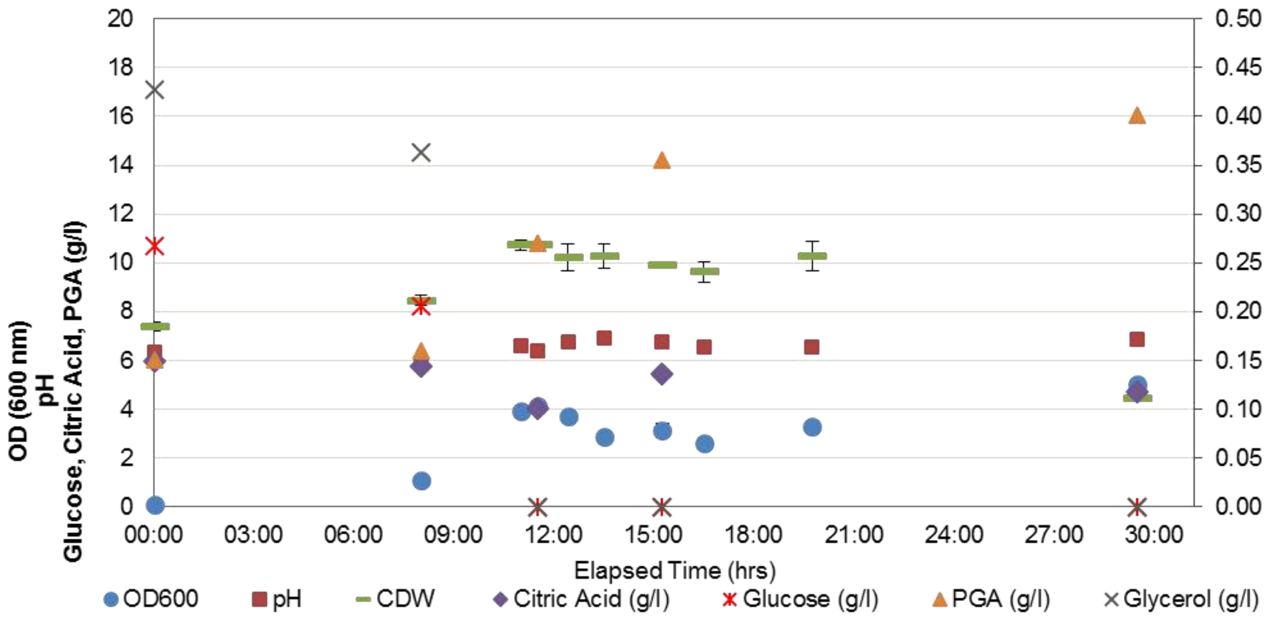


Figure: D-15: Graphical summary of growth data, substrate utilisation and PGA production in Batch Reactor 1

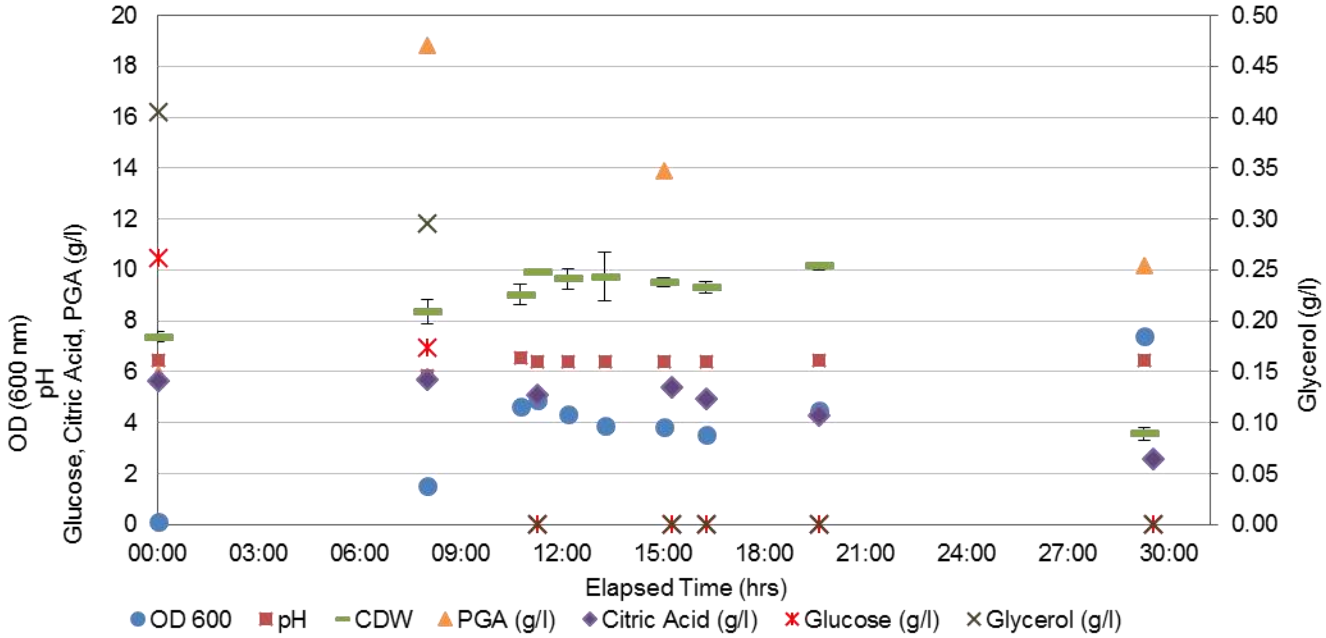


Figure: D-16: Graphical summary of growth data, substrate utilisation and PGA production in Batch Reactor 2

Batch Reactor 1 and 2 showed similar trends in their growth and substrate utilisation rates with glucose and glycerol being depleted at around 12 hours. Minor differences could be expected as slightly larger impellers were used in Batch Reactor 2. The results after 30 hours are reported in Table: D-3.

Table: D-3: Summary of yield coefficients, biokinetic constants and concentrations achieved after 30 hours

	Batch Reactor 1	**Batch Reactor 2**
OD600 after 30 hours	5.01 ± 0.08	7.38 ± 0.02
CDW (g/l) after 30 hours	4.40 ± 0.12	3.57 ± 0.25
PGA production (g/l) after 30 hours	16.04 ± 0.30	10.18 ± 0.20
μmax (hrs-1)	0.111	0.113
Yx/ Max	0.27	0.26
Yp/s Max	0.94	1.23

D.5 Recommendations for further study

Due to the fast consumption of the substrate, it is recommended that the batch reactor study be shortened to just over 12 hours, once the glucose and glycerol has been consumed. It will also be valuable to repeat the batch reactor and inoculate it early in the morning, as opposed to late at night. This will ensure that more data is collected during the exponential growth phase than the stationary phase.

Thereafter, the batch scenario should be turned into an exponential fed batch system at an appropriate feed rate to ensure there is no glucose starved scenarios. The fed batch data will provide valuable insight into the behaviour of this organism and γ-PGA production under semi-continuous conditions, in preparation for the continuous reactor work.

E PRELIMINARY EXPERIMENTAL EVALUATION OF SELECTED BACTERIAL BIOREACTORS

Following the assessment of bioreactor types and designs in Chapter 6 an experimental study began for the further evaluation of the specific design parameters for each of the selected bioreactor types. Ultimately a comparison of the performance of the three types of bioreactor will be conducted with respect to the production of PGA. This study will then inform the final choice of bioreactor type and design parameters for the bacterial bioreactor train in an experimental integrated WWBR system.

E.1 Bioreactor Design and Commissioning

The three bioreactors selected as the most promising in the bioreactor review reported in Chapter 6 were the moving bed bioreactor (MBBR), the aerobic granular sludge operated as a sequencing batch reactor (AGS-SBR), and the rotating biological contactor (RBC). These systems have been designed at the 5 to 20 litre scale for construction from simplified material on a low cost basis. The first of these, the MBBR, has been constructed and commissioned; the other two bioreactors are under construction, but due to time constraints could not be commissioned during this project.

E.1.1 Moving Bed Biofilm Reactor

Description of the MBBR

The Moving Bed Biofilm Reactor (MBBR) is based on attached growth biofilm principles of biological wastewater treatment. The core of the process is the biofilm carrier elements. While the biofilm is fixed to the carrier, the media is thoroughly mixed and retained within a reactor using effluent screens. Carrier circulation within the bioreactor is provided by the aeration system (Grady, et al., 2011).

The MBBR for this investigation was designed to ensure that the results from all the reactor experiments are comparable. Since both the existing AGS Reactor and the stirred tank Chemap reactor used as a base case have a 7 litre volume, it was decided to keep volume a fixed variable. The specifications of the MBBR are given in Table: E-1 . Figure: E-1 shows the basic reactor design and dimensions of the reactor that has been constructed and commissioned. Full CAD drawings will be provided in the final report.

Table: E-1: Specifications for lab-scale MBBR

	Description
Total Volume	7 litres
Working Volume	5 litres
Container	Lid and base: 10 mm clear Plexiglas Body: 8mm clear Plexiglas
Dimensions	Height: 310 mm Base: 150 x 150 mm
Inflow	Peristaltic Pump set at appropriate flowrate to achieve desired HRTs. The influent will come through the lid of the reactor,
Outflow	Peristaltic Pump. There are three effluent ports, spaced equidistant from the base of the reactor, to the height of the liquid level at 5 litres. This will allow for change in liquid heights as sampling occurs, and should a different working volume be needed. Meshing will cover the outlets to prevent biomass and media carriers from exiting the reactor.

	Description
Aeration	A 'grill' shaped sparger (shown in the figure below) is being constructed from ¼" stainless steel piping with a bend radius of 31 mm that will be placed horizontally in the base of the reactor. 1mm holes with a 10 mm pitch will be drilled along the length of the piping. Mass transfer studies will be performed to ensure that this aeration design provides sufficient mixing and dissolved oxygen to the system.
Biofilm carriers	Literature shows that the use of polyethylene carriers with a density of 0.95 to 0.99 g/cm^3 and height inner surface area (~ 300 m^2/m^3) are ideal, since they become neutrally buoyant once covered in biomass (WEF, 2010; Wang, et al., 2005; Grady, et al., 2011). MBBR carriers were purchased from Ecotao, a company in Kwa-Zulu Natal. They have a surface area of 600 m^2/m^3 with 6 internal spaces.

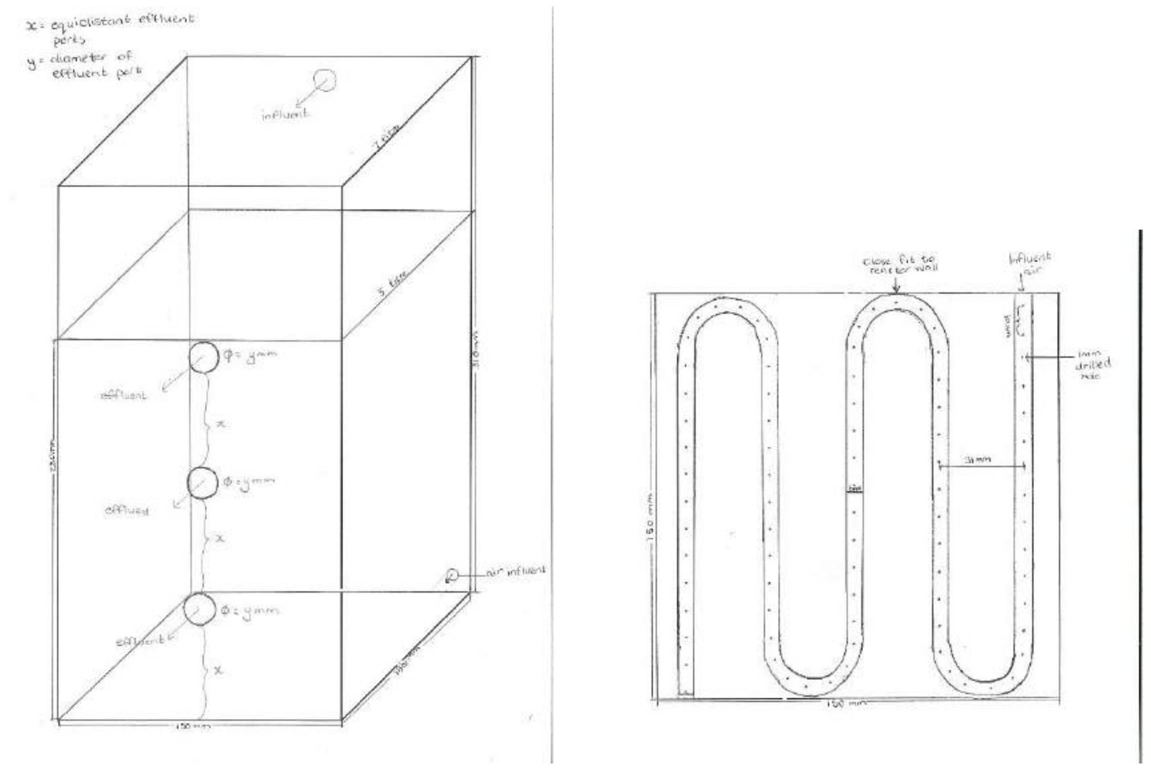


Figure: E-1: Preliminary construction sketches of lab-scale MBBR

Assembly of the MBBR

The MBBR has been constructed, as shown in the photographs in Figure: E-2, Figure: E-3 and Figure: E-4. The reactor was commissioned in November 2015 for preliminary experiments to ensure colonisation of the carriers. Since the initial comissioning of the reactor, modifications have been made to the reactor. These include housing for the pH and DO probes in the lid of the reactor, as well as a flap onto the lid to allow for sampling of carriers. Data logging will be done through an analogue transmitter to a computer, and will include the pH, DO and temperature of the system at chosen time intervals.The materials used to construct the reactor are listed in Table: E-2.

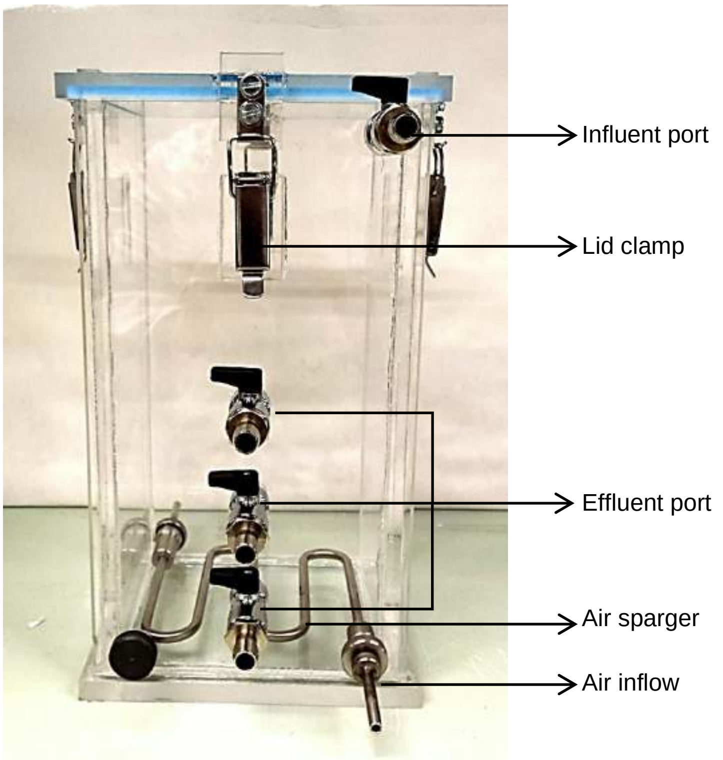


Figure: E-2: Front view of MBBR

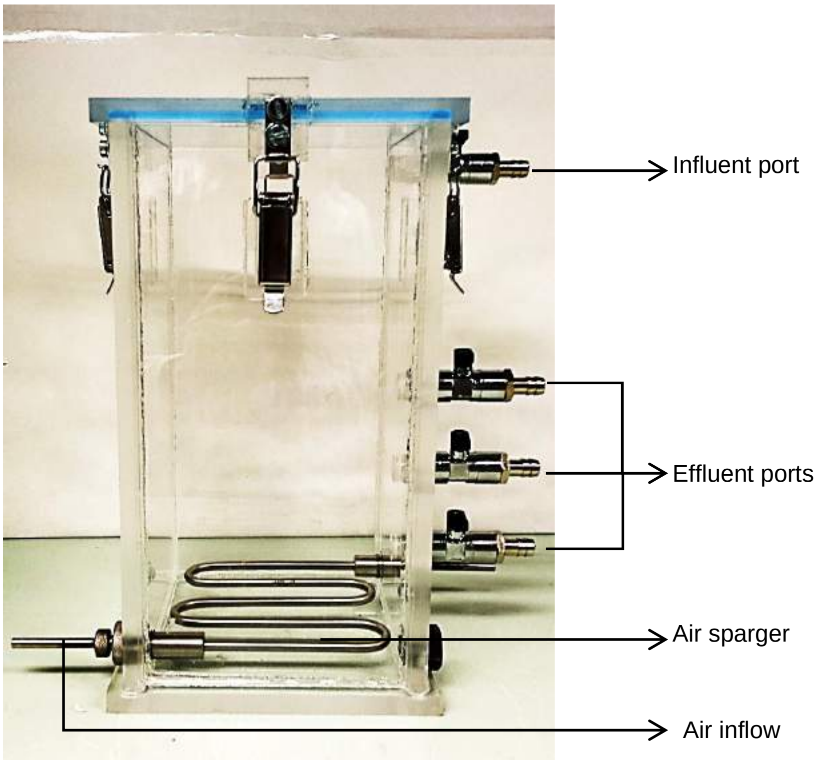


Figure: E-3: Left side view of MBBR

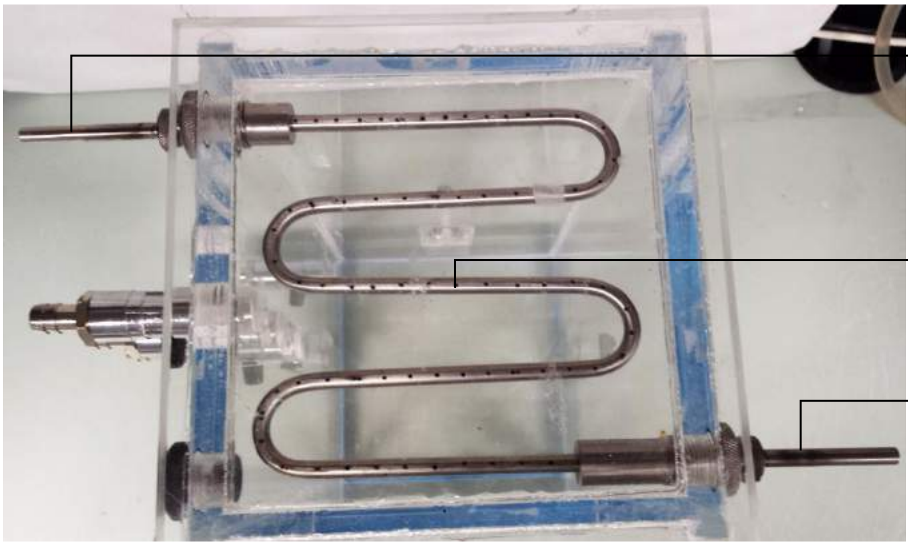


Figure: E-4: Bottom view of MBBR. This figure shows the design of the aeration as shown in Figure: E-1

Table: E-2: List of materials used to construct MBBR

Part	Material	Source
Reactor Shell	Plexiglass, clear Base and lid: 10 mm thickness Walls 8 mm	Maizey
Air sparger	¼ " stainless steel pipe	Lab stock
Ports	Stainless steel	Gripper and Co

E.1.2 Aerobic Granular Sludge in an SBR

Description of AGS bioreactor

The AGS reactor is a sequencing batch reactor, operated with filling, mixing, aeration, settling, extraction and idling phases. However it can be operated as a simulated continuous-flow activated sludge process. The different stages allow for the formation of the aerobic granules that settle rapidly, potential product formation and wastewater treatment.

For the purposes of this experiment, the same Sequential Batch Reactor that was constructed and used in Verster et al. (2013), is used with minor modifications described in Table: E-3.

Assembly of AGS bioreactor

The specifications of the AGS is given in Table: E-3, with modifications. Figure: E-5 shows the AGS reactor from Verster et al. (2013) with modifications for this investigation.

Table: E-3: Specifications for lab-scale AGS reactor

	Current Setup	Modifications
Total Volume	7 litres	None
Working Volume	5 litres	None
Container	Acrylic 100 mm inner diameter tube, Perspex covers machined in UCT Chemical Engineering Workshop	None
H/D Ratio	Between 5 and 7	None
Inflow	Fish tank pump to pump media from a storage container	Peristaltic pump set at flowrate to achieve desired HRTs

	Current Setup	Modifications
Outflow	GSR Force pilot operated solenoid valve 1/2 " 220 VAC Brass GSR D43231001. With no current it is closed, opening when current is passed through. Outflow limited through a narrow aperture.	Peristaltic pump with the outflow limited though a ball valve. Sample ports will also be installed in the side of the reactor at varying heights.
Aeration	Fish tank aerator coupled to a sparger, inserted into the base of the reactor	A circular sparger made from ¼" stainless steel tubing, will be placed onto the inside of the reactor base to help improve aeration and mixing issues experienced in the previous setup.

Figure: E-5: The laboratory scale AGS setup to be used, with modifications.

E.1.3 Rotating Biological Contactor

Description of the RBC

The RBC is a type of static biofilm reactor using attached growth. It typically includes a trough shaped vessel with closely packed circular disks, spaced evenly along a motor driven shaft. The disks are submerged typically to 40% and are rotated slowly. Literature shows that the speed of rotation can vary from 1 to 20 rpm (Chavan & Mukherji, 2010; Grady, et al., 2011; Malandra, et al., 2003). The medium flows perpendicular to the shaft. (WEF, 2010)

As discussed with respect to the design of the MBBR (Section E.1.1), it was decided to keep volume a fixed variable. Therefore the RBC will also be designed to have a total volume of 7 litres, with a working volume of 5 litres.

Traditionally, the RBC is an open system, only occasionally requiring covers (Grady, et al., 2011). The laboratory scale reactor is designed as a closed reactor to minimise exposure of the microorganism to the atmosphere of the laboratory. *Bacillus* is a sporulating microorganism (Madonsela, 2013) and a potential contaminant, thus extra care is required to ensure the design is a closed system to prevent contaminants and nuisance organisms while mimicking the function of an open system.

