Exploring the factors at play to make wastewater biorefineries a reality

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in fulfilment of the requirements for the degree of
Doctor of Philosophy

by
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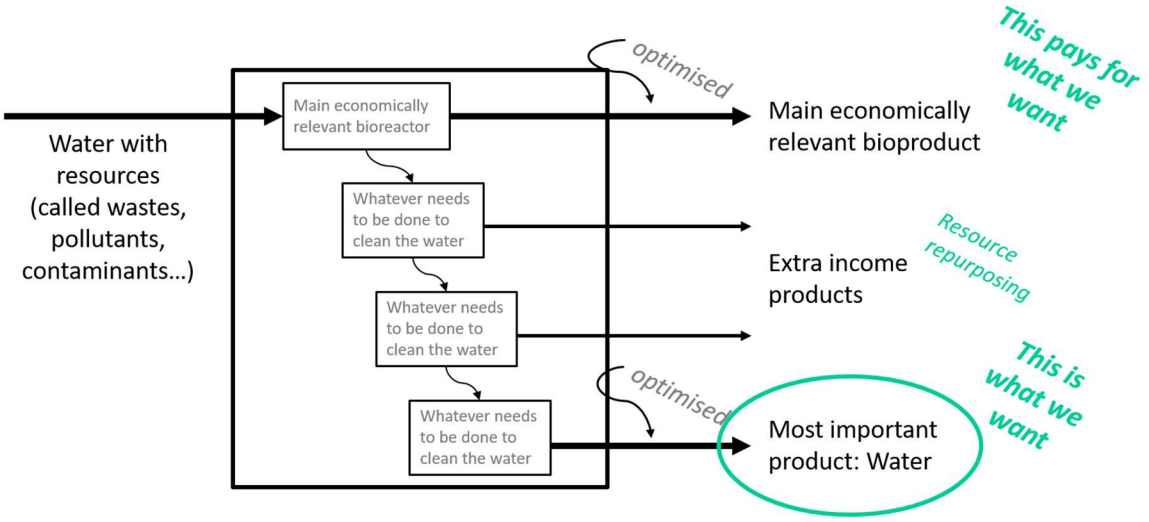
ABSTRACT

This thesis concerns the topic of wastewater biorefineries (WWBR), in which wastewater is not seen simply as a waste stream to be cleaned but as a valuable material flow to be converted into bioproducts, while still meeting discharge limits at the end. To set the scene, similar developing approaches to valorise wastewaters globally are reviewed. Wastewaters in South Africa are reviewed and categorised with regards to their potential to serve as raw material, in terms of their volume, concentration and complexity. Bioproducts possible from wastewater is reviewed and evaluated. The wastewater biorefinery is conceptualised in the context of current wastewater treatment technologies and a set of evaluation criteria is developed. A multi-reactor setup is suggested in which wastewater is used, in series, as substrate by heterotrophic microbes like bacteria, photo-mixotrophic organisms like algae, macrophytes and fungi. Each reactor group is considered in detail and evaluated with regards to its suitability to the wastewater biorefinery, leading to selection of appropriate reactor designs. Stoichiometric mass balances of all unit operations are established, showing the material value flows, and combined to model this multi-bioreactor approach. Subsequently the model is tested against literature data. Finally, the applicability of the wastewater biorefinery concept for certain waste streams is assessed.