The reactor design specifications are provided in Table: E-4 and Figure: E-6, showing the dimensions of the troughs and a preliminary design. The motor and shaft system are designed as a fixed unit. To improve the flexibility of the experiments, variable submergence is a design requirement, implying that the rotating shaft needs to be movable. Construction will commence on finalisation of the design.

Assembly of the RBC

Table: E-4: Specifications for lab-scale RBC

	Description
Total Volume	7 litres
Working Volume	5 litres
Container	Rectangular shaped, with triangular prisms inserted into the base of the vessel to reduce the dead space.
Dimensions	To be confirmed once a finalised closed design is obtained
Inflow	Peristaltic Pump set at appropriate flowrate to achieve desired HRTs.
Outflow	Peristaltic Pump.
Aeration	This is naturally achieved through the rotation of the shafts. Mass Transfer studies will need to be performed to ensure that sufficient mass transfer is achieved. Since the laboratory setup will need to be a closed system, air will be flushed into the headspace.
Circular Disks	The material used for these disks varies. High-density polyethylene and polystyrene are two examples of materials used. Polystyrene foam disks were used by Tawfik, et al. (2001), Costley & Wallis (2001), and Malandra, et al. (2003). Acrylic disks were used by Chavan & Mukherji (2010).
Variable Motor	One of the threats outlined is that the shaft system is susceptible to failure due to an inadequate shaft-motor design. To prevent this, the torque will be calculated by modelling the system as a rotating disk through a fluid, with the following equation: $$T = N\frac{4\pi\mu\Omega}{S}\int_0^R r^3\,dr$$ where μ is bulk liquid viscosity, Ω is angular velocity of the disks and S is clearance between disks and N is the number of disks and R is the disk radius. (Mourtos, 2015) By knowing the torque, a correctly specified motor can be purchased. A speed controller circuit will be built to vary the shaft speed.

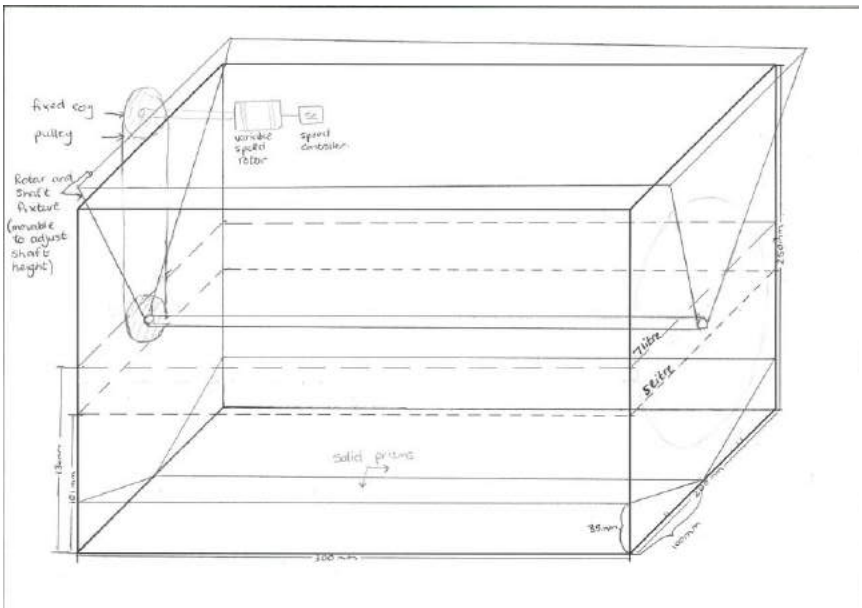


Figure: E-6: Preliminary sketch of RBC design

E.2 Initial Performance Studies for the Moving Bed Bioreactor

E.2.1 Tracer Tests and Mixing Studies

Mixing time studies were completed for the MBBR by observing the colour change using phenolphthalein indicator and monitoring the pH when acid was added as a pulse input to an alkaline solution. The tests were performed in a batch scenario, since it was assumed that continuous flow would aid the mixing and thus batch tests are sufficient as an initial test of mixing.

The reactor was filled with a 0.001 M solution of sodium hydroxide and 10 mℓ of phenolphthalein was added. Ten millilitre pulses of a 1 M HCl were added, slightly to the right of centre at the top of the reactor. The addition continued to excess to ensure neutralisation of the solution. Different air flowrates were selected, and the mixing time was determined both visually when the pink colour was removed and by recording the pH, assessing when it had reached steady state. The experiment was done both in the absence and presence of biofilm carriers. The studies were done in triplicate for each flowrate.

Table: E-5: Mixing time for different air flowrates for laboratory scale MBBR, without carriers

Air flowrate (l.min-1)	Mixing time (s)
0.1	50
0..2	35
0.5	40
1	25
5	7

Table: E-5 and Table: E-6 show that with an increase in flowrate, the mixing time decreases, as expected. For missing without carriers, a flowrate above 5 $l.min^{-1}$ was not selected due to the very fast mixing time. However with carriers, it was evident that flowrates of less than 5 ℓ.min^{-1} resulted in poor circulation of the biofilm carriers.

Table: E-6: Mixing time for different air flowrates for laboratory scale MBBR, with carriers

Air flowrate ℓ.min-1	Mixing time (s)
1	35
5	11
7	8
10	9
15	10
20	7

The photographs (Figure: E-7 and Figure: E-8) represent variable intervals when the most noticeable changes occurred, starting from zero seconds to the mixing time once pH had stabilised. The mixing patterns were similar for the various flowrates.

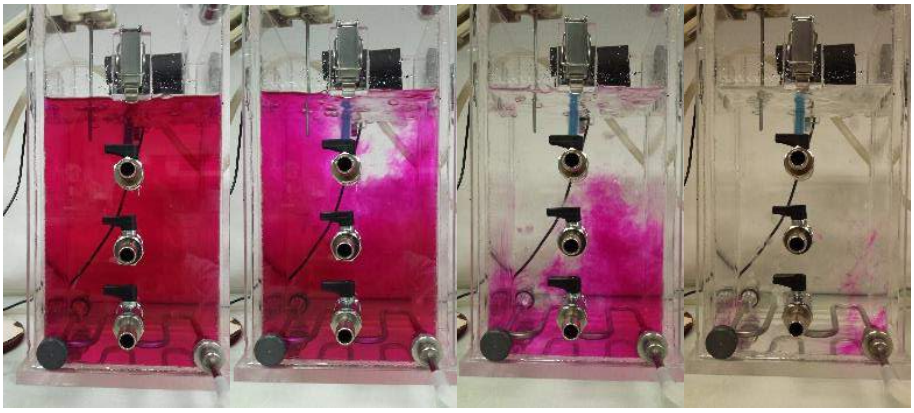


Figure: E-7: Images of phenolphthalein mixing over time for laboratory scale MBBR, without carriers

Figure: E-8: Images of phenolphthalein mixing over time for laboratory scale MBBR, with carriers

The images for mixing without carriers (Figure: E-7) show that are is a dead zone in the bottom right corner. However, with the carriers (Figure: E-8) the right hand side of the reactor became clear first and a smaller dead zone was apparent in the bottom left corner. This is likely due to the fact that two air sources needed to be used in order to achieve a high flowrate of air in the case of the carriers – splitting the air from one source to each of the inlets was inadequate. Thus, two separate rotameters are needed. It is important to note that due to the density of the carriers being less than water, they will float when there is no bacterial attachment. Thus, mixing studies with beads that are not acclimatised, will show slightly different mixing patterns. Once the carriers have bacterial attachment, they circulate well when aerated.

Before the MBBR is commissioned fully, optimisation studies will be done to find the ideal carrier fill rate and aeration flowrate to prevent high shear as well as tracer and mixing studies under continuous flow conditions at the retention times to be used in the experimental work. These mixing studies should also be conducted with carriers and without.

E.2.2 Initial commissioning of MBBR

The reactor drainage batch and fed batch operations was used to colonise the biofilm carriers.

The fresh biofilm carriers were placed in 5 ℓ Erlenmeyer flasks, along with the reactor drainage and stirred at a slow rate to encourage attachment. The media was refreshed and the flasks inoculated with fresh cells every 2 weeks approximately.

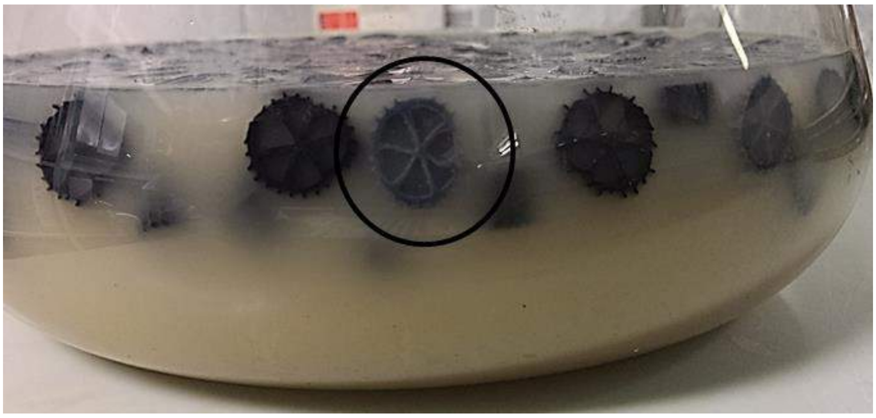


Figure: E-9: Colonisation on the biofilm carriers after approximately 6 weeks in 5L Erlenmeyer flasks

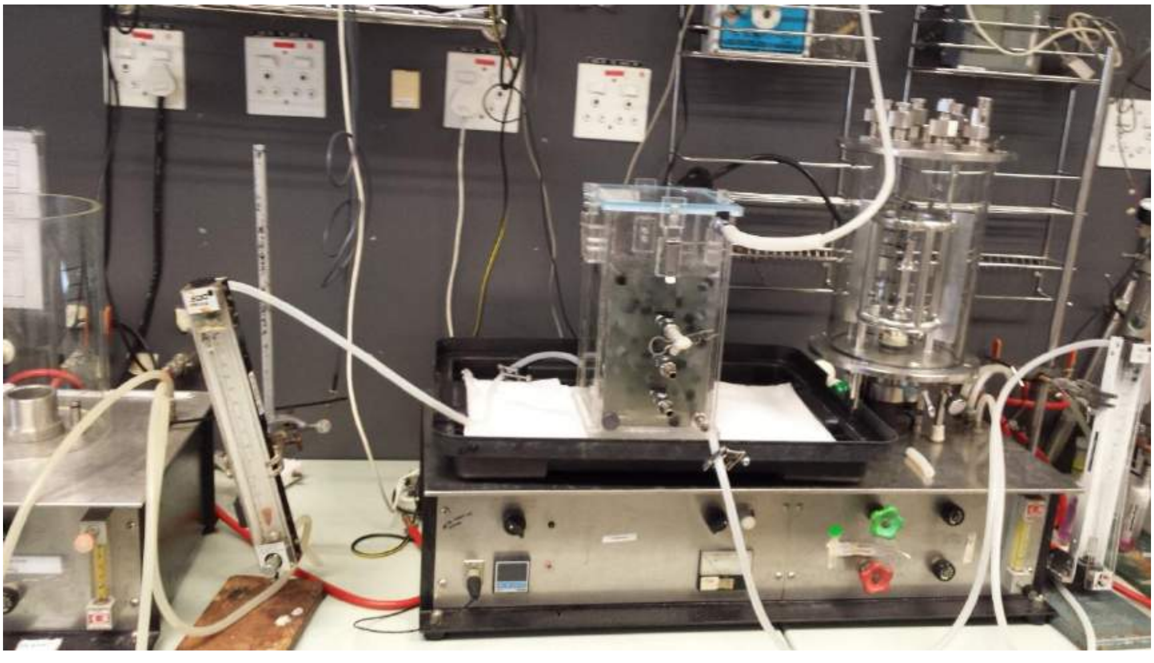
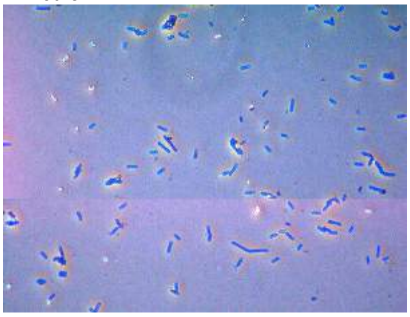
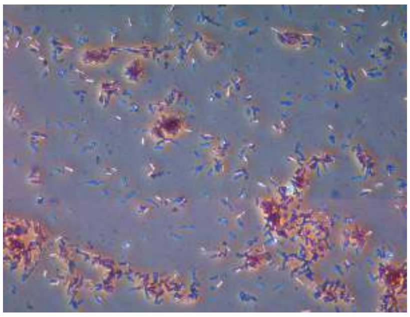


Figure: E-10: Experimental setup of MBBR for preliminary experiments

The MBBR was commissioned and inoculated to obtain preliminary results and further colonise the beads in the reactor. The experimental setup is shown in Figure: E-10. Following inoculation, two samples per day were taken as *Bacillus* grows more slowly at room temperature (Madonsela, 2013). Over the first 24 hours, the OD_{600} increased from 0.894 to 1.066. Foaming was observed in the reactor on the second day. Table: E-7 shows the preliminary results from the first three data points obtained, for OD, pH, PGA production, glucose utilisation by DNS assay and gram stain.

Table: E-7: Preliminary results from first run in MBBR

	OD	**pH**	**Temperature (°C)**	**γ-PGA (g/ℓ)**	**Glucose (g/ℓ)**
0 hours	0.88 ± 0.07	6.44 ± 0.38	25	6.28 +-0.11	15.3 +- 0.025
24 hours 	1.08 ± 0.02	6.41 ± 0.01	22.9	6.77 +-0.1	10.35 +- 0.55
30 hours 	1.696 ± 0.02	5.99	22	5.36 +- 0.06	7.43 +- 0.31

From Table: E-7 it is evident that there is γ-PGA is present in the culture broth. However, no trend can be observed as only three sample points were taken. There is some indication that metabolism of PGA may occur during active exponential growth in balanced media. Further investigation will be required. The decrease in glucose is expected, as the bacteria started growing exponentially when the foaming was observed and a drop in pH. Conclusions and recommendations will be presented once more data is available.

E.3 Current Experimental Work

Two problems became apparent during the first experimental work on the MBBR. The first was that the carriers were inadequate. This has been addressed and work has begun with improved carriers (see Section E.3.1). The second was the need for a laboratory space where experiments could be performed without concern for contamination of other experiments (a "dirty lab"). This is currently being addressed with the commissioning of a new laboratory, "The Water Quality Laboratory", as part of the ongoing enhancement of UCT's Chemical Engineering and Civil Engineering Departments involvement in wastewater remediation research (see SectionE.4).

E.3.1 Acquiring new carriers

Following the initial commissioning of the MBBR, it was determined that the carriers that were obtained were of an inferior quality and showed poor attachment. Two carriers with high surface areas and widely used in biological WWT applications globally, were then selected. These were AnoxKaldnes carriers from Veolia, specifically K3 and BiofilmChip^{TM} P as shown in Figure: E-11 and Table: E-8. These are specifically designed for large scale moving bed reactors.

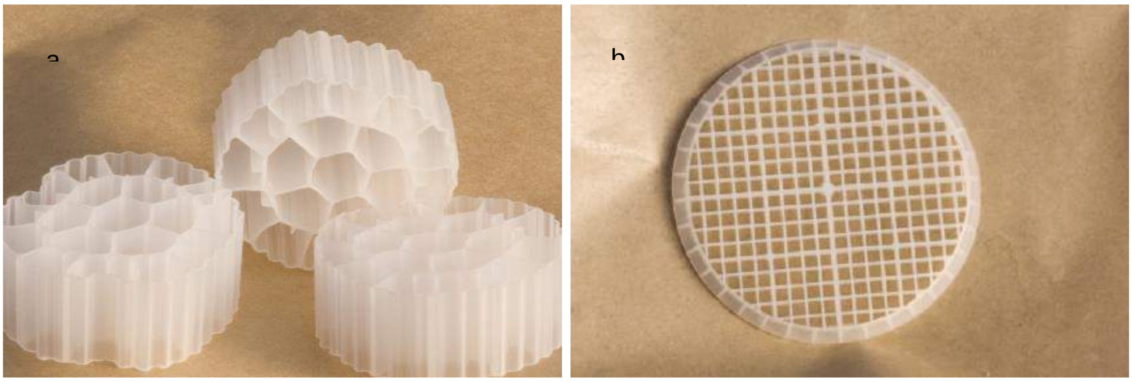


Figure: E-11: Close up images of the AnoxKaldnes carriers (a) K3 carriers (b) BiofilmChipTM P carriers

Table: E-8: Properties of the selected carriers AnoxKaldnes K3 and BiofilmChip^{TM} P

	K3 Kaldnes	**BiofilmChipTM P**
Material	High Density Polyethylene	High-density polyethylene or polypropylene
Surface Area (m^2/m^3)	500 ± 1 %	900 ± 1 %
Density (kg/dm^3)	0.95 ± 0.02 %	0.96 – 1.02 ± 0.1 %
Dimensions	Width: 10 mm Diameter: 25 mm	Width: 3 mm Diameter: 45 mm

E.3.2 Acclimatisation of K3 carriers

The acclimatisation of the K3 carriers was commissioned in early February soon after the shipment arrived. An airtight 6 litre round bottomed flask was used, with a fine bubble air curtain for aeration.

Figure: E-12: (L) the fine bubble air curtain used in the round bottom flask for carrier acclimatisation (R) setup showing the syringe sampling and effluent drainage

To start the acclimatisation, the flask was filled with 3 litres of medium and an inoculum of *Bacillus subtilis.* The system was fed daily with 1.5 litres of half strength MME (see Appendix A for composition) and the pH is maintained between 6.5 and 7.0 with 1M H_2SO_4 as this is the ideal growth condition for this organism (Madonsela, 2013). Gram staining and microscope images were taken every few days to monitor the growth, sterility and system changes.

After the carriers had been acclimatising for four weeks, SEM was done to check for bacterial attachment. The results showed thick attachment of rod-shaped bacteria, consistent with *B. subtilis.*

Figure: E-13: Acclimatising vessel after three weeks

Standard protocol for the preparation of the samples for SEM was followed. The carriers were fixed by placing them in 2.5 % glutaraldehyde in the fridge at 4 °C for 8 hours. They were then rinsed with buffer and then distilled water. Thereafter the dehydration process was done, involving soaking the carriers in serially increasing concentrations of ethanol, consisting of 30 %, 50 %, 70 %, 90 %, 95 % and 100 %, for 10 minutes at each concentration. A small section of the carrier was then cut out and mounted onto a stub with carbon glue, dried with HMDS and sputter coated with gold palladium alloy. Figure E-14 below shows the carriers after the alcohol dehydration steps

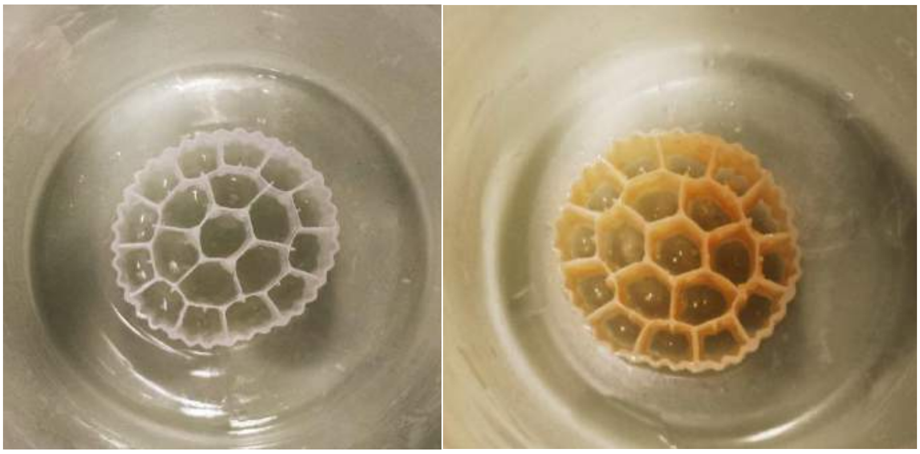


Figure: E-14: (L) Control: carrier with no attachment (R) Acclimatised carrier: after acclimatisation lasting 4 weeks

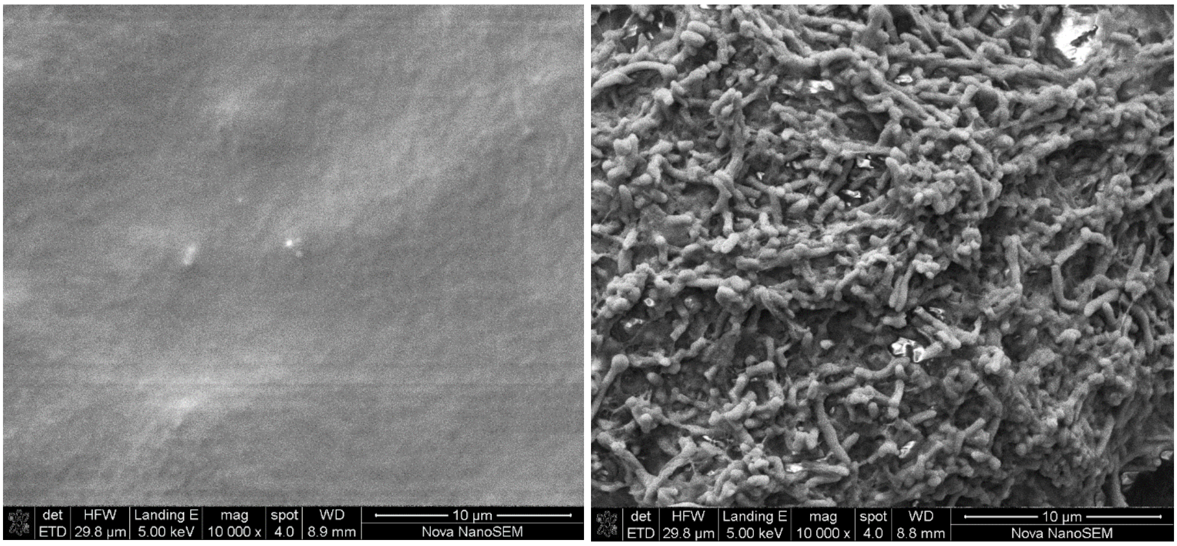


Figure: E-15: SEM images, magnification 10 000 x: (L) control carrier with no attachment (R) carrier with thick bacterial attachment of rod shaped bacteria

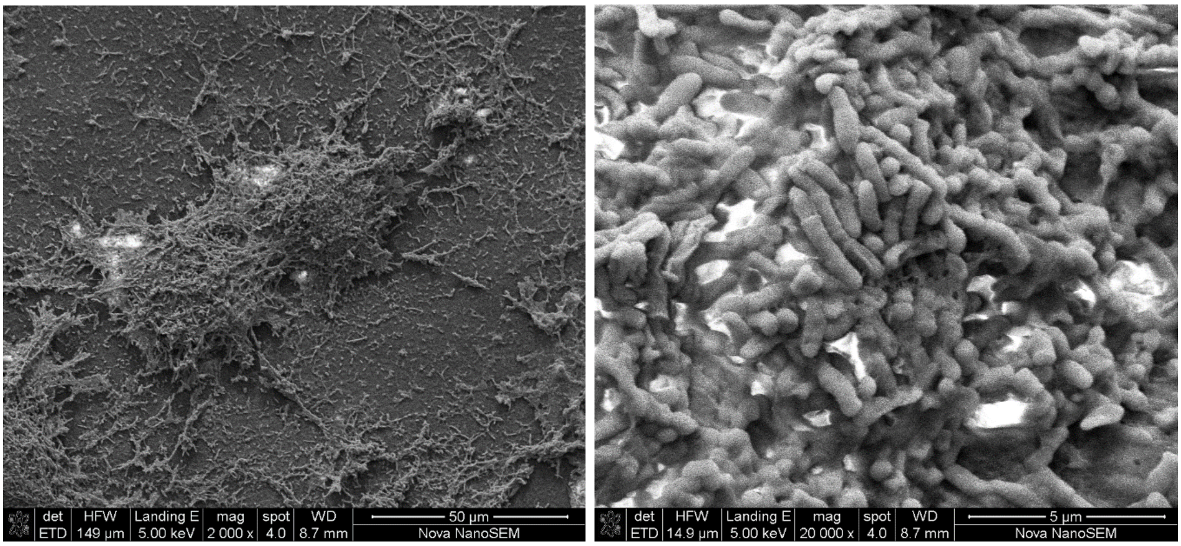


Figure: E-16: SEM images: (L) bacterial attachment, magnification 2 000 x (R) bacterial attachment, magnification 20 000 x, showing individual cells

Currently, this system is currently being maintained with daily feeding until the suitable laboratory space is ready and the MBBR can be fully commissioned.

E.4 Ongoing Experimental Work

Due to the sporulating nature of *Bacillus subtilis*, the commissioning of the reactors in the CeBER labs is not possible as it poses a high risk of contamination of ongoing pure culture work. The MBBR and AGS reactor, were not able to be designed to be fully airtight while still being able to sample adequately thus the need for a laboratory space where air contamination is not a problem. The new Water Quality Laboratory in the New Engineering Building has been identified as the appropriate space to run the future experiments. Unfortunately health and safety and infrastructure delays have prevented the commissioning of the reactors in the first half of 2016. However it will be possible to commission the bioreactors soon.

The experimental work in the MBBR will entail varying the retention times from just below the μ_{max} to a minimum value, at half strength MME. This will be repeated for half strength medium and a lower dilution. Repeatability will be tested by returning the system to lower and higher retention times and monitoring the steady state conditions.

The analyses of the system will involve the pH, OD_{600} and CDW of the planktonic and carrier cells as immediate tests. The substrate utilisation will be analysed for glucose, glycerol and citric acid by HPLC and the γ-PGA production will be monitored with the UV-Scan method developed in Appendix D. The protein and carbohydrate composition of the extracted γ-PGA will also be analysed with Bradford's Assay and Phenol-Sulphuric Acid Assay respectively.

Carriers will be continuously acclimatised alongside the reactor to replace those removed for sampling. SEM will also be done on the carriers to monitor the attachment over time.

F SUPPLEMENTARY MASS BALANCE TABLES FOR BIOREACTOR TRAINS

F.1 Nitrogen and phosphorus mass balances

Table: F-1: Relative weight fractions of nitrogen and phosphorus normalised to carbon for various stream components

Conversion description	Unit	Symbol of factor
Relative Mass fraction of Nitrogen (normalised to Carbon) for Biomass:		
N/C for Bacterial Biomass	Mass % N / Mass % C	$F(X_{Bact})_{N/C}$
N/C for Algal Biomass	Mass % N / Mass % C	$F(X_{Algal})_{N/C}$
N/C for Macrophyte Biomass	Mass % N / Mass % C	$F(X_{Mac})_{N/C}$
Relative Mass fraction of Phosphorous (normalised to Carbon) for Biomass:		
P/C for Bacterial Biomass	Mass % P / Mass % C	$F(X_{Bact})_{P/C}$
P/C for Algal Biomass	Mass % P / Mass % C	$F(X_{Algal})_{P/C}$
P/C for Macrophyte Biomass	Mass % P / Mass % C	$F(X_{Mac})_{P/C}$
Relative Mass fraction of Nitrogen (normalised to Carbon) for Products:		
N/C for Product V1	Mass % N / Mass % C	$F(V1)_{N/C}$
N/C for Product W1	Mass % N / Mass % C	$F(W1)_{N/C}$
N/C for Product X1 and Product X2	Mass % N / Mass % C	$F(X1)_{N/C}$
N/C for Product X3	Mass % N / Mass % C	$F(X3)_{N/C}$
N/C for Product Y1	Mass % N / Mass % C	$F(Y1)_{N/C}$
N/C for Product Y2	Mass % N / Mass % C	$F(Y2)_{N/C}$
N/C for Product Y3	Mass % N / Mass % C	$F(Y3)_{N/C}$
Relative Mass fraction of Phosphorous (normalised to Carbon) for Products:		
P/C for Product V1	Mass % P / Mass % C	$F(V1)_{P/C}$
P/C for Product W1	Mass % P / Mass % C	$F(W1)_{P/C}$
P/C for Product X1 and X2	Mass % P / Mass % C	$F(X1)_{P/C}$
P/C for Product X3	Mass % P / Mass % C	$F(X3)_{P/C}$
P/C for Product Y1	Mass % P / Mass % C	$F(Y1)_{P/C}$
P/C for Product Y2	Mass % P / Mass % C	$F(Y2)_{P/C}$
P/C for Product Y3	Mass % P / Mass % C	$F(Y3)_{P/C}$

Notes:
Solids reactor biomass all goes to Product Y1 and Product Y3
Product W2 does not have a N/C or P/C conversion as algal oil does not contain N or P
Product W3 is Algal biomass
Product Y4 is Compost and does not have a specified CNP ratio

F.2 Mass Balance for Algal Bioreactor

The algal bioreactor train flowsheet can be found in Section 7.5.1, with descriptions of units and related overall mass balance equations presented and the streams described.

Table: F-2: Mass balance for Unit 2.0 Mixing Tank: Algal Bioreactor inflow

Carbon, Nitrogen, Phosphorous and Water Mass Balance: Unit 2.0: Mixing Tank				
Fraction	**D1: Improved Compliance Effluent**	**D2: Settled Wastewater** BYPASS	**D3-5 Supplement Streams**	**D: Inflow to Algal Bioreactor**
Total Carbon	$N_{C(D1)} =$ $X_{C(D1)} + P_{V1,C(D1)} + P_{VFA,C(D1)} + IN_{C(D1)}$	$N_{C(D2)} = N_{C(A)} * (1 - r_{B1})$	$N_{C(D3-5)} = Q_{(D3)} * C_{C(D3)} + Q_{(D4)} * C_{C(D4)} + Q_{(D5)} * C_{C(D5)}$	$N_{C(D)} = N_{C(D1)} + N_{C(D2)} + N_{C(D3-5)}$
Total Nitrogen	$N_{N(D1)} =$ $X_{N(D1)} + P_{V1,N(D1)} + P_{VFA,N(D1)} + IN_{N(D1)}$	$N_{N(D2)} = N_{N(A)} * (1 - r_{B1})$	$N_{N(B2-4)} = Q_{(B2)} * C_{N(B2)} + Q_{(B3)} * C_{N(B4)} + Q_{(B5)} * C_{N(B5)}$	$N_{N(D)} = N_{N(D1)} + N_{N(D2)} + N_{N(D3-5)}$
Total Phosphorous	$N_{P(D1)} =$ $X_{P(D1)} + P_{V1,P(D1)} + P_{VFA,P(D1)} + IN_{P(D1)}$	$N_{P(D2)} = N_{P(A)} * (1 - r_{B1})$	$N_{P(B2-4)} = Q_{(B2)} * C_{P(B2)} + Q_{(B3)} * C_{P(B4)} + Q_{(B5)} * C_{P(B5)}$	$N_{P(D)} = N_{P(D1)} + N_{P(D2)} + N_{P(D3-5)}$
Total Water	$N_{W(D1)} = N_{W(C1)} - N_{W(C2)}$	$N_{W(D2)} = N_{W(A)} * (1 - r_{B1})$	$N_{W(D3-5)} = N_{W(D3)} + N_{W(D4)} + N_{W(D5)}$	$N_{W(D)} = N_{W(D1)} + N_{W(D2)} + N_{W(D3-5)}$
Checks: Total stream amounts: $(N_{C(D1)} + N_{C(D2)} + N_{C(D3-5)}) - (N_{C(D)}) = 0$ $(N_{N(D1)} + N_{N(D2)} + N_{N(D3-5)}) - (N_{N(D)}) = 0$ $(N_{P(D1)} + N_{P(D2)} + N_{P(D3-5)}) - (N_{P(D)}) = 0$ $(N_{W(D1)} + N_{W(D2)} + N_{W(D3-5)}) - (N_{W(D)}) = 0$ The Substrate Streams D3, D4 and D5 are assumed to have negligible solids components.				

Table: F-3: Mass balance for Unit 2.1 Algal Bioreactor

Carbon Mass Balance: Unit 1.1: Algal Bioreactor				
Carbon Fraction	**D: Inflow to Algal Bioreactor**	**E1: Algal Broth**	**E5: CO_2**	**E6: H_2O**
Biomass X_{Algal} (including P_{W3})		$X_{C(E1)} = N_{C(D)} * Y_{XAlgal/C}$		
Product P_{W1}		$P_{W1,C(E1)} = N_{C(D)} * Y_{P,W1/C}$		
Product P_{W2}		$P_{W2,C(E1)} = N_{C(D)} * Y_{P,W2/C}$		
Carbon Dioxide CO_{2Algal}			$CO_{2C,Algal(E5)} = N_{C(D)} * Y_{CO2Algall/C}$	
Unconverted Carbon	$IN_{C(D)} = N_{C(D)} = N_{C(D1)} + N_{C(D2)} + N_{C(D3-5)}$	$IN_{C(E1)} = N_{C(D)} * (1- (Y_{XAlgal/C} + Y_{P,W1/C} + Y_{P,W2/C} + Y_{CO2Algal/C}))$		
Totals	$N_{C(D)} = IN_{C(D)}$	$N_{C(E1)} = X_{C(E1)} + P_{W1,C(E1)} + P_{W2,C(E1)} + IN_{C(E1)}$	$N_{C(E5)} = CO_{2Algal(E5)}$	$N_{C(E6)} = 0$
Checks: Total stream amounts: $(N_{C(D)} + N_{C(E5)} + N_{C(E6)}) - (N_{C(E1)}) = 0$				

Nitrogen Mass Balance: Unit 2.1: Algal Bioreactor				
Nitrogen Fraction	**D: Inflow to Algal Bioreactor**	**E1: Algal Broth**	**E5: CO_2**	**E6: H_2O**
Biomass X_{Algal} (including P_{W3})		$X_{N(E1)} = X_{C(E1)} * f(X_{Alg})_{N/C}$		
Product P_{W1}		$P_{W1,N(E1)} = P_{W1,C(E1)} * f(W1)_{N/C}$		
Product P_{W2}		0		
0Unconverted Nitrogen	$IN_{N(D)} = N_{N(D)} = N_{N(D1)} + N_{N(D2)} + N_{(D3-5)}$	$IN_{N(E1)} = IN_{N(D)} - X_{N(E1)} – P_{W1,N(E1)}$		
Totals	$N_{N(D)} = IN_{N(D)}$	$N_{N(E1)} = X_{N(E1)} + P_{W1,N(E1)} + IN_{N(C1)}$	$N_{N(E5)} = 0$	$N_{N(E6)} = 0$
Checks: Total stream amounts: $N_{N(D)} - N_{N(C1)} = 0$ Product W2 is Algal oil and contains no N or P.				

Phosphorous Mass Balance: Unit 2.1: Algal Bioreactor				
Phosphorous Fraction	**D: Inflow to Algal Bioreactor**	**E1: Algal Broth**	**E5: CO_2**	**E6: H_2O**
Biomass X_{Algal} (including P_{W3})		$X_{P(E1)} = X_{C(E1)} * f(X_{Alg})_{P/C}$		
Product P_{W1}		$P_{W1,P(E1)} = P_{W1,C(E1)} * f(W1)_{P/C}$		
Product P_{W2}		0		
Unconverted Phosphorous	$IN_{P(D)} = N_{P(D)} = N_{P(D1)} + N_{P(D2)} + N_{P(D3-5)}$	$IN_{P(E1)} = IN_{P(D)} - X_{P(E1)} – P_{W1,P(E1)}$		
Totals	$N_{P(D)} = IN_{P(D)}$	$N_{P(E1)} = X_{P(E1)} + P_{W1,P(E1)} + IN_{P(E1)}$	$N_{P(E5)} = 0$	$N_{P(E6)} = 0$
Checks: Total stream amounts: $(N_{P(B)} + N_{P(C4)}) – (N_{P(C1)}) = 0$ Product W2 is Algal oil and contains no N or P.				

Water Mass Balance: Unit 2.1: Algal Bioreactor				
	D: Inflow to Algal Bioreactor	**E1: Algal Broth**	**E5: CO_2**	**E6: H_2O**
Total Water	$N_{W(D)}$	$N_{W(E1)} = N_{W(D)} + N_{W(E6)}$		$N_{W(E6)} = N_{W(D)}*(F_{precip} - F_{evap})$
$(N_{W(D)} + N_{W(E6)}) – (N_{W(E1)}) = 0$				

Table: F-4: Mass balance for Unit 2.2 Separator: algal biomass & algal products from almost compliant effluent

Carbon Mass Balance: Unit 2.2: Separator			
Carbon Fraction	**E1: Algal Broth outflow**	**E2: Biomass & Product**	**F1: Almost Compliant Effluent**
Biomass X_{Algal} (including P_{W3})	$X_{C(E1)} = N_{C(D)} * Y_{XAlgal/C}$	$X_{C(E2)} = X_{C(E1)} * \text{eff}_{E2}$	$X_{C(F1)} = X_{C(E1)} * (1 - \text{eff}_{E2})$
Product P_{W1}	$P_{W1,C(E1)} = N_{C(D)} * Y_{P,W1/C}$	$P_{W1,C(E2)} = P_{W1,C(E1)} * \text{eff}_{E2}$	$P_{W1,C(E1)} = P_{W1,C(E1)} * (1 - \text{eff}_{E2})$
Product P_{W2}	$P_{W2,C(E1)} = N_{C(D)} * Y_{P,W2/C}$	$P_{W2,C(E2)} = P_{W2,C(E1)} * \text{eff}_{E2}$	$P_{W2,C(E1)} = P_{W2,C(E1)} * (1 - \text{eff}_{E2})$
Unconverted Carbon	$IN_{C(E1)} = N_{C(D)} * (1-(Y_{XAlgal/C} + Y_{P,W1/C} + Y_{P,W2/C} + Y_{CO2Algal/C}))$	$IN_{C(E2)} = IN_{C(E1)} * (N_{W(E2)}/N_{W(E1)})$	$IN_{C(F1)} = IN_{C(E1)} * (N_{W(F1)}/N_{W(E1)})$
Totals	$N_{C(E1)} = X_{C(E1)} + P_{W1,C(E1)} + P_{W2,C(E1)} + IN_{C(E1)}$	$N_{C(E2)} = X_{C(E2)} + P_{W1,C(E2)} + P_{W2,C(E2)} + IN_{C(E2)}$	$N_{C(F1)} = X_{C(F1)} + P_{W1,C(F1)} + P_{W2,C(F1)} + IN_{C(F1)}$
Checks: Total stream amounts: $(N_{C(E1)}) - (N_{C(E2)} + N_{C(F1)}) = 0$ The fraction dissolved components (e.g. unconverted Carbon) depends on the water split, which depends on the solids content (SC) of the bottoms stream.			

Nitrogen Mass Balance: Unit 2.2: Separator			
Nitrogen Fraction	**E1: Algal Broth outflow**	**E2: Biomass & Product**	**F1: Almost Compliant Effluent**
Biomass X_{Algal} (including P_{W3})	$X_{N(E1)} = X_{C(E1)} * f(X_{Alg})_{N/C}$	$X_{N(E2)} = X_{N(E1)} * \text{eff}_{E2}$	$X_{N(F1)} = X_{N(E1)} * (1 - \text{eff}_{E2})$
Product P_{W1}	$P_{W1,N(E1)} = P_{W1,C(E1)} * f(W1)_{N/C}$	$P_{W1,N(E2)} = P_{W1,N(E1)} * \text{eff}_{E2}$	$P_{W1,N(E1)} = P_{W1,N(E1)} * (1 - \text{eff}_{E2})$
Product P_{W2}	0	0	0
Unconverted Nitrogen	$IN_{N(E1)} = IN_{N(D)} - X_{N(E1)} - P_{W1,N(E1)}$	$IN_{N(E2)} = IN_{N(E1)} * (N_{W(E2)}/N_{W(E1)})$	$IN_{N(F1)} = IN_{N(E1)} * (N_{W(F1)}/N_{W(E1)})$
Totals	$N_{N(E1)} = X_{N(E1)} + P_{W1,N(E1)} + IN_{N(C1)}$	$N_{N(E2)} = X_{N(E2)} + P_{W1,N(E2)} + IN_{N(E2)}$	$N_{N(F1)} = X_{N(F1)} + P_{W1,N(F1)} + IN_{N(F1)}$
Checks: Total stream amounts: $(N_{N(E1)}) - (N_{N(F1)} + N_{N(E2)}) = 0$			

Phosphorous Mass Balance: Unit 2.2: Separator			
Phosphorous Fraction	**E1: Algal Broth outflow**	**E2: Biomass & Product**	**F1: Almost Compliant Effluent**
Biomass X^{Algal} (including P_{W3})	$X_{P(E1)} = X_{C(E1)} * f_{(XAlg)P/C}$	$X_{P(E2)} = X_{P(E1)} * \text{eff}_{E2}$	$X_{P(F1)} = X_{P(E1)} * (1 - \text{eff}_{E2})$
Product P_{W1}	$P_{W1,P(E1)} = P_{W1,C(E1)} * f_{(W1)P/C}$	$P_{W1,P(E2)} = P_{W1,P(E1)} * \text{eff}_{E2}$	$P_{W1,P(E1)} = P_{W1,P(E1)} * (1 - \text{eff}_{E2})$
Product P_{W2}	0	0	0
Unconverted Phosphorous	$IN_{P(E1)} = IN_{P(D)} - X_{P(E1)} - P_{W1,P(E1)}$	$IN_{P(E2)} = IN_{P(E1)} * (N_{W(E2)}/N_{W(E1)})$	$IN_{P(F1)} = IN_{P(E1)} * (N_{W(F1)}/N_{W(E1)})$
Totals	$N_{P(E1)} = X_{P(E1)} + P_{W1,P(E1)} + IN_{P(E1)}$	$N_{P(E2)} = X_{P(E2)} + P_{W1,P(E2)} + IN_{P(E2)}$	$N_{P(F1)} = X_{P(F1)} + P_{W1,P(F1)} + IN_{P(F1)}$
Checks: Total stream amounts: $(N_{P(E1)}) - (N_{P(F1)} + N_{P(E2)}) = 0$			

Water Mass Balance: Unit 2.2: Separator			
	E1: Algal Broth outflow	**E2: Biomass & Product**	**F1: Almost Compliant Effluent**
Total Water	$N_{W(E1)} = N_{W(D)} + N_{W(E6)}$	$N_{W(E2)} = (N_{C(E2)}/C_{comp,algal}) * ((1-SC_{E2})/SC_{E2})$	$N_{W(F1)} = N_{W(E1)} - N_{W(E2)}$
Checks: Total stream amounts: $(N_{W(E1)}) - (N_{W(F1)} + N_{W(E2)}) = 0$ The value of the total solids content of stream E2 is estimated by dividing the kg carbon in stream E2 ($N_{C(E2)}$) by the carbon composition of algal biomass. This is an overestimation but is simplified from using the compositions of the product streams.			

Table: F-5: Mass balance for Unit 2.3 Separator: algal biomass from algal products

Carbon Mass Balance: Unit 2.3: Separator			
Carbon Fraction	**E2: Biomass & Product**	**E3: Algal Product Stream**	**E4: Biomass**
Biomass X_{Algal} (including P_{W3})	$X_{C(E2)} = X_{C(E1)} * eff_{E2}$	$X_{C(E3)} = X_{C(E2)} * (1 - eff_{E4})$	$X_{C(E4)} = X_{C(E2)} * eff_{E4}$
Product P_{W1}	$P_{W1,C(E2)} = P_{W1,C(E1)} * eff_{E2}$	$P_{W1,C(E3)} = P_{W1,C(E2)} * eff_{E3}$	$P_{W1,C(E4)} = P_{W1,C(E2)} * (1- eff_{E3})$
Product P_{W2}	$P_{W2,C(E2)} = P_{W2,C(E1)} * eff_{E2}$	$P_{W2,C(E3)} = P_{W1,C(E2)} * eff_{E3}$	$P_{W1,C(E4)} = P_{W1,C(E2)} * (1- eff_{E3})$
Unconverted Carbon	$IN_{C(E2)} = IN_{C(E1)} * (N_{W(E2)}/N_{W(E1)})$	$IN_{C(E3)} = IN_{C(E2)} * (N_{W(E3)}/N_{W(E2)})$	$IN_{C(E4)} = IN_{C(E2)} * (N_{W(E4)}/N_{W(E2)})$
Totals	$N_{C(E2)} = X_{C(E2)} + P_{W1,C(E2)} + P_{W2,C(E2)} + IN_{C(E2)}$	$N_{C(E3)} = X_{C(E3)} + P_{W1,C(E3)} + P_{W2,C(E3)} + IN_{C(E3)}$	$N_{C(E4)} = X_{C(E4)} + P_{W1,C(E4)} + P_{W2,C(E4)} + IN_{C(E4)}$
Checks: Total stream amounts: $(N_{C(E2)}) - (N_{C(E3)} + N_{C(E4)}) = 0$			

Nitrogen Mass Balance: Unit 2.3: Separator			
Nitrogen Fraction	**E2: Biomass & Product**	**E3: Algal Product Stream**	**E4: Biomass**
Biomass X_{Algal} (including P_{W3})	$X_{N(E2)} = X_{N(E1)} * eff_{E2}$	$X_{N(E3)} = X_{N(E2)} * (1 - eff_{E4})$	$X_{N(E4)} = X_{N(E2)} * eff_{E4}$
Product P_{W1}	$P_{W1,N(E2)} = P_{W1,N(E1)} * eff_{E2}$	$P_{W1,N(E3)} = P_{W1,N(E2)} * eff_{E3}$	$P_{W1,N(E4)} = P_{W1,N(E2)} * (1- eff_{E3})$
Product P_{W2}	0	0	0
Unconverted Nitrogen	$IN_{N(E2)} = IN_{N(E1)} * (N_{W(E2)}/N_{W(E1)})$	$IN_{N(E3)} = IN_{N(E2)} * (N_{W(E3)}/N_{W(E2)})$	$IN_{N(E4)} = IN_{N(E2)} * (N_{W(E4)}/N_{W(E2)})$
Totals	$N_{N(E2)} = X_{N(E2)} + P_{W1,N(E2)} + IN_{N(E2)}$	$N_{N(E3)} = X_{N(E3)} + P_{W1,N(E3)} + IN_{N(E3)}$	$N_{N(E4)} = X_{N(E4)} + P_{W1,N(E4)} + IN_{N(E4)}$
Checks: Total stream amounts: $(N_{N(E2)}) - (N_{N(E3)} + N_{N(E4)}) = 0$			

Phosphorous Mass Balance: Unit 2.3: Separator			
Phosphorous Fraction	**E2: Biomass & Product**	**E3: Algal Product Stream**	**E4: Biomass**
Biomass X_{Algal} (including P_{W3})	$X_{P(E2)} = X_{P(E1)} * eff_{E2}$	$X_{P(E3)} = X_{P(E2)} * (1 - eff_{E4})$	$X_{P(E4)} = X_{P(E2)} * eff_{E4}$
Product P_{W1}	$P_{W1,P(E2)} = P_{W1,P(E1)} * eff_{E2}$	$P_{W1,P(E3)} = P_{W1,P(E2)} * eff_{E3}$	$P_{W1,P(E4)} = P_{W1,P(E2)} * (1- eff_{E3})$
Product P_{W2}	0	0	0
Unconverted Phosphorous	$IN_{C(E2)} = IN_{C(E1)} * (N_{W(E2)}/N_{W(E1)})$	$IN_{P(E3)} = IN_{P(E2)} * (N_{W(E3)}/N_{W(E2)})$	$IN_{P(E4)} = IN_{P(E2)} * (N_{W(E4)}/N_{W(E2)})$
Totals	$N_{C(E2)} = X_{C(E2)} + P_{W1,C(E2)} + IN_{C(E2)}$	$N_{P(E3)} = X_{P(E3)} + P_{W1,P(E3)} + IN_{P(E3)}$	$N_{P(E4)} = X_{P(E4)} + P_{W1,P(E4)} + IN_{P(E4)}$
Checks: Total stream amounts: $(N_{P(E2)}) - (N_{P(E3)} + N_{P(E4)}) = 0$			

Water Mass Balance: Unit 2.3: Separator			
	E2: Biomass & Product	**E3: Algal Product Stream**	**E4: Biomass**
Total Water	$N_{W(E2)} = (N_{C(E2)}/C_{comp,algal}) * ((1-SC_{E2})/SC_{E2})$	$N_{W(E3)} = N_{W(E2)} - N_{W(E4)}$	$N_{W(E4)} = (N_{C(E4)}/C_{comp,algal})*((1-SC_{E4})/SC_{E4})$
Checks: Total stream amounts: $(N_{W(E2)}) - (N_{W(E3)} + N_{W(E4)}) = 0$ The value of the total solids content of stream E4 is estimated by dividing the kg carbon in stream E4 ($N_{C(E4)}$) by the carbon composition of algal biomass .			

Table: F-6: Mass balance for Unit 2.4 Separator: algal bioproduct W1 from algal oil product W2

Carbon Mass Balance: Unit 2.4: Separator

Carbon Fraction	E3: Algal Product Stream	W1: Algal Bioproduct Stream	W2: Algal Oil Stream
Biomass X_{Algal}	$X_{C(E3)} = X_{C(E2)} * (1 - eff_{E4})$	$X_{C(W1)} = X_{C(E3)} * eff_{W1}$	$X_{C(W2)} = X_{C(E3)} * (1 - eff_{W1})$
Product P_{W1}	$P_{W1,C(E3)} = P_{W1,C(E2)} * eff_{E3}$	$P_{W1,C(W1)} = P_{W1,C(E3)} * eff_{W1}$	$P_{W1,C(W2)} = P_{W1,C(E3)} * (1 - eff_{W1})$
Product P_{W2}	$P_{W2,C(E3)} = P_{W1,C(E2)} * eff_{E3}$	$P_{W2,C(W1)} = P_{W1,C(E3)} * (1 - eff_{W2})$	$P_{W2,C(W2)} = P_{W1,C(E3)} * eff_{W2}$
Unconverted Carbon	$IN_{C(E3)} = IN_{C(E2)} * (N_{W(E3)}/N_{W(E2)})$	$IN_{C(W1)} = IN_{C(E3)} * (N_{W(W1)}/N_{W(E3)})$	$IN_{C(W2)} = IN_{C(E3)} * (N_{W(W2)}/N_{W(E3)})$
Totals	$N_{C(E3)} = X_{C(E3)} + P_{W1,C(E3)} + P_{W2,C(E3)} + IN_{C(E3)}$	$N_{C(W1)} = X_{C(W1)} + P_{W1,C(W1)} + P_{W2,C(W1)} + IN_{C(W1)}$	$N_{C(W2)} = X_{C(W2)} + P_{W1,C(W2)} + P_{W2,C(W2)} + IN_{C(W2)}$

Checks: Total stream amounts:
$(N_{C(E3)}) – (N_{C(W1)} + N_{C(W2)}) = 0$
The emphasis is on the purity of the algal oil, product W2. The biomass fraction is assumed to be separated with product W1, and so uses the same efficiency, eff_{W1}.
SC_{W2} is the “non-water” content (normally the solids” content), which in this case refers to the oil content in stream W2. The contaminating moisture would be 1 – SC = LC.

Nitrogen Mass Balance: Unit 2.4: Separator

Nitrogen Fraction	E3: Algal Product Stream	W1: Algal Bioproduct Stream	W2: Algal Oil Stream
Biomass X_{Algal}	$X_{N(E3)} = X_{N(E2)} * (1 - eff_{E4})$	$X_{N(W1)} = X_{N(E3)} * eff_{W1}$	$X_{N(W2)} = X_{N(E3)} * (1 - eff_{W1})$
Product P_{W1}	$P_{W1,N(E3)} = P_{W1,N(E2)} * eff_{E3}$	$P_{W1,N(W1)} = P_{W1,N(E3)} * eff_{W1}$	$P_{W1,N(W2)} = P_{W1,N(E3)} * (1 - eff_{W1})$
Product P_{W2}	0	0	0
Unconverted Nitrogen	$IN_{N(E3)} = IN_{N(E2)} * (N_{W(E3)}/N_{W(E2)})$	$IN_{N(W1)} = IN_{N(E3)} * (N_{W(W1)}/N_{W(E3)})$	$IN_{N(W2)} = IN_{N(E3)} * (N_{W(W2)}/N_{W(E3)})$
Totals	$N_{N(E3)} = X_{N(E3)} + P_{W1,N(E3)} + IN_{N(E3)}$	$N_{N(W1)} = X_{N(W1)} + P_{W1,N(W1)} + IN_{N(W1)}$	$N_{N(W2)} = X_{N(W2)} + P_{W1,N(W2)} + IN_{N(W2)}$

Checks: Total stream amounts:
$(N_{N(E3)}) – (N_{N(W1)} + N_{N(W2)}) = 0$

Phosphorous Mass Balance: Unit 2.4: Separator

Phosphorous Fraction	E3: Algal Product Stream	W1: Algal Bioproduct Stream	W2: Algal Oil Stream
Biomass X_{Algal}	$X_{P(E3)} = X_{P(E2)} * (1 - eff_{E4})$	$X_{P(W1)} = X_{P(E3)} * eff_{W1}$	$X_{P(W2)} = X_{P(E3)} * (1 - eff_{W1})$
Product P_{W1}	$P_{W1,P(E3)} = P_{W1,P(E2)} * eff_{E3}$	$P_{W1,P(W1)} = P_{W1,P(E3)} * eff_{W1}$	$P_{W1,P(W2)} = P_{W1,P(E3)} * (1 - eff_{W1})$
Product P_{W2}	0	0	0
Unconverted Phosphorous	$IN_{P(E3)} = IN_{P(E2)} * (N_{W(E3)}/N_{W(E2)})$	$IN_{P(W1)} = IN_{P(E3)} * (N_{W(W1)}/N_{W(E3)})$	$IN_{P(W2)} = IN_{P(E3)} * (N_{W(W2)}/N_{W(E3)})$
Totals	$N_{P(E3)} = X_{P(E3)} + P_{W1,P(E3)} + IN_{P(E3)}$	$N_{P(W1)} = X_{P(W1)} + P_{W1,P(W1)} + IN_{P(W1)}$	$N_{P(W2)} = X_{P(W2)} + P_{W1,P(W2)} + IN_{P(W2)}$

Checks: Total stream amounts:
$(N_{P(E3)}) – (N_{P(W1)} + N_{P(W2)}) = 0$

Water Mass Balance: Unit 2.4: Separator

	E3: Algal Product Stream	W1: Algal Bioproduct Stream	W2: Algal Oil Stream
Total Water	$N_{W(E3)} = N_{W(E2)} - N_{W(E4)}$	$N_{W(W1)} = N_{W(E3)} - N_{W(W2)}$	$N_{W(W2)} = N_{C(W2)}/C_{comp,ProductW2})*((1-SC_{W2})/SC_{W2})$

Checks: Total stream amounts:
$(N_{W(E3)}) – (N_{W(W1)} + N_{W(W2)}) = 0$

Table: F-7: Mass balance for Unit 2.5 Splitter: algal biomass to biomass product W3 and bottoms

Carbon, Nitrogen, Phosphorous and Water Mass Balance: Unit 2.5: Splitter			
Fraction	**E4: Biomass**	**W3: Algal Biomass Stream** "Digestable Waste"	**U3: Algal Bottoms**
Total Carbon	$N_{C(E4)} = X_{C(E4)} + P_{W1,C(E4)} + P_{W2,C(E4)} + IN_{C(E4)}$	$N_{C(W3)} = N_{C(E4)} * r_{W3}$	$N_{C(U3)} = N_{C(E4)} * (1 - r_{W3})$
Total Nitrogen	$N_{N(E4)} = X_{N(E4)} + P_{W1,N(E4)} + IN_{N(E4)}$	$N_{N(W3)} = N_{N(E4)} * r_{W3}$	$N_{N(U3)} = N_{N(E4)} * (1 - r_{W3})$
Total Phosphorous	$N_{P(E4)} = X_{P(E4)} + P_{W1,P(E4)} + IN_{P(E4)}$	$N_{P(W3)} = N_{P(E4)} * r_{W3}$	$N_{P(U3)} = N_{P(E4)} * (1 - r_{W3})$
Total Water	$N_{W(E4)} = (N_{C(E4)}/C_{comp,algal})*((1-SC_{E4})/SC_{E4})$	$N_{W(W3)} = N_{W(E4)} * r_{W3}$	$N_{W(U3)} = N_{W(E4)} * (1 - r_{W3})$
Checks: Total stream amounts: $(N_{C(E4)}) - (N_{C(W3)} + N_{C(U3)}) = 0$ $(N_{N(E4)}) - (N_{N(W3)} + N_{N(U3)}) = 0$ $(N_{P(E4)}) - (N_{P(W3)} + N_{P(U3)}) = 0$ $(N_{W(E4)}) - (N_{W(W3)} + N_{W(U3)}) = 0$			

F.3 Mass Balance for Macrophyte Bioreactor

The macrophyte bioreactor train flowsheet is found in Section 7.5.2 and the units with the corresponding overall mass balance equations and stream descriptions are presented there.

Table: F-8: Mass balance for Unit 3.0 Mixing Tank: macrophyte bioreactor inflow

Carbon, Nitrogen, Phosphorous and Water Mass Balance: Unit 1.0: Mixing tank			
Fraction	**F1: Almost Compliant Effluent**	**F2-4: Supplement Streams**	**F: Inflow to Macrophyte Bioreactor**
Total Carbon	$N_{C(F1)}$	$N_{C(F2-4)} = Q_{(F2)}* C_{C(F2)} + Q_{(F3)}* C_{C(F4)} + Q_{(F5)}* C_{C(F5)}$	$N_{C(F)} = N_{C(F1)} + N_{C(F2-4)}$
Total Nitrogen	$N_{N(F1)}$	$N_{N(F2-4)} = Q_{(F2)}* C_{N(F2)} + Q_{(F3)}* C_{N(F4)} + Q_{(F5)}* C_{N(F5)}$	$N_{N(F)} = N_{N(F1)} + N_{N(F2-4)}$
Total Phosphorous	$N_{P(F1)}$	$N_{P(F2-4)} = Q_{(F2)}* C_{P(F2)} + Q_{(F3)}* C_{P(F4)} + Q_{(F5)}* C_{P(F5)}$	$N_{P(F)} = N_{P(F1)} + N_{P(F2-4)}$
Total Water	$N_{W(F1)}$	$N_{W(F2-4)} = N_{W(F2)} + N_{W(F3)} + N_{W(F4)}$	$N_{W(F)} = N_{W(F1)} + N_{W(F2-4)}$
Checks: Total stream amounts: $(N_{C(F1)} + N_{C(F2-4)}) - (N_{C(F)}) = 0$ $(N_{N(F1)} + N_{N(F2-4)}) - (N_{N(F)}) = 0$ $(N_{P(F1)} + N_{P(F2-4)}) - (N_{P(F)}) = 0$ $(N_{W(F1)} + N_{W(F2-4)}) - (N_{W(F)}) = 0$ The Substrate Streams F2, F3 and F4 are assumed to have negligible solids component.			

Table: F-9: Mass balance for Unit 3.1 Macrophyte Bioreactor

Carbon Mass Balance: Unit 3.1: Macrophyte Bioreactor				
Carbon Fraction	**F: Inflow to Macrophyte Bioreactor**	**G1: Wet Biomass**	**G6: CO_2 Release = Outflow**	**G7: H_2O**
Biomass $X_{Macrophyte}$		$X_{C(G1)} = CO_{2C,Macrophyte(G6)}$		
Carbon Dioxide $CO_{2,Macrophyte}$			$CO_{2C,Macrophyte(G6)} = (Y_{macrophyte} * C_{macrophyte} * N_{W(F)}) / 365$	
Biomass $X_{S,Bacterial}$ (to sediment)		$X_{C,S,Bact(G1)} = IN_{C(F)} * Y_{X,S,Bact/C}$		
Unconverted Carbon	$IN_{C(F)} = N_{C(F)} = N_{C(F1)} + N_{C(F2-4)}$	$IN_{C(G1)} = IN_{C(F)} - X_{C,S,Bact(G1)}$		
Totals	$N_{C(F)} = IN_{C(F)}$	$N_{C(G1)} = X_{C(G1)} + X_{C,S,Bact(G1)} + IN_{C(G1)}$	$N_{C(G5)} = CO_{2Macrophyte(G6)}$	$N_{C(G7)} = 0$
Checks: Total stream amounts: $(N_{C(F)} + N_{C(G6)}) - (N_{C(G1)}) = 0$				

Nitrogen Mass Balance: Unit 3.1: Macrophyte Bioreactor				
Nitrogen Fraction	**F: Inflow to Macrophyte Bioreactor**	**G1: Wet Biomass**	**G6: CO_2 Release = Outflow**	**G7: H_2O**
Biomass $X_{Macrophyte}$		$X_{N(G1)} = X_{C(G1)} * f(X_{macrophyte})_{N/C}$		
Biomass $X_{S,Bacterial}$ (to sediment)		$X_{N,S,Bact(G1)} = X_{C,S,Bact(G1)} * f(X_{bacterial})_{N/C}$		
Unconverted Nitrogen	$IN_{N(F)} = N_{N(F)} = N_{N(F1)} + N_{(F2-4)}$	$IN_{N(G1)} = IN_{N(F)} - X_{N(G1)} - X_{N,S,Bact(G1)}$		
Totals	$N_{N(F)} = IN_{N(F)}$	$N_{N(G1)} = X_{N(G1)} + X_{N,S,Bact(G1)} + IN_{N(G1)}$	$N_{P(C5)} = 0$	$N_{P(C6)} = 0$
Checks: Total stream amounts: $N_{N(F)} - N_{N(G1)} = 0$				

Phosphorous Mass Balance: Unit 3.1: Macrophyte Bioreactor				
Phosphorous Fraction	**F: Inflow to Macrophyte Bioreactor**	**G1: Wet Biomass**	**G6: CO_2 Release = Outflow**	**G7: H_2O**
Biomass $X_{Macrophyte}$		$X_{P(G1)} = X_{C(G1)} * f(X_{Macrophyte})_{P/C}$		
Biomass $X_{S,Bacterial}$ (to sediment)		$X_{P,S,Bact(G1)} = X_{C,S,Bact(G1)} * f(X_{bacterial})_{P/C}$		
Unconverted Phosphorous	$IN_{P(F)} = N_{P(F)} = N_{P(F1)} + N_{P(F2-4)}$	$IN_{P(G1)} = IN_{P(F)} - X_{P(G1)} - X_{P,S,Bact(G1)}$		
Totals	$N_{P(F)} = IN_{P(F)}$	$N_{P(G1)} = X_{P(G1)} + X_{P,S,Bact(G1)} + IN_{N(G1)}$	$N_{P(G6)} = 0$	$N_{P(G7)} = 0$
Checks: Total stream amounts: $N_{P(F)} - N_{P(G1)} = 0$				

Water Mass Balance: Unit 3.1: Macrophyte Bioreactor				
	F: Inflow to Macrophyte Bioreactor	**G1: Wet Biomass**	**G6: CO_2 Release = Outflow**	**G7: H_2O**
Total Water	$N_{W(F)}$	$N_{W(G1)} = N_{W(F)} + N_{W(G7)}$		$N_{W(G7)} = N_{W(F)} * (F_{precip} - F_{evap})$
$(N_{W(F)} + N_{W(G7)}) - (N_{W(G1)}) = 0$				

Table: F-10: Mass balance for Unit 3.2 Separator: solids from compliant effluent

Carbon Mass Balance: Unit 3.2: Separator			
Carbon Fraction	**G1: Wet Biomass**	**G2: Solids**	**Z: Compliant Effluent**
Biomass, including solids $X_{Macrophyte}$	$X_{C(G1)} = C_{,CO2Macrophyte(G6)}$	$X_{C(G2)} = X_{C(G1)} * eff_{G2}$	$X_{C(Z)} = X_{C(G1)} * (1 - eff_{G2})$
Biomass $X_{S,Bacterial}$ (to sediment)	$X_{C,S,Bact(G1)} = IN_{C(F)} * Y_{X,S,Bact/C}$	$X_{C,S,Bact(G2)} = X_{C,S,Bact(G1)}) * eff_{G2}$	$X_{C,S,Bact(Z)} = X_{C,S,Bact(G1)}) * (1 - eff_{G2})$
Unconverted Carbon	$IN_{C(G1)} = IN_{C(F)} - X_{C,S,Bact(G1)}$	$IN_{C(G2)} = IN_{C(G1)} * (N_{W(G2)}/N_{W(G1)})$	$IN_{C(Z)} = IN_{C(G1)} * (N_{W(Z)}/N_{W(G1)})$
Totals	$N_{C(G1)} = X_{C(G1)} + X_{C,S,Bact(G1)} + IN_{C(G1)}$	$N_{C(G2)} = X_{C(G2)} + X_{C,S,Bact(G2)} + IN_{C(G2)}$	$N_{C(Z)} = X_{C(Z)} + X_{C,S,Bact(Z)} + IN_{C(Z)}$
Checks: Total stream amounts: $(N_{C(G1)}) - (N_{C(G2)} + N_{C(Z)}) = 0$ The fraction dissolved components (e.g. unconverted Carbon) depend on the water split, which depends on the solids content (SC) of the bottoms stream.			

Nitrogen Mass Balance: Unit 3.2: Separator			
Nitrogen Fraction	**G1: Wet Biomass**	**G2: Solids**	**Z: Compliant Effluent**
Biomass, including solids $X_{Macrophyte}$	$X_{N(G1)} = X_{C(G1)} * f(X_{macrophyte})_{N/C}$	$X_{N(G2)} = X_{N(G1)} * eff_{G2}$	$X_{N(Z)} = X_{N(G1)} * (1 - eff_{G2})$
Biomass $X_{S,Bacterial}$ (to sediment)	$X_{N,S,Bact(G1)} = X_{C,S,Bact(G1)} * f(X_{bacterial})_{N/C}$	$X_{N,S,Bact(G2)} = X_{N,S,Bact(G1)}) * eff_{G2}$	$X_{N,S,Bact(Z)} = X_{N,S,Bact(G1)}) * (1 - eff_{G2})$
Unconverted Nitrogen	$IN_{N(G1)} = IN_{N(F)} - X_{N(G1)} - X_{N,S,Bact(G1)}$	$IN_{N(G2)} = IN_{N(G1)} * (N_{W(G2)}/N_{W(G1)})$	$IN_{N(Z)} = IN_{N(G1)} * (N_{W(Z)}/N_{W(G1)})$
Totals	$N_{N(G1)} = X_{N(G1)} + X_{N,S,Bact(G1)} + IN_{N(G1)}$	$N_{N(G2)} = X_{N(G2)} + X_{N,S,Bact(G2)} + IN_{N(G2)}$	$N_{N(Z)} = X_{N(G1)} + X_{N,S,Bact(Z)} + IN_{N(G1)}$
Checks: Total stream amounts: $(N_{N(G1)}) - (N_{N(G2)} + N_{N(Z)}) = 0$			

Phosphorous Mass Balance: Unit 3.2: Separator			
Phosphorous Fraction	**G1: Wet Biomass**	**G2: Solids**	**Z: Compliant Effluent**
Biomass, including solids $X_{Macrophyte}$	$X_{P(G1)} = X_{C(G1)} * f(X_{Macrophyte})_{P/C}$	$X_{P(G2)} = X_{P(G1)} * eff_{G2}$	$X_{C(Z)} = X_{C(G1)} * (1 - eff_{G2})$
Biomass $X_{S,Bacterial}$ (to sediment)	$X_{P,S,Bact(G1)} = X_{C,S,Bact(G1)} * f(X_{bacterial})_{P/C}$	$X_{P,S,Bact(G2)} = X_{P,S,Bact(G1)}) * eff_{G2}$	$X_{P,S,Bact(Z)} = X_{P,S,Bact(G1)}) * (1 - eff_{G2})$
Unconverted Phosphorous	$IN_{P(G1)} = IN_{P(F)} - X_{P(G1)} - X_{P,S,Bact(G1)}$	$IN_{P(G2)} = IN_{P(G1)} * (N_{W(G2)}/N_{W(G1)})$	$IN_{P(Z)} = IN_{P(G1)} * (N_{W(Z)}/N_{W(G1)})$
Totals	$N_{P(G1)} = X_{P(G1)} + X_{P,S,Bact(G1)} + IN_{N(G1)}$	$N_{P(G2)} = X_{P(G2)} + X_{P,S,Bact(G2)} + IN_{P(G2)}$	$N_{P(Z)} = X_{P(G1)} + X_{P,S,Bact(Z)} + IN_{P(G1)}$
Checks: Total stream amounts: $(N_{P(G1)}) - (N_{P(C2)} + N_{P(Z)}) = 0$			

Water Mass Balance: Unit 3.2: Separator			
	G1: Wet Biomass	**G2: Solids**	**Z: Compliant Effluent**
Total Water	$N_{W(G1)} = N_{W(F)} + N_{W(G7)}$	$N_{W(G2)} = (N_{C(G2)}/C_{comp,macrophyte}) * ((1-SC_{G2})/SC_{G2})$	$N_{W(Z)} = N_{W(G1)} - N_{W(G2)}$
Checks: Total stream amounts: $(N_{W(G1)}) – (N_{W(G2)} + N_{W(Z)}) = 0$ The value of the total solids content of stream G2 is estimated by dividing the total kg Carbon in stream G2 ($N_{C(G2)}$) by the Carbon composition of macrophyte biomass. This is slightly inaccurate as the composition of macrophyte and bacterial biomass differs, but is sufficient for a first order estimate			

Table: F-11: Mass balance for Unit 3.3 Separator: macrophyte sediment from biomass & fibre

Carbon Mass Balance: Unit 3.3: Separator			
Carbon Fraction	**G2: Solids**	**G3: Fibrous Biomass**	**G4: Sediment**
Biomass $X_{Macrophyte}$	$X_{C(G2)} = X_{C(G1)} * eff_{G2}$	$X_{C(G3)} = X_{C(G2)} * eff_{G3}$	$X_{C(G4)} = X_{C(G2)} * (1 - eff_{G3})$
Biomass $X_{S,Bacterial}$ (to sediment)	$X_{C,S,Bact(G2)} = X_{C,S,Bact(G1)}$) * eff_{G2}	$X_{C,S,Bact(G3)} = X_{C,S,Bact(G2)} * (1 - eff_{G4})$	$X_{C,S,Bact(G4)} = X_{C,S,Bact(G2)} * eff_{G4}$
Unconverted Carbon	$IN_{C(G2)} = IN_{C(G1)} * (N_{W(G2)}/N_{W(G1)})$	$IN_{C(G3)} = IN_{C(G2)} * (N_{W(G3)}/N_{W(G2)})$	$IN_{G(G4)} = IN_{C(G2)} * (N_{W(G4)}/N_{W(G2)})$
Totals	$N_{C(G2)} = X_{C(G2)} + X_{C,S,Bact(G2)} + IN_{C(G2)}$	$N_{C(G3)} = X_{C(G3)} + X_{C,S,Bact(G3)} + IN_{C(G3)}$	$N_{C(G4)} = X_{C(G4)} + X_{C,S,Bact(G4)} + IN_{C(G4)}$
Checks: Total stream amounts: $(N_{C(G2)}) – (N_{C(G3)} + N_{C(G4)}) = 0$			
Nitrogen Mass Balance: Unit 3.3: Separator			
Nitrogen Fraction	**G2: Solids**	**G3: Fibrous Biomass**	**G4: Sediment**
Biomass $X_{Macrophyte}$	$X_{N(G2)} = X_{N(G1)} * eff_{G2}$	$X_{N(G3)} = X_{N(G2)} * eff_{G3}$	$X_{N(G4)} = X_{N(G2)} * (1 - eff_{G3})$
Biomass $X_{S,Bacterial}$ (to sediment)	$X_{N,S,Bact(G2)} = X_{N,S,Bact(G1)}$) * eff_{G2}	$X_{N,S,Bact(G3)} = X_{N,S,Bact(G2)} * (1 - eff_{G4})$	$X_{N,S,Bact(G4)} = X_{N,S,Bact(G2)} * eff_{G4}$
Unconverted Nitrogen	$IN_{N(G2)} = IN_{N(G1)} * (N_{W(G2)}/N_{W(G1)})$	$IN_{N(G3)} = IN_{N(G2)} * (N_{W(G3)}/N_{W(G2)})$	$IN_{N(G4)} = IN_{N(G2)} * (N_{W(G4)}/N_{W(G2)})$
Totals	$N_{N(G2)} = X_{N(G2)} + X_{N,S,Bact(G2)} + IN_{N(G2)}$	$N_{N(G3)} = X_{N(G3)} + X_{N,S,Bact(G3)} + IN_{N(G3)}$	$N_{N(G4)} = X_{N(G4)} + X_{N,S,Bact(G4)} + IN_{N(G4)}$
Checks: Total stream amounts: $(N_{N(G2)}) – (N_{N(G3)} + N_{N(G4)}) = 0$			
Phosphorous Mass Balance: Unit 3.3: Separator			
Phosphorous Fraction	**G2: Solids**	**G3: Fibrous Biomass**	**G4: Sediment**
Biomass $X_{Macrophyte}$	$X_{P(G2)} = X_{P(G1)} * eff_{G2}$	$X_{P(G3)} = X_{P(G2)} * eff_{G3}$	$X_{P(G4)} = X_{P(G2)} * (1 - eff_{G3})$
Biomass $X_{S,Bacterial}$ (to sediment)	$X_{P,S,Bact(G2)} = X_{P,S,Bact(G1)}$) * eff_{G2}	$X_{P,S,Bact(G3)} = X_{P,S,Bact(G2)} * (1 - eff_{G4})$	$X_{P,S,Bact(G4)} = X_{P,S,Bact(G2)} * eff_{G4}$
Unconverted Phosphorous	$IN_{P(G2)} = IN_{P(G1)} * (N_{W(G2)}/N_{W(G1)})$	$IN_{P(G3)} = IN_{P(G2)} * (N_{W(G3)}/N_{W(G2)})$	$IN_{P(G4)} = IN_{P(G2)} * (N_{W(G4)}/N_{W(G2)})$
Totals	$N_{P(G2)} = X_{P(G2)} + X_{P,S,Bact(G2)} + IN_{P(G2)}$	$N_{P(G3)} = X_{P(G3)} + X_{P,S,Bact(G3)} + IN_{P(G3)}$	$N_{P(G4)} = X_{P(G4)} + X_{P,S,Bact(G4)} + IN_{P(G4)}$
Checks: Total stream amounts: $(N_{P(G2)}) – (N_{P(G3)} + N_{P(G4)}) = 0$			

Water Mass Balance: Unit 3.3: Separator			
	G2: Solids	**G3: Fibrous Biomass**	**G4: Sediment**
Total Water		$N_{W(G3)} = (N_{C(G3)}/C_{comp, macrophyte})*((1-SC_{G3})/SC_{G3})$	$N_{W(G4)} = N_{W(G2)} - N_{W(G3)}$
Checks: Total stream amounts: $(N_{W(G2)}) - (N_{W(G3)} + N_{W(G4)}) = 0$ The value of the total solids content of stream G3 is estimated by dividing the kg Carbon in stream G3 ($N_{C(G3)}$) by the Carbon composition of macrophyte biomass. In a stream separating two solids, the solid content specification of the stream with the highest priority liquid content is used to specify the water split.			

Table: F-12: Mass balance for Unit 3.4 Separator: macrophyte fibre bioproduct X1 from cellulosic biomass

Carbon Mass Balance: Unit 3.4: Separator			
Carbon Fraction	**G3: Fibrous Biomass**	**G5: Cellulosic Biomass Stream**	**X1: Fibre Product Stream**
Biomass $X_{Macrophyte}$	$X_{C(G3)} = X_{C(G2)} * eff_{G3}$	$X_{C(G5)} = X_{C(G3)} * (1 - eff_{X1})$	
Product P_{X1}			$P_{X1,C(X1)} = X_{C(G3)} * eff_{X1}$
Biomass $X_{S,Bacterial}$ (to sediment)	$X_{C,S,Bact(G3)} = X_{C,S,Bact(G2)} * (1 - eff_{G4})$	$X_{C,S,Bact(G5)} = X_{C,S,Bact(G3)}$	0
Unconverted Carbon	$IN_{C(G3)} = IN_{C(G2)} * (N_{W(G3)}/N_{W(G2)})$	$IN_{C(G5)} = IN_{C(G3)} * (N_{W(G5)}/N_{W(G3)})$	$IN_{C(X1)} = IN_{C(G3)} * (N_{W(X1)}/N_{W(G3)})$
Totals	$N_{C(G3)} = X_{C(G3)} + X_{C,S,Bact(G3)} + IN_{C(G3)}$	$N_{C(G5)} = X_{C(G5)} + X_{C,S,Bact(G5)} + IN_{C(G5)}$	$N_{C(X1)} = P_{X1,C(X1)} + IN_{C(X1)}$
Checks: Total stream amounts: $(N_{C(G3)}) - (N_{C(G5)} + N_{C(X1)}) = 0$			

Nitrogen Mass Balance: Unit 3.4: Separator			
Nitrogen Fraction	**G3: Fibrous Biomass**	**G5: Cellulosic Biomass Stream**	**X1: Fibre Product Stream**
Biomass $X_{Macrophyte}$	$X_{N(G3)} = X_{N(G2)} * eff_{G3}$	$X_{N(G5)} = X_{N(G3)} * (1 - eff_{X1})$	
Product P_{X1}			$P_{X1,N(X1)} = X_{N(G3)} * eff_{X1}$
Biomass $X_{S,Bacterial}$ (to sediment)	$X_{N,S,Bact(G3)} = X_{N,S,Bact(G2)} * (1 - eff_{G4})$	$X_{N,S,Bact(G5)} = X_{N,S,Bact(G3)}$	0
Unconverted Nitrogen	$IN_{N(G3)} = IN_{N(G2)} * (N_{W(G3)}/N_{W(G2)})$	$IN_{N(G5)} = IN_{N(G3)} * (N_{W(G5)}/N_{W(G3)})$	$IN_{N(X1)} = IN_{N(G3)} * (N_{W(X1)}/N_{W(G3)})$
Totals	$N_{N(G3)} = X_{N(G3)} + X_{N,S,Bact(G3)} + IN_{N(G3)}$	$N_{N(G5)} = X_{N(G5)} + X_{N,S,Bact(G5)} + IN_{N(G5)}$	$N_{N(X1)} = P_{X1,N(X1)} + IN_{N(X1)}$
Checks: Total stream amounts: $(N_{N(G3)}) – (N_{N(G5)} + N_{N(X1)}) = 0$			

Phosphorous Mass Balance: Unit 3.4: Separator			
Phosphorous Fraction	**G3: Fibrous Biomass**	**G5: Cellulosic Biomass Stream**	**X1: Fibre Product Stream**
Biomass $X_{Macrophyte}$	$X_{P(G3)} = X_{P(G2)} * eff_{G3}$	$X_{P(G5)} = X_{P(G3)} * (1 - eff_{X1})$	
Product P_{X1}			$P_{X1,P(X1)} = X_{P(G3)} * eff_{X1}$
Biomass $X_{S,Bacterial}$ (to sediment)	$X_{P,S,Bact(G3)} = X_{P,S,Bact(G2)} * (1 - eff_{G4})$	$X_{P,S,Bact(G5)} = X_{P,S,Bact(G3)}$	0
Unconverted Phosphorous	$IN_{P(G3)} = IN_{P(G2)} * (N_{W(G3)}/N_{W(G2)})$	$IN_{P(G5)} = IN_{P(G3)} * (N_{W(G5)}/N_{W(G3)})$	$IN_{P(X1)} = IN_{P(G3)} * (N_{W(X1)}/N_{W(G3)})$
Totals	$N_{P(G3)} = X_{P(G3)} + X_{P,S,Bact(G3)} + IN_{P(G3)}$	$N_{P(G5)} = X_{P(G5)} + X_{P,S,Bact(G5)} + IN_{P(G5)}$	$N_{P(X1)} = P_{X1,P(X1)} + IN_{P(X1)}$
Checks: Total stream amounts: $(N_{P(G3)}) – (N_{P(G5)} + N_{P(X1)}) = 0$			

Water Mass Balance: Unit 3.4: Separator			
	G3: Fibrous Biomass	**G5: Cellulosic Biomass Stream**	**X1: Fibre Product Stream**
Total Water	$N_{W(G3)} = (N_{C(G3)}/C_{comp, macrophyte})*((1-SC_{G3})/SC_{G3})$	$N_{W(G5)} = N_{C(G3)} - N_{W(X1)}$	$N_{W(X1)} = (N_{C(X1)}/C_{comp, macrophyte})*((1-SC_{X1})/SC_{X1})$
Checks: Total stream amounts: $(N_{W(G3)}) – (N_{W(G5)} + N_{W(X1)}) = 0$ The value of the total solids content of stream G5 is estimated by dividing the kg Carbon in stream G5 ($N_{C(G5)}$) by the Carbon composition of macrophyte biomass .			

Table: F-13: Mass balance for Unit 3.5 Splitter: macrophyte cellulosic biomass to product stream X2 and bottoms

Carbon, Nitrogen, Phosphorous and Water Mass Balance: Unit 3.5: Splitter			
Fraction	**G5: Cellulosic Biomass Stream**	**X2: Cellulosic Biomass Product Stream**	**U4: Macrophyte Bottoms**
Total Carbon	$N_{C(G5)}$	$N_{C(X2)} = N_{C(G5)} * r_{X2}$	$N_{C(U4)} = N_{C(G5)} * (1 - r_{X2})$
Total Nitrogen	$N_{N(G5)}$	$N_{N(X2)} = N_{N(G5)} * r_{X2}$	$N_{N(U4)} = N_{N(G5)} * (1 - r_{X2})$
Total Phosphorous	$N_{P(G5)}$	$N_{P(X2)} = N_{P(G5)} * r_{X2}$	$N_{P(U4)} = N_{P(G5)} * (1 - r_{X2})$
Total Water	$N_{W(G5)}$	$N_{W(X2)} = N_{W(G5)} * r_{X2}$	$N_{W(U4)} = N_{W(G5)} * (1 - r_{X2})$
Checks: Total stream amounts: $(N_{C(G5)}) - (N_{C(X2)} + N_{C(U4)}) = 0$ $(N_{N(G5)}) - (N_{N(X2)} + N_{N(U4)}) = 0$ $(N_{P(G5)}) - (N_{P(X2)} + N_{P(U4)}) = 0$ $(N_{W(G5)}) - (N_{W(X2)} + N_{W(U4)}) = 0$			

Table: F-14: Mass balance for Unit 3.6 Splitter: macrophyte sediment to product stream X3 and bottoms

Carbon, Nitrogen, Phosphorous and Water Mass Balance: Unit 3.6: Splitter			
Fraction	**G4: Sediment**	**X3: Sediment Product Stream**	**U5: Macrophyte Bottoms**
Total Carbon	$N_{C(G4)}$	$N_{C(X3)} = N_{C(G4)} * r_{X3}$	$N_{C(U5)} = N_{C(G4)} * (1 - r_{X3})$
Total Nitrogen	$N_{N(G4)}$	$N_{N(X3)} = N_{N(G4)} * r_{X3}$	$N_{N(U5)} = N_{N(G4)} * (1 - r_{X3})$
Total Phosphorous	$N_{P(G4)}$	$N_{P(X3)} = N_{P(G4)} * r_{X3}$	$N_{P(U5)} = N_{P(G4)} * (1 - r_{X3})$
Total Water	$N_{W(G4)}$	$N_{W(X3)} = N_{W(G4)} * r_{X3}$	$N_{W(U5)} = N_{W(G4)} * (1 - r_{X3})$
Checks: Total stream amounts: $(N_{C(G4)}) - (N_{C(X3)} + N_{C(U5)}) = 0$ $(N_{N(G4)}) - (N_{N(X3)} + N_{N(U5)}) = 0$ $(N_{P(G4)}) - (N_{P(X3)} + N_{P(U5)}) = 0$ $(N_{W(G4)}) - (N_{W(X3)} + N_{W(U5)}) = 0$			

F.4 Mass Balance for Solids Bioreactor

In Section 7.5.3 the detailed flowsheet for the solids bioreactor train is given with a list of units and overall mass balance equations and a list of stream descriptions.

Table: F-15: Mass balance for Unit 4.0 Mixing Tank: solids bioreactor inflow

Carbon, Nitrogen, Phosphorous and Water Mass Balance: Unit 4.0: Mixing tank			
Fraction	**U1: PST Bottoms**	**U2: Bacterial Bottoms**	**U3: Algal Bottoms**
Total Carbon	$N_{C(U1)} = IN_{C(U1)liq} + IN_{C(U1)sol}$	$N_{C(U2)} = N_{C(C3)} * (1 - r_{C4})$	$N_{C(U3)} = N_{C(E4)} * (1 - r_{W3})$
Total Nitrogen	$N_{N(U1)} = IN_{N(U1)liq} + IN_{N(U1)sol}$	$N_{N(U2)} = N_{N(C3)} * (1 - r_{C4})$	$N_{N(U3)} = N_{N(E4)} * (1 - r_{W3})$
Total Phosphorous	$N_{P(U1)} = IN_{P(U1)liq} + IN_{P(U1)sol}$	$N_{P(U2)} = N_{P(C3)} * (1 - r_{C4})$	$N_{P(U3)} = N_{P(E4)} * (1 - r_{W3})$
Total Water	$N_{W(U1)} = N_{TOTAL(A1-4)sol} * ((1-SC_{U1})/SC_{U1})$	$N_{W(U2)} = N_{W(C3)} * (1 - r_{C4})$	$N_{W(U3)} = N_{W(E4)} * (1 - r_{W3})$

Carbon, Nitrogen, Phosphorous and Water Mass Balance: Unit 4.0: Mixing tank			
Fraction	**U4 & 5: Macrophyte Bottoms**	**U6-8 Supplement Streams**	**U: Inflow to Solids Bioreactor**
Total Carbon	$N_{C(U4)} = N_{C(G5)} * (1 – r_{X2})$ $N_{C(U5)} = N_{C(G4)} * (1 – r_{X3})$	$N_{C(U6-8)} = Q_{(U6)} * C_{C(U6)} + Q_{(U7)} * C_{C(U7)} + Q_{(U8)} * C_{C(U8)}$	$N_{C(U)} = N_{C(U1)} + N_{C(U2)} + N_{C(U3)} + N_{C(U4-5)} + N_{C(U6-8)}$
Total Nitrogen	$N_{N(U4)} = N_{N(G5)} * (1 – r_{X2})$ $N_{N(U5)} = N_{N(G4)} * (1 – r_{X3})$	$N_{N(U6-8)} = Q_{(U6)} * C_{N(U6)} + Q_{(U7)} * C_{N(U7)} + Q_{(U8)} * C_{N(U8)}$	$N_{N(U)} = N_{N(U1)} + N_{N(U2)} + N_{N(U3)} + N_{N(U4-5)} + N_{N(U6-8)}$
Total Phosphorous	$N_{P(U4)} = N_{P(G5)} * (1 – r_{X2})$ $N_{P(U5)} = N_{P(G4)} * (1 – r_{X3})$	$N_{P(U6-8)} = Q_{(U6)} * C_{N(U6)} + Q_{(U7)} * C_{N(U7)} + Q_{(U8)} * C_{N(U8)}$	$N_{P(U)} = N_{P(U1)} + N_{P(U2)} + N_{P(U3)} + N_{P(U4-5)} + N_{P(U6-8)}$
Total Water	$N_{W(U4)} = N_{W(G5)} * (1 – r_{X2})$ $N_{W(U5)} = N_{W(G4)} * (1 – r_{X3})$	$N_{W(U6-8)} = Q_{(U6)} * C_{W(U6)} + Q_{(U7)} * C_{W(U7)} + Q_{(U8)} * C_{W(U8)}$	$N_{W(U)} = N_{W(U1)} + N_{W(U2)} + N_{W(U3)} + N_{W(U4-5)} + N_{W(U6-8)}$
Checks: Total stream amounts: $(N_{C(U1)} + N_{C(U2)} + N_{C(U3)} + N_{C(U4-5)} + N_{C(U6-8)}) – (N_{C(U)}) = 0$ $(N_{N(U1)} + N_{N(U2)} + N_{N(U3)} + N_{N(U4-5)} + N_{N(U6-8)}) – (N_{N(U)}) = 0$ $(N_{P(U1)} + N_{P(U2)} + N_{P(U3)} + N_{P(U4-5)} + N_{P(U6-8)}) – (N_{P(U)}) = 0$ $(N_{W(U1)} + N_{W(U2)} + N_{W(U3)} + N_{W(U4-5)} + N_{W(U6-8)}) – (N_{W(U)}) = 0$ The Substrate Streams U6, U7 and U8 are assumed to have negligible solids component.			

Table: F-16: Mass balance for Unit 4.1 Solids Bioreactor

Carbon Mass Balance: Unit 4.1: Solids Bioreactor				
Carbon Fraction	**U: Inflow to Solids Bioreactor**	**H1: Solids Matrix**	**H4: CO_2 Release = Outflow**	**H5: H_2O**
Biomass X_{Solids}		$X_{C(H1)} = N_{C(U)} * Y_{Xsolids/C}$		
Product P_{Y1}		$P_{Y1,C(H1)} = N_{C(U)} * Y_{P,Y1/C}$		
Product P_{Y2}		$P_{Y2,C(H1)} = N_{C(U)} * Y_{P,Y2/C}$		
Product P_{Y3}		$P_{Y3,C(H1)} = N_{C(U)} * Y_{P,Y3/C}$		
Carbon Dioxide $CO_{2Solids}$			$CO_{2C,Solids(H4)} = N_{C(U)} * Y_{CO2Solids/C}$	
Unconverted Carbon	$IN_{C(U)} = N_{C(U)} = N_{C(U1)} + N_{C(U2)} + N_{C(U3)} + N_{C(U4)} + N_{C(U5)} + N_{C(U6-8)}$	$IN_{C(H1)} = N_{C(U)} * (1- (Y_{XSolids/C} + Y_{P,Y1/C} + Y_{P,Y2/C} + Y_{P,Y3/C} + Y_{CO2Solids/C}))$		
Totals	$N_{C(U)} = IN_{C(U)}$	$N_{C(H1)} = X_{C(H1)} + P_{Y1,C(H1)} + P_{Y2,C(H1)} + P_{Y3,C(H1)} + IN_{C(H1)}$	$N_{C(H4)} = CO_{2Solids(H4)}$	$N_{C(H5)} = 0$
Checks: Total stream amounts: $(N_{C(U)} + N_{C(H4)}) – (N_{C(H1)}) = 0$				
Nitrogen Mass Balance: Unit 4.1: Solids Bioreactor				
Nitrogen Fraction	**U: Inflow to Solids Bioreactor**	**H1: Solids Matrix**	**H4: CO_2 Release = Outflow**	**H5: H_2O**
Biomass X_{Solids}		$X_{N(H1)} = X_{C(H1)} * f(X_{Solids})_{N/C}$		
Product P_{Y1}		$P_{Y1,N(H1)} = P_{Y1,C(H1)} * f(Y1)_{N/C}$		
Product P_{Y2}		$P_{Y2,N(H1)} = P_{Y2,C(H1)} * f(Y2)_{N/C}$		
Product P_{Y3}		$P_{Y3,N(H1)} = P_{Y3,C(H1)} * f(Y3)_{N/C}$		
Unconverted Nitrogen	$IN_{N(U)} = N_{N(U)} = N_{N(U1)} + N_{N(U2)} + N_{N(U3)} + N_{N(U4)} + N_{N(U5)} + N_{N(U6-8)}$	$IN_{N(H1)} = IN_{N(U)} - X_{N(H1)} – P_{Y1,N(H1)} – P_{Y2,N(H1)} – P_{Y3,N(H1)}$		
Totals	$N_{N(U)} = IN_{N(U)}$	$N_{N(H1)} = X_{N(H1)} + P_{Y1,N(H1)} + P_{Y2,N(H1)} + P_{Y3,N(H1)} + IN_{N(H1)}$		
Checks: Total stream amounts: $N_{N(U)} – N_{N(H1)} = 0$				

Phosphorous Mass Balance: Unit 4.1: Solids Bioreactor				
Phosphorous Fraction	**U: Inflow to Solids Bioreactor**	**H1: Solids Matrix**	**H4: CO_2 Release = Outflow**	**H5: H_2O**
Biomass X_{Solids}		$X_{P(H1)} = X_{C(H1)} * f(X_{Solids})_{P/C}$		
Product P_{Y1}		$P_{Y1,P(H1)} = P_{Y1,C(H1)} * f(Y1)_{P/C}$		
Product P_{Y2}		$P_{Y2,P(H1)} = P_{Y2,C(H1)} * f(Y2)_{P/C}$		
Product P_{Y3}		$P_{Y3,P(H1)} = P_{Y3,C(H1)} * f(Y3)_{P/C}$		
Unconverted Phosphorous	$IN_{P(U)} = N_{P(U)} = N_{P(U1)} + N_{P(U2)} + N_{P(U3)} + N_{P(U4)} + N_{P(U5)} + N_{P(U6-8)}$	$IN_{P(H1)} = IN_{P(U)} - X_{P(H1)} - P_{Y1,P(H1)} - P_{Y2,P(H1)} - P_{Y3,P(H1)}$		
Totals	$N_{P(U)} = IN_{P(U)}$	$N_{P(H1)} = X_{P(H1)} + P_{Y1,P(H1)} + P_{Y2,P(H1)} + P_{Y3,P(H1)} + IN_{P(H1)}$		
Checks: Total stream amounts: $N_{P(U)} - N_{P(H1)} = 0$				

Water Mass Balance: Unit 4.1: Solids Bioreactor				
	U: Inflow to Solids Bioreactor	**H1: Solids Matrix**	**H4: CO_2 Release = Outflow**	**H5: H_2O**
Total Water	$N_{W(U)} = N_{W(U1)} + N_{W(U2)} + N_{W(U3)} + N_{W(U4)} + N_{W(U5)} + N_{W(U6-8)}$	$N_{W(H1)} = N_{W(U)} + N_{W(H5)}$		$N_{W(H5)} = N_{W(U)} *(F_{precip} - F_{evap})$
$(N_{W(U)} + N_{W(H5)}) - (N_{W(H1)}) = 0$				

Table: F-17: Mass balance for Unit 4.2 Separator: surface related solids bioreactor product Y2 from wet subsurface matrix

Carbon Mass Balance: Unit 4.2: Separator			
Carbon Fraction	**H1 :Solids matrix**	**H2: Wet subsurface matrix**	**Y1: Crust related product**
Biomass X_{Solids}	$X_{C(H1)} = N_{C(U)} * Y_{Xsolids/C}$	$X_{C(H2)} = X_{C(H1)} * eff_{H2}$	$X_{C(Y1)} = X_{C(H1)} * (1 - eff_{H2})$
Product P_{Y1}	$P_{Y1,C(H1)} = N_{C(U)} * Y_{P,Y1/C}$	$P_{Y1,C(H2)} = P_{Y1,C(H1)} * (1 - eff_{Y1})$	$P_{Y1,C(Y1)} = P_{Y1,C(H1)} * eff_{Y1}$
Product P_{Y2}	$P_{Y2,C(H1)} = N_{C(U)} * Y_{P,Y2/C}$	$P_{Y2,C(H2)} = P_{Y2,C(H1)} * (N_{W(H2)}/N_{W(H1)})$	$P_{Y2,C(Y1)} = P_{Y2,C(H1)} * (N_{W(Y1)}/N_{W(H1)})$
Product P_{Y3}	$P_{Y3,C(H1)} = N_{C(U)} * Y_{P,Y3/C}$	$P_{Y3,C(H2)} = P_{Y3,C(H1)} * eff_{H2}$	$P_{Y3,C(Y1)} = P_{Y3,C(H1)} * (1 - eff_{H2})$
Unconverted Carbon	$IN_{C(H1)} = N_{C(U)} * (1- (Y_{XSolids/C} + Y_{P,Y1/C} + Y_{P,Y2/C} + Y_{P,Y3/C} + Y_{CO2Solids/C}))$	$IN_{C(H2)} = IN_{C(H1)} * (N_{W(H2)}/N_{W(H1)})$	$IN_{C(Y1)} = IN_{C(H1)} * (N_{W(Y1)}/N_{W(H1)})$
Totals	$N_{C(H1)} = X_{C(H1)} + P_{Y1,C(H1)} + P_{Y2,C(H1)} + P_{Y3,C(H1)} + IN_{C(H1)}$	$N_{C(H2)} = X_{C(H2)} + P_{Y1,C(H2)} + P_{Y2,C(H2)} + P_{Y3,C(H2)} + IN_{C(H2)}$	$N_{C(Y1)} = X_{C(Y1)} + P_{Y1,C(Y1)} + P_{Y2,C(Y1)} + P_{Y3,C(Y1)} + IN_{C(Y1)}$
Checks: Total stream amounts: $(N_{C(H1)}) - (N_{C(H2)} + N_{C(Y1)}) = 0$ The fraction dissolved components (e.g. unconverted Carbon) depend on the water split, which depends on the solids content (SC) of the bottoms stream.			

Nitrogen Mass Balance: Unit 4.2: Separator			
Nitrogen Fraction	**H1 :Solids matrix**	**H2: Wet subsurface matrix**	**Y1: Crust related product**
Biomass X_{Solids}	$X_{N(H1)} = X_{C(H1)} * f(X_{Solids})_{N/C}$	$X_{N(H2)} = X_{N(H1)} * eff_{H2}$	$X_{N(Y1)} = X_{N(H1)} * (1 - eff_{H2})$
Product P_{Y1}	$P_{Y1,N(H1)} = P_{Y1,C(H1)} * f(Y1)_{N/C}$	$P_{Y1,N(H2)} = P_{Y1,N(H1)} * (1 – eff_{Y1})$	$P_{Y1,N(Y1)} = P_{Y1,N(H1)} * eff_{Y1}$
Product P_{Y2}	$P_{Y2,N(H1)} = P_{Y2,C(H1)} * f(Y2)_{N/C}$	$P_{Y2,N(H2)} = P_{Y2,N(H1)} * (N_{W(H2)}/N_{W(H1)})$	$P_{Y2,N(Y1)} = P_{Y2,N(H1)} * (N_{W(Y1)}/N_{W(H1)})$
Product P_{Y3}	$P_{Y3,N(H1)} = P_{Y3,C(H1)} * f(Y3)_{N/C}$	$P_{Y3,N(H2)} = P_{Y3,N(H1)} * eff_{H2}$	$P_{Y3,N(Y1)} = P_{Y3,N(H1)} * (1 - eff_{H2})$
Unconverted Nitrogen	$IN_{N(H1)} = IN_{N(U)} - X_{N(H1)} – P_{Y1,N(H1)} – P_{Y2,N(H1)} – P_{Y3,N(H1)}$	$IN_{N(H2)} = IN_{N(H1)} * (N_{W(H2)}/N_{W(H1)})$	$IN_{C(Y1)} = IN_{C(H1)} * (N_{W(Y1)}/N_{W(H1)})$
Totals	$N_{N(H1)} = X_{N(H1)} + P_{Y1,N(H1)} + P_{Y2,N(H1)} + P_{Y3,N(H1)} + IN_{N(H1)}$	$N_{N(H2)} = X_{N(H2)} + P_{Y1,N(H2)} + P_{Y2,N(H2)} + P_{Y3,N(H2)} + IN_{N(H2)}$	$N_{N(Y1)} = X_{N(Y1)} + P_{Y1,N(Y1)} + P_{Y2,N(Y1)} + P_{Y3,N(Y1)} + IN_{N(Y1)}$
Checks: Total stream amounts: $(N_{N(H1)}) – (N_{N(H2)} + N_{N(Y1)}) = 0$			

Phosphorous Mass Balance: Unit 4.2: Separator			
Phosphorous Fraction	**H1 :Solids matrix**	**H2: Wet subsurface matrix**	**Y1: Crust related product**
Biomass X_{Solids}	$X_{P(H1)} = X_{C(H1)} * f(X_{Solids})_{P/C}$	$X_{P(H2)} = X_{P(H1)} * eff_{H2}$	$X_{P(Y1)} = X_{P(H1)} * (1 - eff_{H2})$
Product P_{Y1}	$P_{Y1,P(H1)} = P_{Y1,C(H1)} * f(Y1)_{P/C}$	$P_{Y1,P(H2)} = P_{Y1,P(H1)} * (1 – eff_{Y1})$	$P_{Y1,P(Y1)} = P_{Y1,P(H1)} * eff_{Y1}$
Product P_{Y2}	$P_{Y2,P(H1)} = P_{Y2,C(H1)} * f(Y2)_{P/C}$	$P_{Y2,P(H2)} = P_{Y2,P(H1)} * (N_{W(H2)}/N_{W(H1)})$	$P_{Y2,P(Y1)} = P_{Y2,P(H1)} * (N_{W(Y1)}/N_{W(H1)})$
Product P_{Y3}	$P_{Y3,P(H1)} = P_{Y3,C(H1)} * f(Y3)_{P/C}$	$P_{Y3,P(H2)} = P_{Y3,P(H1)} * eff_{H2}$	$P_{Y3,P(Y1)} = P_{Y3,P(H1)} * (1 - eff_{H2})$
Unconverted Phosphorous	$IN_{P(H1)} = IN_{P(U)} - X_{P(H1)} – P_{Y1,P(H1)} – P_{Y2,P(H1)} – P_{Y3,P(H1)}$	$IN_{P(H2)} = IN_{P(H1)} * (N_{W(H2)}/N_{W(H1)})$	$IN_{P(Y1)} = IN_{P(H1)} * (N_{W(Y1)}/N_{W(H1)})$
Totals	$N_{P(H1)} = X_{P(H1)} + P_{Y1,P(H1)} + P_{Y2,P(H1)} + P_{Y3,P(H1)} + IN_{P(H1)}$	$N_{P(H2)} = X_{P(H2)} + P_{Y1,P(H2)} + P_{Y2,P(H2)} + P_{Y3,P(H2)} + IN_{P(H2)}$	$N_{P(Y1)} = X_{P(Y1)} + P_{Y1,P(Y1)} + P_{Y2,P(Y1)} + P_{Y3,P(Y1)} + IN_{P(Y1)}$
Checks: Total stream amounts: $(N_{P(H1)}) – (N_{P(H2)} + N_{P(Y1)}) = 0$			

Water Mass Balance: Unit 4.2: Separator			
	H1 :Solids matrix	**H2: Wet subsurface matrix**	**Y1: Crust related product**
Total Water	$N_{W(H1)} = N_{W(U)} + N_{W(H5)}$	$N_{W(H2)} = N_{W(H1)} - N_{W(Y1)}$	$N_{W(Y1)} = (N_{C(Y1)}/C_{comp,solids}) * ((1-SC_{Y1})/SC_{Y1})$
Checks: Total stream amounts: $(N_{W(H1)}) – (N_{W(H2)} + N_{W(Y1)}) = 0$			

Table: F-18: Mass balance for Unit 4.3 Separator: liquor related solids bioreactor product Y2 from pressed cake

Carbon Mass Balance: Unit 4.3: Separator			
Carbon Fraction	**H2: Wet subsurface matrix**	**H3: Pressed cake**	**Y2: Liquor related product stream**
Biomass X_{Solids}	$X_{C(H2)} = X_{C(H1)} * eff_{H2}$	$X_{C(H3)} = X_{C(H2)} * eff_{H3}$	$X_{C(H3)} = X_{C(H2)} * (1 - eff_{H3})$
Product P_{Y1}	$P_{Y1,C(H2)} = P_{Y1,C(H1)} * (1 – eff_{Y1})$	$P_{Y1,C(H3)} = P_{Y1,C(H3)} * eff_{H3}$	$P_{Y1,C(H3)} = P_{Y1,C(H3)} * (1 - eff_{H3})$
Product P_{Y2}	$P_{Y2,C(H2)} = P_{Y2,C(H1)} * eff_{H2}$	$P_{Y2,C(H3)} = P_{Y2,C(H2)} * (N_{W(H3)}/N_{W(H2)})$	$P_{Y2,C(Y2)} = P_{Y2,C(H2)} * (N_{W(Y2)}/N_{W(H2)})$
Product P_{Y3}	$P_{Y3,C(H2)} = P_{Y3,C(H1)} * eff_{H2}$	$P_{Y3,C(H3)} = P_{Y3,C(H3)} * eff_{H3}$	$P_{Y3,C(H3)} = P_{Y3,C(H3)} * (1 - eff_{H3})$
Unconverted Carbon	$IN_{C(H2)} = IN_{C(H1)} * (N_{W(H2)}/N_{W(H1)})$	$IN_{C(H3)} = IN_{C(H2)} * (N_{W(H3)}/N_{W(H2)})$	$IN_{C(Y2)} = IN_{C(H2)} * (N_{W(Y2)}/N_{W(H2)})$
Totals	$N_{C(H2)} = X_{C(H2)} + P_{Y1,C(H2)} + P_{Y2,C(H2)} + P_{Y3,C(H2)} + IN_{C(H2)}$	$N_{C(H3)} = X_{C(H3)} + P_{Y1,C(H3)} + P_{Y2,C(H3)} + P_{Y3,C(H3)} + IN_{C(H3)}$	$N_{C(Y2)} = X_{C(Y2)} + P_{Y1,C(Y2)} + P_{Y2,C(Y2)} + P_{Y3,C(Y2)} + IN_{C(Y2)}$
Checks: Total stream amounts: $(N_{C(H2)}) – (N_{C(H3)} + N_{C(Y2)}) = 0$			
Nitrogen Mass Balance: Unit 4.3: Separator			
Nitrogen Fraction	**H2: Wet subsurface matrix**	**H3: Pressed cake**	**Y2: Liquor related product stream**
Biomass X_{Solids}	$X_{N(H2)} = X_{N(H1)} * eff_{H2}$	$X_{N(H3)} = X_{N(H2)} * eff_{H3}$	$X_{N(H3)} = X_{N(H2)} * (1 - eff_{H3})$
Product P_{Y1}	$P_{Y1,N(H2)} = P_{Y1,N(H1)} * (1 – eff_{Y1})$	$P_{Y1,N(H3)} = P_{Y1,N(H3)} * eff_{H3}$	$P_{Y1,N(H3)} = P_{Y1,N(H3)} * (1 - eff_{H3})$
Product P_{Y2}	$P_{Y2,N(H2)} = P_{Y2,N(H1)} * eff_{H2}$	$P_{Y2,N(H3)} = P_{Y2,N(H2)} * (N_{W(H3)}/N_{W(H2)})$	$P_{Y2,N(Y2)} = P_{Y2,N(H2)} * (N_{W(Y2)}/N_{W(H2)})$
Product P_{Y3}	$P_{Y3,N(H2)} = P_{Y3,N(H1)} * eff_{H2}$	$P_{Y3,N(H3)} = P_{Y3,N(H3)} * eff_{H3}$	$P_{Y3,N(H3)} = P_{Y3,N(H3)} * (1 - eff_{H3})$
Unconverted Nitrogen	$IN_{N(H2)} = IN_{N(H1)} * (N_{W(H2)}/N_{W(H1)})$	$IN_{N(H3)} = IN_{N(H2)} * (N_{W(H3)}/N_{W(H2)})$	$IN_{N(Y2)} = IN_{N(H2)} * (N_{W(Y2)}/N_{W(H2)})$
Totals	$N_{N(H2)} = X_{N(H2)} + P_{Y1,N(H2)} + P_{Y2,N(H2)} + P_{Y3,N(H2)} + IN_{N(H2)}$	$N_{N(H3)} = X_{N(H3)} + P_{Y1,N(H3)} + P_{Y2,N(H3)} + P_{Y3,N(H3)} + IN_{N(H3)}$	$N_{N(Y2)} = X_{N(Y2)} + P_{Y1,N(Y2)} + P_{Y2,N(Y2)} + P_{Y3,N(Y2)} + IN_{N(Y2)}$
Checks: Total stream amounts: $(N_{N(H2)}) – (N_{N(H3)} + N_{N(Y2)}) = 0$			
Phosphorous Mass Balance: Unit 4.3: Separator			
Phosphorous Fraction	**H2: Wet subsurface matrix**	**H3: Pressed cake**	**Y2: Liquor related product stream**
Biomass X_{Solids}	$X_{P(H2)} = X_{P(H1)} * eff_{H2}$	$X_{P(H3)} = X_{P(H2)} * eff_{H3}$	$X_{P(H3)} = X_{P(H2)} * (1 - eff_{H3})$
Product P_{Y1}	$P_{Y1,P(H2)} = P_{Y1,P(H1)} * (1 – eff_{Y1})$	$P_{Y1,P(H3)} = P_{Y1,P(H3)} * eff_{H3}$	$P_{Y1,P(H3)} = P_{Y1,P(H3)} * (1 - eff_{H3})$
Product P_{Y2}	$P_{Y2,P(H2)} = P_{Y2,P(H1)} * eff_{H2}$	$P_{Y2,P(H3)} = P_{Y2,P(H2)} * (N_{W(H3)}/N_{W(H2)})$	$P_{Y2,P(Y2)} = P_{Y2,P(H2)} * (N_{W(Y2)}/N_{W(H2)})$
Product P_{Y3}	$P_{Y3,P(H2)} = P_{Y3,P(H1)} * eff_{H2}$	$P_{Y3,P(H3)} = P_{Y3,P(H3)} * eff_{H3}$	$P_{Y3,P(H3)} = P_{Y3,P(H3)} * (1 - eff_{H3})$
Unconverted Phosphorous	$IN_{P(H2)} = IN_{P(H1)} * (N_{W(H2)}/N_{W(H1)})$	$IN_{P(H3)} = IN_{P(H2)} * (N_{W(H3)}/N_{W(H2)})$	$IN_{P(Y2)} = IN_{P(H2)} * (N_{W(Y2)}/N_{W(H2)})$
Totals	$N_{P(H2)} = X_{P(H2)} + P_{Y1,P(H2)} + P_{Y2,P(H2)} + P_{Y3,P(H2)} + IN_{P(H2)}$	$N_{P(H3)} = X_{P(H3)} + P_{Y1,P(H3)} + P_{Y2,P(H3)} + P_{Y3,P(H3)} + IN_{P(H3)}$	$N_{PY2} = X_{P(Y2)} + P_{Y1,P(Y2)} + P_{Y2,P(Y2)} + P_{Y3,P(Y2)} + IN_{P(Y2)}$
Checks: Total stream amounts: (NP(H2)) – (NP(H3) + NP(Y2)) = 0			

Water Mass Balance: Unit 4.3: Separator			
	H2: Wet subsurface matrix	**H3: Pressed cake**	**Y2: Liquor related product stream**
Total Water	$N_{W(H2)} = N_{W(H1)} - N_{W(Y1)}$	$N_{W(H3)} = (N_{C(H3)}/C_{comp,solids})*((1-SC_{H3})/SC_{H3})$	$N_{W(Y2)} = N_{W(H2)} - N_{W(H3)}$
Checks: Total stream amounts: $(N_{W(H2)}) – (N_{W(H3)} + N_{W(Y2)}) = 0$			

Table: F-19: Mass balance for Unit 4.4 Separator: cake related solids bioreactor product Y3 from compost Y4

Carbon Mass Balance: Unit 4.4: Separator			
Carbon Fraction	**H3: Pressed cake**	**Y3: Cake related product stream**	**Y4: Compost**
Biomass X_{Solids}	$X_{C(H3)} = X_{C(H2)} * eff_{H3}$	$X_{C(Y3)} = X_{C(H3)} * (1 – eff_{Y4})$	$X_{C(Y4)} = X_{C(H3)} * eff_{Y4}$
Product P_{Y1}	$P_{Y1,C(H3)} = P_{Y1,C(H3)} * eff_{H3}$	$P_{Y1,C(Y3)} = P_{Y1,C(H3)} * (1 - eff_{Y4})$	$P_{Y1,C(Y4)} = P_{Y1,C(H3)} * eff_{Y4}$
Product P_{Y2}	$P_{Y2,C(H3)} = P_{Y2,C(H2)} * (N_{W(H3)}/N_{W(H2)})$	$P_{Y2,C(Y3)} = P_{Y2,C(H3)} * (1 - eff_{Y4})$	$P_{Y2,C(Y4)} = P_{Y2,C(H3)} * eff_{Y4}$
Product P_{Y3}	$P_{Y3,C(H3)} = P_{Y3,C(H3)} * eff_{H3}$	$P_{Y3,C(Y3)} = P_{Y3,C(H3)} *eff_{Y3}$	$P_{Y3,C(Y3)} = P_{Y3,C(H3)} *(1 - eff_{Y3})$
Unconverted Carbon	$IN_{C(H3)} = IN_{C(H2)} * (N_{W(H3)}/N_{W(H2)})$	$IN_{C(Y3)} = IN_{C(H3)} * (N_{W(Y3)}/N_{W(H3)})$	$IN_{C(Y4)} = IN_{C(H3)} * (N_{W(Y4)}/N_{W(H3)})$
Product P_{Y4}			$P_{Y4,C(Y4)} = X_{C(Y4)} + P_{Y1,C(Y4)} + P_{Y2,C(Y4)} + P_{Y3,C(Y4)} + IN_{C(Y4)}$
Totals	$N_{C(H3)} = X_{C(H3)} + P_{Y1,C(H3)} + P_{Y2,C(H3)} + P_{Y3,C(H3)} + IN_{C(H3)}$	$N_{C(Y3)} = X_{C(Y3)} + P_{Y1,C(Y3)} + P_{Y2,C(Y3)} + P_{Y3,C(Y3)} + IN_{C(Y3)}$	$N_{C(Y4)} = P_{Y4,C(Y4)}$
Checks: Total stream amounts: $(N_{C(H3)}) – (N_{C(Y3)} + N_{C(Y4)}) = 0$			
Nitrogen Mass Balance: Unit 4.4: Separator			
Nitrogen Fraction	**H3: Pressed cake**	**Y3: Cake related product stream**	**Y4: Compost**
Biomass X_{Solids}	$X_{N(H3)} = X_{N(H2)} * eff_{H3}$	$X_{N(Y3)} = X_{N(H3)} * (1 – eff_{Y4})$	$X_{N(Y4)} = X_{N(H3)} * eff_{Y4}$
Product P_{Y1}	$P_{Y1,N(H3)} = P_{Y1,N(H3)} * eff_{H3}$	$P_{Y1,N(Y3)} = P_{Y1,N(H3)} * (1 - eff_{Y4})$	$P_{Y1,N(Y4)} = P_{Y1,N(H3)} * eff_{Y4}$
Product P_{Y2}	$P_{Y2,N(H3)} = P_{Y2,N(H2)} * (N_{W(H3)}/N_{W(H2)})$	$P_{Y2,N(Y3)} = P_{Y2,N(H3)} * (1 - eff_{Y4})$	$P_{Y2,N(Y4)} = P_{Y2,N(H3)} * eff_{Y4}$
Product P_{Y3}	$P_{Y3,N(H3)} = P_{Y3,N(H3)} * eff_{H3}$	$P_{Y3,N(Y3)} = P_{Y3,N(H3)} *eff_{Y3}$	$P_{Y3,N(Y3)} = P_{Y3,N(H3)} *(1 - eff_{Y3})$
Unconverted Nitrogen	$IN_{N(H3)} = IN_{N(H2)} * (N_{W(H3)}/N_{W(H2)})$	$IN_{N(Y3)} = IN_{N(H3)} * (N_{W(Y3)}/N_{W(H3)})$	$IN_{N(Y4)} = IN_{N(H3)} * (N_{W(Y4)}/N_{W(H3)})$
Product P_{Y4}			$P_{Y4,N(Y4)} = X_{N(Y4)} + P_{Y1,N(Y4)} + P_{Y2,N(Y4)} + P_{Y3,N(Y4)} + IN_{N(Y4)}$
Totals	$N_{N(H3)} = X_{N(H3)} + P_{Y1,N(H3)} + P_{Y2,N(H3)} + P_{Y3,N(H3)} + IN_{N(H3)}$	$N_{N(Y3)} = X_{N(Y3)} + P_{Y1,N(Y3)} + P_{Y2,N(Y3)} + P_{Y3,N(Y3)} + IN_{N(Y3)}$	$N_{C(Y4)} = P_{Y4,N(Y4)}$
Checks: Total stream amounts: $(N_{N(H3)}) – (N_{N(Y3)} + N_{N(Y4)}) = 0$			

Phosphorous Mass Balance: Unit 4.4: Separator			
Phosphorous Fraction	**H3: Pressed cake**	**Y3: Cake related product stream**	**Y4: Compost**
Biomass X_{Solids}	$X_{P(H3)} = X_{P(H2)} * eff_{H3}$	$X_{P(Y3)} = X_{P(H3)} * (1 – eff_{Y4})$	$X_{P(Y4)} = X_{P(H3)} * eff_{Y4}$
Product P_{Y1}	$P_{Y1,P(H3)} = P_{Y1,P(H3)} * eff_{H3}$	$P_{Y1,P(Y3)} = P_{Y1,P(H3)} * (1 - eff_{Y4})$	$P_{Y1,P(Y4)} = P_{Y1,P(H3)} * eff_{Y4}$
Product P_{Y2}	$P_{Y2,P(H3)} = P_{Y2,P(H2)} * (N_{W(H3)}/N_{W(H2)})$	$P_{Y2,P(Y3)} = P_{Y2,P(H3)} * (1 - eff_{Y4})$	$P_{Y2,P(Y4)} = P_{Y2,P(H3)} * eff_{Y4}$
Product P_{Y3}	$P_{Y3,P(H3)} = P_{Y3,P(H3)} * eff_{H3}$	$P_{Y3,P(Y3)} = P_{Y3,P(H3)} *eff_{Y3}$	$P_{Y3,P(Y3)} = P_{Y3,P(H3)} *(1 - eff_{Y3})$
Unconverted Phosphorous	$IN_{P(H3)} = IN_{P(H2)} * (N_{W(H3)}/N_{W(H2)})$	$IN_{P(Y3)} = IN_{P(H3)} * (N_{W(Y3)}/N_{W(H3)})$	$IN_{P(Y4)} = IN_{P(H3)} * (N_{W(Y4)}/N_{W(H3)})$
Product P_{Y4}			$P_{Y4,P(Y4)} = X_{P(Y4)} + P_{Y1,P(Y4)} + P_{Y2,P(Y4)} + P_{Y3,P(Y4)} + IN_{P(Y4)}$
Totals	$N_{P(H3)} = X_{P(H3)} + P_{Y1,P(H3)} + P_{Y2,P(H3)} + P_{Y3,P(H3)} + IN_{P(H3)}$	$N_{P(Y3)} = X_{P(Y3)} + P_{Y1,P(Y3)} + P_{Y2,P(Y3)} + P_{Y3,P(Y3)} + IN_{P(Y3)}$	$N_{P(Y4)} = P_{Y4,P(Y4)}$
Checks: Total stream amounts: $(N_{P(H3)}) – (N_{P(Y3)} + N_{P(Y4)}) = 0$			

Water Mass Balance: Unit 4.4: Separator			
	H3: Pressed cake	**Y3: Cake related product stream**	**Y4: Compost**
Total Water	$N_{W(H3)} = (N_{C(H3)}/C_{comp, solids})*((1-SC_{H3})/SC_{H3})$	$N_{W(Y3)} = (N_{C(Y3)}/C_{comp, solids})*((1-SC_{Y3})/SC_{Y3})$	$N_{W(Y4)} = N_{C(H3)} - N_{W(Y3)}$
Checks: Total stream amounts: $(N_{W(H3)}) – (N_{W(Y3)} + N_{W(Y4)}) = 0$			

G SUPPLEMENTARY DATA FOR SELECTION OF MASS BALANCE FACTORS

G.1 Supporting data for Section 8.1 Unit Mass Balances

G.1.1 Bacterial Bioreactor Factors for Mass Balances

These factors are all enumerated in Chapter 8 Section 8.1.1 as a full example of the requirements.

G.1.2 Algal Bioreactor Factors for Mass Balances

Table: G-1: Calculation of g-C-algal biomass/g-C-substrate using Bumbak, et al. (2011) values

Algal biomass (g/L)	Total substrate (g/L)	Type of substrate	g C biomass biomass C fraction:	g C substrate	g-C-algal biomass/g-C-substrate
83	217	ethanol	43.16	113.22	0.381
26	82	glucose	13.52	32.80	0.412
116	356	glucose (molasses)	60.32	142.40	0.424
72	178	glucose	37.44	71.20	0.526
116.2	224	glucose	60.42	89.60	0.674
109	182	acetic acid	56.68	72.80	0.779
165.8	253	glucose	86.22	101.20	0.852
109	157	glucose	56.68	62.80	0.903
40	45	glucose	20.80	18.00	1.156
117.2	130	glucose	60.94	52.00	1.172
22.1	22	glucose	11.49	8.80	1.306
51.2	n	glucose	26.62		not used
48	n	glucose	24.96		not used
84	n	glucose	43.68		not used
51.8	n	glucose	26.94		not used
42	n	carob	21.84		not used
48	n	glucose	24.96		not used
39.5	n	ethanol	20.54		not used
Average					

Algal biomass (g/L)	Total substrate (g/L)	Type of substrate	g C biomass biomass C fraction:	g C substrate	g-C-algal biomass/g-C-substrate
74.5					**0.78**
glucose C fraction: 0.40 **ethanol C fraction: 0.52** **acetic acid C fraction : 0.44**					

The tabulated factors for the algal bioreactor mass balances follow. These tables match those in Section 8.1.1and Section 8.1.2 refers.

Table: G-2: Conversion of composition to mass percent for algal biomass

Element	Composition: Normalised to P (Park, et al., 2011) (mol element per mol C in molecule)	Molar mass of element	Mass (g element/mol molecule)	Biomass Composition (mass fraction: g / g total dry biomass) values used in model
C	106	12	1 272	0.520 = TOC algal biomass
N	16	14	224	0.092
P	1	31	31	0.013
H	181	1	181	0.074
O	46	16	736	0.300
Total			**2 444**	**1.00**

Table: G-3: Oil content and lipid productivity of some microalgae species (adapted from Olguín (2012))

Cultivation conditions	Range of oil content (% dry weight) values in literature	Selected factor value for start-point
Freshwater, N starvation	42 - 60	
Freshwater, N deficient	43	
Freshwater, nutrient sufficient	21 - 38	
Heterotrophic culture	20 - 50	20
Marine, N starvation	41- 73	
Marine, nutrient sufficiency	29 - 67	

G.1.3 Macrophyte Bioreactor Factors for Mass Balances

The tabulated factors for the algal bioreactor mass balances follow. These tables match those in Section 8.1.1 and Section 8.1.3 refers.

Table: G-4: Lignin carbon content

	C	H	O	Total	Fraction C (g-C/g-lignin)
molecular mass element	12	1	16	-	
lignin mol formula	11	14	1	$C_9H_{10}O_2$, $C_{10}H_{12}O_3$, $C_{11}H_{14}O$	
lignin mass fraction	132	14	16	162	0.814815

Table: G-5: Macrophyte (flax) carbon content

0.00735	fraction N average flax
0.00023	fraction P grass
assume remainder lignin	
0.99242	lignin
0.814815	C content
0.808639	fraction C

Table: G-6: Macrophyte (flax) plant biomass (Dodkins & Mendzil, 2014b)

shoots	roots	total	g/m^2
86.3	43.4	129.7	
131.4	207.6	339	
121.7	48.1	169.8	
269	58.9	327.9	
72	57.7	129.7	
1 528	329	1 857	
2 350	533	2 883	
1 113	299	1412	
834	184	1 018	
		918.4556	**g/m^2**
		0.92	kg/m^2
		0.1667	m^2 planted area
		0.15	kg per m^3 influent
		2	harvests per year
		0.31	kg per m^3 influent

Table: G-7: Macrophyte (flax) CO_2 uptake

918.4556	g/m^2
0.92	kg/m^2 total biomass
0.1667	m^2 planted area, using a depth of 1.2m and 20% planting cover
2	harvests per year
0.306	kg total plant mass per m^3 influent, per year
0.81	C composition of macrophyte
0.248033	kg C per m^3 influent, per year
365	days per year (averaged growth)
0.0006795	kg C uptake per m^3 influent, per day

G.1.4 Solids Bioreactor Factors for Mass Balances

The tabulated factors for the algal bioreactor mass balances follow. These tables match those in Section 8.1.1 and Section 8.1.4 refers.

Table: G-8: Production of organic acids by solid-state fermentation with different substrates (partial) (Pandey, et al., 2010)

Microorganism	Bioreactor	Substrate/Support	Acid production (g/kg)
Citric Acid			
A. niger LPB 21	Horizontal drum	Treated cassava bagasse	269
A. niger LPB 2001	Packed bed	Cassava bagasse	309
A. niger NRRL 328	Packed-bed column		816
A. niger NRRL 567	(flow-rate of 65mL/min)		771
A. niger LPB 21	Packed bed	Mussel processing wastes (polyurethane foams)	179
Lactic Acid			
Lactobacillus delbrueckii	Erlenmeyer flask	Sugarcane bagasse (cassava bagasse hydrolysate)	249
Oxalic Acid			
A. niger SL 1	Erlenmeyer flask	Sweet potato	26.4
Gluconic Acid			
A. niger ATCC 10577	Erlenmeyer flask	Fig	490

Table: G-9: Comparison of composts from water hyacinth (Lindsey & Hirt, 1999)

Contents	Water hyacinth aerobic compost	Water hyacinth anaerobic compost	Cow dung compost
N	1.1	1.9	0.5
P_2O_5	0.8	1.0	0.3
CaO	3.2	4.6	0.2
K_2O	2.4	2.9	0.3
MgO	1.3	1.8	-
Organic matter	84.2	86.8	89.3

G.2 Supplementary information for Section 8.2

The Mars confection factory wastewater PHA production process

Based on the feast-famine principle to produce PHA a three-step process was proposed by Tamis, et al. (2014):

1. anaerobic fermentation to direct the many organic compounds in wastewater to VFA
2. enrichment of biomass with superior PHA-producing capacity in a selective environment and
3. maximization of the PHA content of the biomass in an accumulation step by feeding the enriched biomass with VFA in fed-batch mode in absence of a nitrogen source

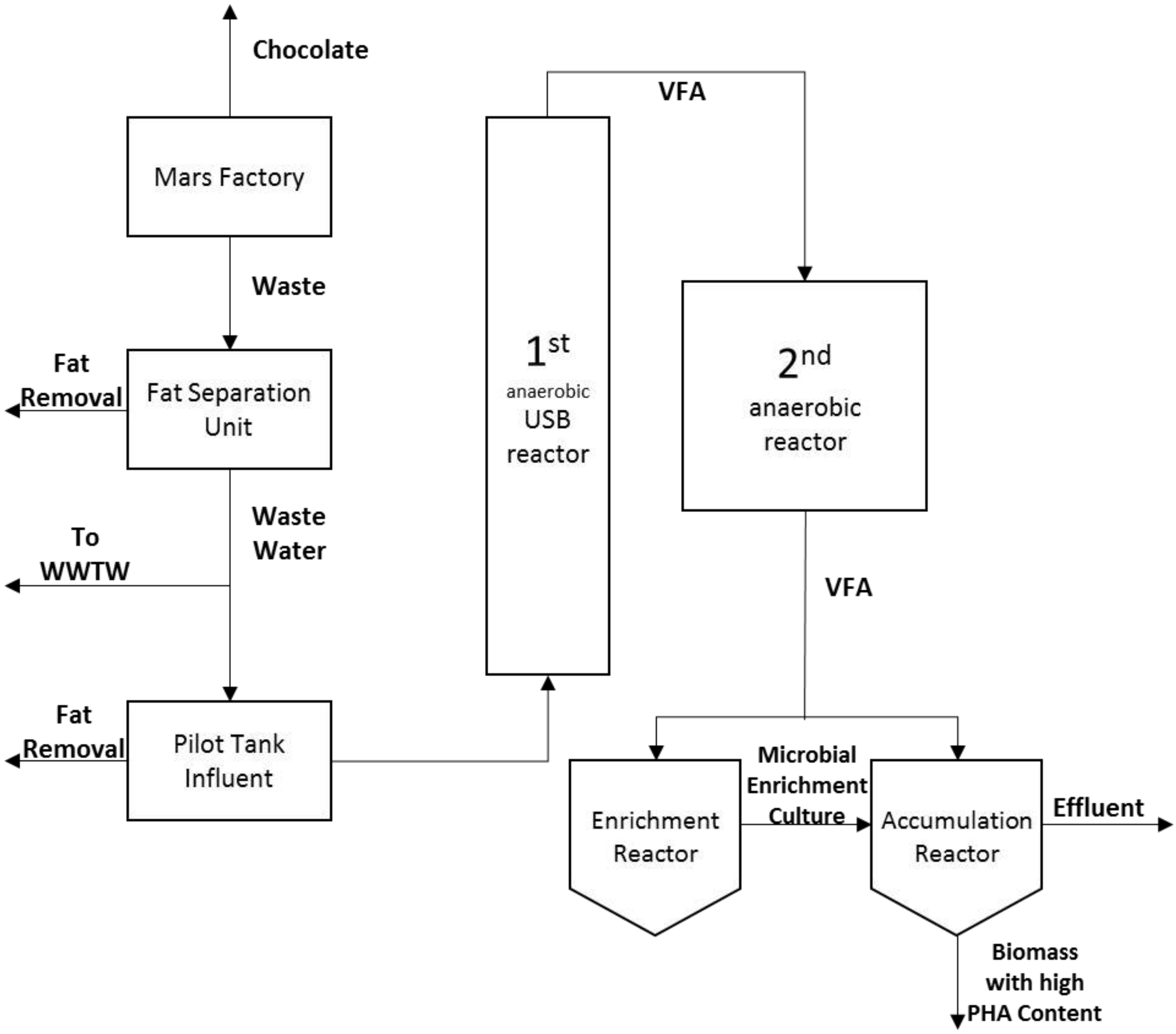


Figure: G-1: Three-step process to produce PHA from Mars factory wastewater (Tamis, et al., 2014)

Thus:

COD → (step 1) → VFA → (step 2) → biomass → (Step 3) → PHB accumulation

Step 1: 0.91 g VFA COD/g-ww-COD

Step 2: Split streams. Biomass yield 0.34 g biomass / g COD, the other stream is fed as substrate to enable PHB accumulation.

Step 3: PHB accumulation, 70wt%, yield 0.44 g-PHA/g COD.

Fernández-Dacosta, et al. (2015) performed a conceptual process design based on data from laboratory and pilot plant scale operations (Tamis, et al., 2014) using real industrial wastewater, and report a PHA yield of 77% dry cell weight. The PHA was polyhydroxybutyrate (PHB), produced in an aerobic conversion reaction using three sequential fermentation steps in a microbial enrichment culture.

The wastewater from the Mars factory was pre-treated in a flotation-based fat separation unit before entering the influent tank of the pilot installation, but no primary settlement of solids was employed. Subsequently, maximization of the VFA concentrations in the wastewater was pursued by application of two anaerobic reactors, operated in series.

Anaerobic fermentation

Firstly, the wastewater was fed to an upflow sludge blanket (USB) type reactor with a working volume of 60 L. The hydraulic retention time (HRT) of the reactor was 4 h and the solid retention time (SRT) was maintained around 4 days by manual sludge removal. To keep the reactor effluent nitrogen depleted (favourable for use in the accumulation reactor later in the process) the target COD:N mass ratio was around 300:1. A nutrient solution containing 3 M nitrogen in the form of urea, 0.3 M phosphate, 0.3 M $MgSO_4$, 0.2 M K_2SO_4, and trace elements (64 mM $FeCl_3$, 3 mM $ZnSO_4$, 2.7 mM H_3BO_3, 2.1 mM $NiCl_2$, 1.5 mM $CoSO_4$, 0.6 mM $CuSO_4$, 0.8 mM Na_2MoO_4) was provided to the reactor.

To buffer the volumes of available VFA substrate for the enrichment and accumulation processes, and to secure full conversion of the fermentable COD to VFA, a second anaerobic fermentation reactor was included in the system, comprising an anaerobic tank with a liquid volume of 1 500 L with a hydraulic retention time of 4 days. After this second step the fermented wastewater was used as a substrate for the enrichment and accumulation reactors.

Enrichment reactor

The enrichment reactor (working volume 200 L) was operated as a sequencing batch reactor (SBR) with a cycle length of 12 h and a solid and liquid retention time of 24 h. The operational cycle consisted of a feed phase, a reaction phase and an effluent phase. During the feed phase 55 L of acidified wastewater (from the second anaerobic fermentation reactor) together with 45 L of clean process water was added using a pH controlled pump. The dilution of the substrate with clean process water was to prevent possible oxygen limitation at high COD concentrations.

The concentration of ammonium was maintained between 10 and 30 mg-N/L at the end of the cycle, through dosing after measurement, if necessary. The resulting COD:N mass ratio in the feed stream was approximately 25:1. It was assumed that ammonium was the limiting growth nutrient with other elements required for microbial growth present in excess.

Accumulation reactor

To maximize the PHA content in the cells, a fed-batch reactor (working volume 200 L) was operated as an accumulation step.

These three steps are seen as a ‘black box’ bioreactor for the purposes of the model. In order to approximate continuous operation a feed and exit-stream rate of 100 L per day is assumed.

The parameters

The average soluble COD (sCOD) of the wastewater that was fed to the anaerobic fermentation varied strongly over time (intrinsic to factory operation, e.g. semi-periodic cleaning of equipment) with an average concentration of 7.8 ± 4.1 g-COD/L (average ± standard deviation over the data set). In addition to soluble COD, a concentration of 0.8 ± 0.5 g-COD/L that could not pass a 0.45 um pore size filter. The soluble nitrogen concentration in the wastewater was negligible (<1 mg/L).

A process yield over the whole process (including anaerobic pre-treatment, enrichment and accumulation steps) of 0.30 ± 0.04 g-COD-PHA/g-COD was established (equal to 0.18 g-PHA/g-COD using 1.7 g-COD/g-PHA). Another significant part of the influent COD (0.11 ± 0.02 g-COD-X/g-COD) was used for biomass production in the enrichment step. No significant COD loss was observed in the anaerobic fermentation steps. The COD can be closed by the amount of COD oxidised in the enrichment and accumulation steps (0.55 ± 0.10 g-COD-oxidised/g-COD-substrate).

Using an initial biomass concentration of 1.5 g/L and a PHA content of 0.7 g-PHA/g-VSS achieved in 4 h, a volumetric productivity of approximately 0.5 g/L/h can be estimated.

Converting this process to the values required by the model, the steps are converted to an overall yield. Product 1 is PHA, Product 2 is unconverted VFA. The purification method used was alkali-surfactant treatment. The authors note that the quality of the produced PHA may not be sufficient for use in thermoplastic application. Nevertheless, the product can be considered as an intermediate for the production of chemical building blocks (for example methyl crotonate and methyl acrylate), where the final quality is not a limiting factor. The total production cost for PHA in this paper came to 1.40 €/kg, with 70% of this cost attributed to the downstream processing components.

Table: G-10: N and P addition through 3M stock solution

300	COD : N	final ratio	
3	M (mol/ℓ) N (urea)		
60	g/mol molar mass of urea		
180	g/ℓ urea		
0.467	ratio N/urea		
84.06	g/ℓ N		
0.3	M PO_4^-		
95	molar mass of PO4		
28.5	g/ℓ PO_4		
0.326	ratio P/PO_4		
9.291	g/ℓ P		
8.6	g(/ℓ) COD incoming		
0.029	g/ℓ N needed		
0.000341	ℓ N solution added per ℓ COD (c1v1 = c2v2, v2 = 1ℓ unit volume)		
0.003168	g/L P delivered with N		

H CREATING SANKEY DIAGRAMS FOR VISUALISATION OF WASTEWATER BIOREFINERY MASS BALANCES

Understanding mass balances is core to good engineering, but being able to visualise them makes it easier to communicate with stakeholders. The Sankey diagram is named after Irish engineer Captain Matthew Henry Phineas Riall Sankey, who used this type of diagram in 1898 to show the energy efficiency of a steam engine (Wikipedia, n.d.).

H.1 Procedure to Display Sankey Files

Sankey diagrams are based on html files, the language of the internet. The diagrams use data that is written in a JavaScript Object Notation (json) format, which is similar to csv files and can be exported from a spreadsheet. This data is then integrated into the html file, using D3, a JavaScript-based tool for loading data into a web page, and generating visuals from that data (JavaScript, n.d.).

The Sankey diagrams in this project are based on a json template from Malcolm MacLean's book (Maclean, n.d.), and uses work from Mike Bostock (2012). A very useful step by step guide is published online by Scott Murray (2013). The diagrams do not need the internet to be displayed, as long as the required background information is made available. In order to do this, the web browser needs to know where to access the local information on the local computer, called a local host.

One way to achieve this is to direct the command window of the computer (called a terminal, Figure: H-1). This example uses Ubuntu, and the information is stored in a folder called “sankey” in a folder called “project_d3” which lives in the “Desktop” folder:

> cd ~/Desktop/project_d3/sankey

> python -m SimpleHTTPServer 8888 &

Or on Windows 10:

> cd Desktop/project_d3/sankey

> py -m http.server 8888 &r 8888 &

Then the file can be opened in the web browser, for example:

file:///home/indiebio/Desktop/project_d3/sankey/Sankey_Bacterial_C_4may16.htm

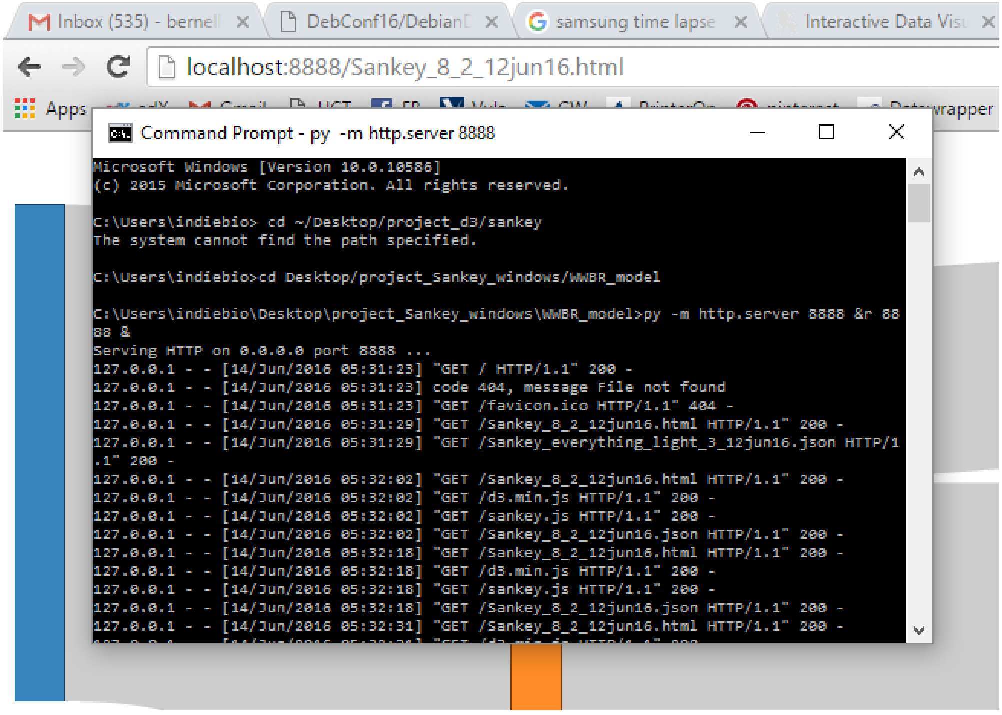


Figure: H-1: Screenshot of the terminal command to use the web browser with the local computer

H.2 Code Used to Import Data into Sankey (.json file)

This is the file that brings the output from the spreadsheet into the Sankey diagram. It is exported (currently manually) from the relevant sheet into a .csv file and renamed as (or with copy and paste into) a .json file, which is then called in the .html file. Therefore it is important that the file names match perfectly.

For the overall model, the nodes are labelled to be consistent with the stream numbers in the detailed flowsheet, with the carbon values allocated the 0 – 99 block, the nitrogen values the 100 – 199 block, the phosphate values the 200 – 299 block and water the 300 – 399 block. Only the first 50 of each block is allocated to streams, with the remaining 50 of each block merely being placeholders to allow the code to function properly. This huge number of nodes may look intimidating, but it actually increases ease of use.

The example shown below uses the data for the single bioreactor train (Section 8.2), which is simpler than the data for the integrated WWBR (Sections 8.3 and 8.4), but follows the same format. The links contain the information about the relationship between the nodes, and form the output values from the spreadsheet.

Filename: `Sankey_8_2_12jun16.json`


```
{
"nodes":[
{"node":0,"name":"A. Incoming wastewater , C"},
{"node":1,"name":"B2. Supplementary substrate, C"},
{"node":2,"name":"1.1. Bacterial bioreactor, C"},
{"node":3,"name":"C5: CO2, C"},
{"node":4,"name":"C6: H2O, C"},
{"node":5,"name":"Separation 1.2, C"},
{"node":6,"name":"Separation 1.3, C"},
{"node":7,"name":"D1: Almost compliant effluent, C"},
{"node":8,"name":"V1: Bacterial product stream, C"},
{"node":9,"name":"U2: Biosolids from bacterial reactor ,
C"},
{"node":10,"name":"A. Incoming wastewater , N"},
{"node":11,"name":"B2. Supplementary substrate, N"},
{"node":12,"name":"1.1. Bacterial bioreactor, N"},
{"node":13,"name":"C5: CO2, N"},
{"node":14,"name":"C6: H2O, N"},
{"node":15,"name":"Separation 1.2, N"},
{"node":16,"name":"Separation 1.3, N"},
{"node":17,"name":"D1: Almost compliant effluent, N"},
{"node":18,"name":"V1: Bacterial product stream, N"},
{"node":19,"name":"U2: Biosolids from bacterial reactor ,
N"},
{"node":20,"name":"A. Incoming wastewater , P"},
```

```
{"node":21,"name":"B2. Supplementary substrate, P"},
{"node":22,"name":"1.1. Bacterial bioreactor, P"},
{"node":23,"name":"C5: CO2, P"},
{"node":24,"name":"C6: H2O, P"},
{"node":25,"name":"Separation 1.2, P"},
{"node":26,"name":"Separation 1.3, P"},
{"node":27,"name":"D1: Almost compliant effluent, P"},
{"node":28,"name":"V1: Bacterial product stream, P"},
{"node":29,"name":"U2: Biosolids from bacterial reactor , P"}

],
"links":[

{"source":0,"target":2,"value":3210},
{"source":1,"target":2,"value":147.6},
{"source":2,"target":3,"value":1336.3248},
{"source":2,"target":4,"value":0},
{"source":2,"target":5,"value":2021.2752},
{"source":5,"target":6,"value":1010.6376},
{"source":5,"target":7,"value":1010.6376},
{"source":6,"target":8,"value":724.65402},
{"source":6,"target":9,"value":285.98358},
{"source":10,"target":12,"value":0},
{"source":11,"target":12,"value":344.4},
{"source":12,"target":13,"value":0},
{"source":12,"target":14,"value":0},
{"source":12,"target":15,"value":344.400096881293},
{"source":15,"target":16,"value":179.457634877693},
{"source":15,"target":17,"value":164.9424620036},
{"source":16,"target":18,"value":117.119976269872},
{"source":16,"target":19,"value":62.3376586078212},

{"source":20,"target":22,"value":0},
{"source":21,"target":22,"value":38.13},
{"source":22,"target":23,"value":0},
{"source":22,"target":24,"value":0},
{"source":22,"target":25,"value":38.1300968812927},
{"source":25,"target":26,"value":13.2877019011627},
{"source":25,"target":27,"value":24.8423949801301},
{"source":26,"target":28,"value":6.67695947736777},
{"source":26,"target":29,"value":6.61074242379491}

]}
```

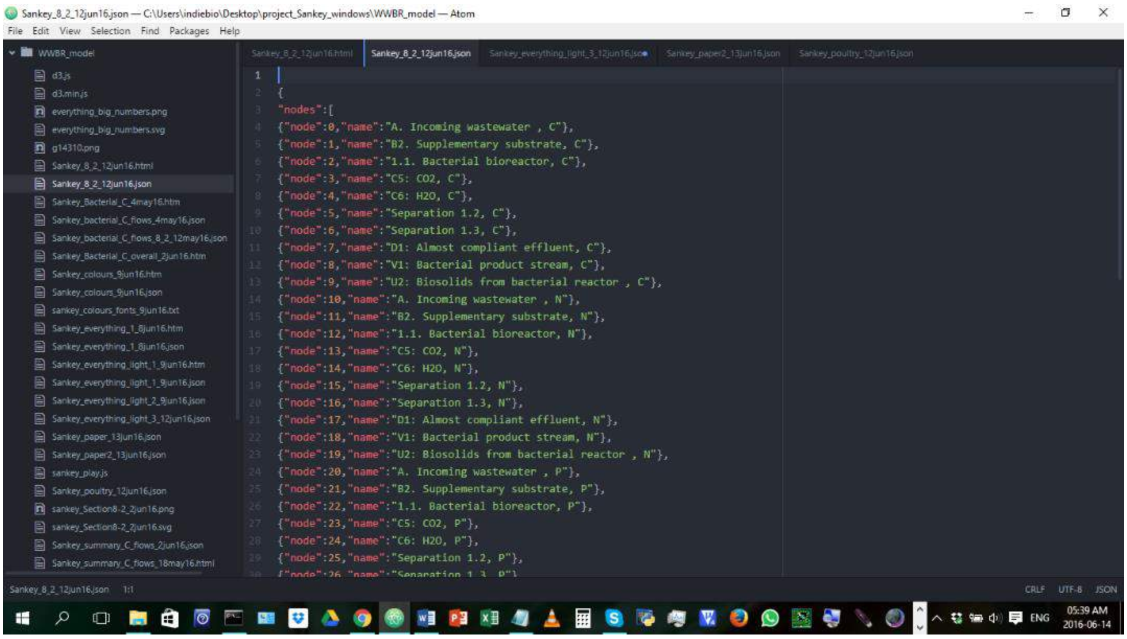



Figure: H-2: Screenshot of what the .json code looks like in an html text editor

H.3 Sankey Code Used to Process Data into Diagram (.html file)

The code below is what takes the data code (Section H.2) and uses it to create the Sankey diagram. (See Sections 8.2.2, 8.3.3, 8.4.2 and 8.4.3 for examples of the diagrams.)

```
<!DOCTYPE html>
<meta charset="utf-8">
<title>SANKEY_C_Bacterial_flows
4may16</title>
<style>

.node rect {

  cursor: move;
  fill-opacity: .9;
  shape-rendering: crispEdges;
}

.node text {
  pointer-events: none;
  text-shadow: 0 1px 0 #fff;
  font: 24px sans-serif;
}

.link {
  fill: none;
  stroke: #000;
  stroke-opacity: .2;
}

.link:hover {
  stroke-opacity: .5;
}

</style>
<header>
  <a
href="http://localhost:8888/">proje
ct d3</a>
  <span class="date">4 May
2016</span>
</header>

<h1>Wastewater Biorefinery Sankey
diagram: Bacterial Bioreactor
Carbon flows</h1>
<p>From formatted JSON, using
Malcolm MacLean's book.<br>
</p>
<body>

<p id="chart">

<script src="d3.min.js"></script>
<script src="sankey.js"></script>
<script> // These are the files you
draw the design from - they have to
be in the folder. These are not the
data (mass flow) files.

var units = "kg C/day";

var margin = {top: 10, right: 10,
bottom: 10, left: 10},
    width = 1200 - margin.left -
margin.right,
    height = 600 - margin.top -
margin.bottom;

var formatNumber =
d3.format(",.0f"),    // zero
decimal places
    format = function(d) { return
formatNumber(d) + " " + units; },
    color = d3.scale.category20();

// append the svg canvas to the page
var svg =
d3.select("#chart").append("svg")
    .attr("width", width +
margin.left + margin.right)
    .attr("height", height +
margin.top + margin.bottom)
  .append("g")
    .attr("transform",
          "translate(" +
margin.left + "," + margin.top +
")");

// Set the sankey diagram properties
var sankey = d3.sankey()
    .nodeWidth(36)
    .nodePadding(40)
    .size([width, height]);

var path = sankey.link();

// load the data (these are your
unit processes.)
// TO DO: Write a macro that can
create this file with the correct
syntax from the excel sheet.)
d3.json("Sankey_8_2_12jun16.json",
function(error, graph) {

  sankey
      .nodes(graph.nodes)
      .links(graph.links)
      .layout(32);

// add in the links
  var link =
svg.append("g").selectAll(".link")
      .data(graph.links)
    .enter().append("path")
      .attr("class", "link")
      .attr("d", path)
      .style("stroke-width",
function(d) { return Math.max(1,
d.dy); })
      .sort(function(a, b) { return
b.dy - a.dy; });

// add the link titles
  link.append("title")
        .text(function(d) {
                    return
d.source.name + " → " +
                d.target.name +
"\n" + format(d.value); });

// add in the nodes
  var node =
svg.append("g").selectAll(".node")
      .data(graph.nodes)
    .enter().append("g")
      .attr("class", "node")
      .attr("transform",
function(d) {
                return
"translate(" + d.x + "," + d.y + ")";
})
    .call(d3.behavior.drag()
      .origin(function(d) { return
d; })
      .on("dragstart", function() {

this.parentNode.appendChild(this);
})
      .on("drag", dragmove));

// add the rectangles for the nodes
  node.append("rect")
      .attr("height", function(d) {
return d.dy; })
      .attr("width",
sankey.nodeWidth())
      .style("fill", function(d) {
                return
d.color = color(d.name.replace(/
.*/, "")); })
      .style("stroke", function(d)
{
                return
d3.rgb(d.color).darker(2); })
    .append("title")
      .text(function(d) {
                return d.name
+ "\n" + format(d.value); });

// add in the title for the nodes
  node.append("text")
      .attr("x", -6)
      .attr("y", function(d) {
return d.dy / 2; })
      .attr("dy", ".35em")
      .attr("text-anchor", "end")
      .attr("transform", null)
      .text(function(d) { return
d.name; })
    .filter(function(d) { return
d.x < width / 2; })
      .attr("x", 6 +
sankey.nodeWidth())
      .attr("text-anchor",
"start");

// the function for moving the nodes
  function dragmove(d) {

d3.select(this).attr("transform",
        "translate(" + d.x + "," +
(
                d.y = Math.max(0,
Math.min(height - d.dy,
d3.event.y))
            ) + ")");
    sankey.relayout();
    link.attr("d", path);
  }

});
</script>

</body>
</html>
```

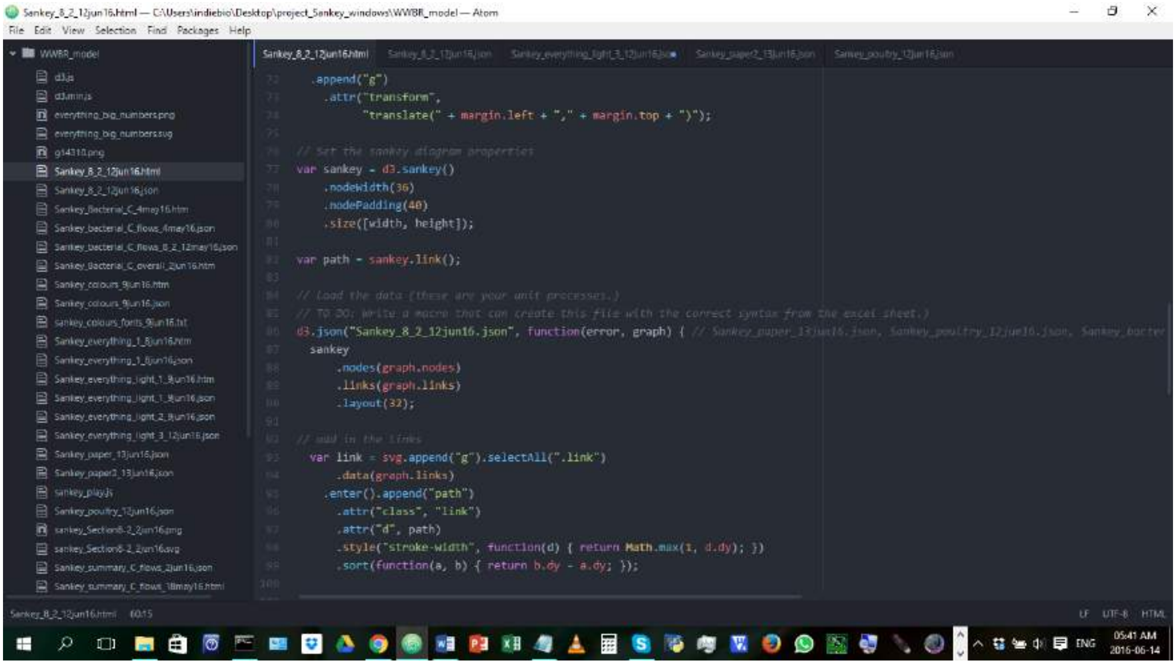


Figure: H-3: Screenshot of what .html file looks like in the "Atom" html editor

H.4 Supporting data that can be accessed from the internet

To run Sankey visualisations, two files are needed in addition to the json (H.2) and html (H.3) files, d3.js (or a smaller version, d3.min.js), and sankey.js:

```
<script src="d3.min.js"></script>

<script src="sankey.js"></script>

<script> // These are the files you draw the design from - they have to be in the folder. These are not the data (mass flow) files.
```

These files can be downloaded at https://d3js.org/

The power of this visualisation is that it is interactive, but a static version is needed for printed reports. The Sankey web image can be exported as a .svg vector graphic, which can then be saved as a .png file at any scale required. The tool used for this is still in beta version: http://nytimes.github.io/svg-crowbar/

Data driven documentation (D3) is a fairly new development, with good documentation existing from about 2011 (for example Murray (2013)). It is therefore still a new field, and the best way to learn is by doing. Sankey diagrams are only one way of many to visualise and interact with data. Some very helpful guides and beautiful visualisations are listed below. Python (https://www.python.org/) is a very useful programming language which makes developing these diagrams more user friendly, and is useful for general engineering (and other) programming too.

Guides

Scott Murray. Interactive Data Visualization for the Web.

http://chimera.labs.oreilly.com/books/1230000000345/index.html

Malcolm Maclean. D3 Tips and Tricks: Interactive Data Visualization in a Web Browser.

https://leanpub.com/D3-Tips-and-Tricks

Visualisations and source code:

https://d3js.org

https://bost.ocks.org/mike/sankey/

http://www.informationisbeautiful.net/