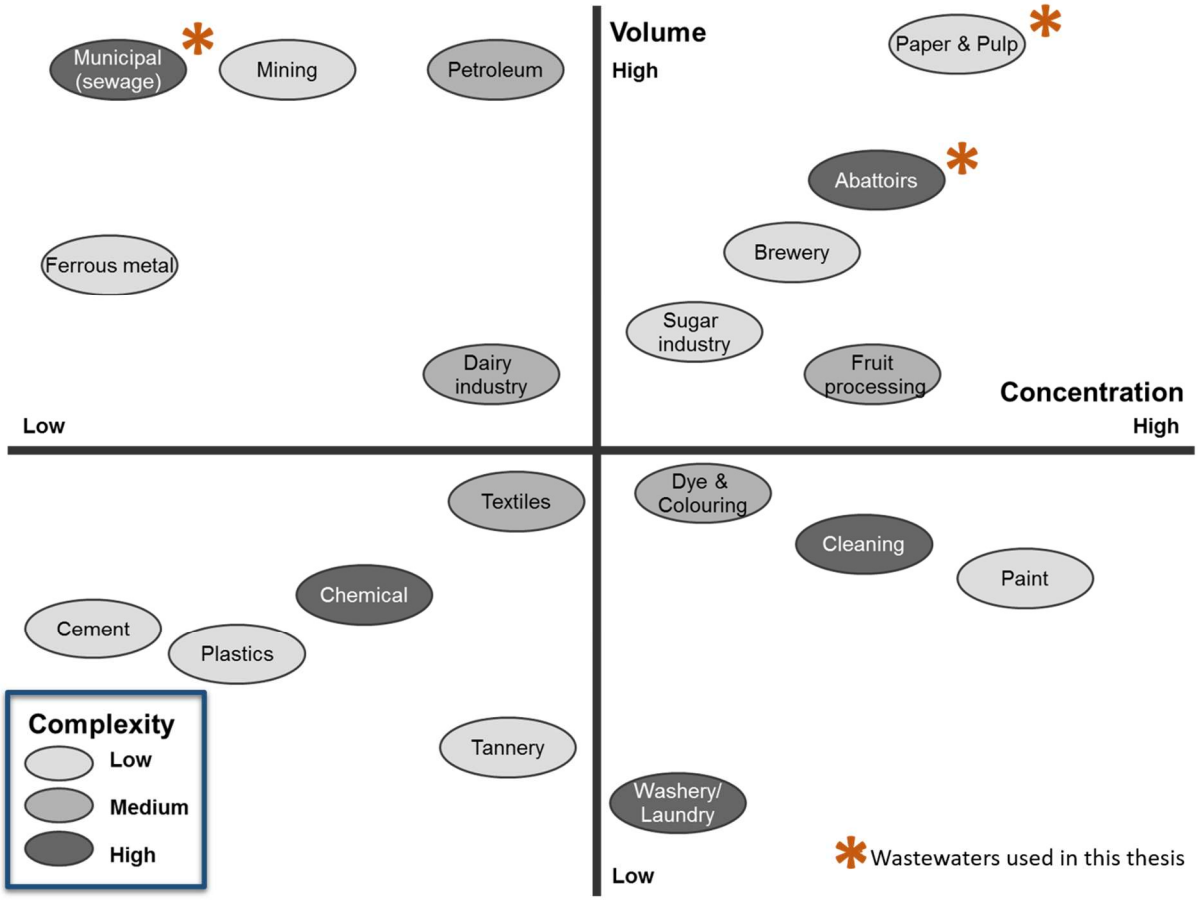


The thesis contributes to the current body of knowledge in the following ways:

1. Introduction of the concept of the wastewater biorefinery (WWBR)
2. Provision of a potential preliminary guide for classification of wastewaters for use in the WWBR
3. Development of criteria for reactor evaluation for use in the WWBR
4. Development of an integrated model to interrogate bioproduction from wastewater and determine product yields associated with wastewater treatment
5. Creation of new knowledge through the interpretation of the model on different wastewater systems.

The wastewater biorefinery is defined as a bioproduction system that integrates multiple unit operations to deliver compliant water as well as a bioproduct or bioproducts. It is approached through the concepts of industrial metabolism and the circular economy. Wastewater biorefineries are shown in this work to be a viable approach to improving resource efficiency while ensuring the better ecological functioning of humans within “greater than human” systems. The work places emphasis on the recovery of bioproducts that conserve molecular complexity but acknowledges that energy production for use on site and in the immediate surroundings is always an important factor in the WWBR.

This thesis introduces the need to include a qualitative way to evaluate the complexity of wastewater, in addition to standard classification of volume and concentration of components. Complexity includes both composition of potentially problematic compounds and how unpredictably it changes over time.



In this approach, it is preferable to generate three types of products: products of sufficient value to be economically viable; products of variable value with concomitant assimilation of major contaminants; and clean water as a product, typically through multiple unit operations, allowing multi-criteria optimisation. Through this approach, multiple criteria can be met.

Function-based products specific to niche industries, particularly those which produced the wastewater of interest, are of substantive interest owing to their streamlined market uptake. This thesis explores the requirements of the products that can be produced from wastewater in a non-sterile context and suggests product groupings that meet these requirements. Products secreted into the bulk volume are difficult to recover, leading preference to biomass associated and intracellular products. The product needs to offer a selective advantage to the organisms producing it to facilitate enrichment through, ecological selection of the microbial consortium with simultaneous cell retention through reactor design and operation.

Four groupings of unit operations were reviewed in detail and evaluated with regards to their suitability to the wastewater biorefinery, using a two-part set of evaluation criteria that was developed in this work, considering the reactor design, and its operation.

	#	Challenge that wastewater poses	Design requirement
Design Priority	1	Wastewater as feedstock: large volume, dilute concentration	Decoupling of hydraulic and solid retention times
	2	Wastewater as feedstock: continuous but variable inflow of wastewater	Continuous or semi-continuous process
	3	Dilute medium: cost of downstream processing for product recovery	Product formation in different phase
	4	Complex, variable medium: biomass retention and multiple constituents complicate product recovery	Facilitation of product recovery
Operational Priority	5	Wastewater remediation: need to use the entire wastewater flow for bioproduction	Think big! Commodity rather than niche
	6	Complexity and volume of feedstock: energy for sterilization unfeasible, need robust biocatalysts	Selecting for/enriching microbial community under non-sterile operation, including biocontrol considerations
	7	Complex, variable feedstock: cannot maintain a monoculture	Ecological selection to maintain desired cultures and give advantage to product
	8	Wastewater remediation: non-negotiable production of ecologically compliant effluent	Production of water fit for use or release into environment

The four unit operations each contribute a specific role to the functioning of the WWBR as a system. It is acknowledged that not all units are commercially important, and that the concept of diminishing returns should be kept in mind. 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. Existing heterotrophic microbial reactor systems like the aerobic granular sludge system (AGS) exist that suit the wastewater biorefinery approach particularly well, while activated sludge along with biological nutrient removal (BNR), the most commonly used reactor system in South Africa, is the least suitable to the WWBR. The photo-mixotrophic reactor represented by the algal bioreactor is helpful to scavenge high proportions of nutrients, particularly nitrogen and phosphorus. The algal bioreactor is also known to produce commodity products. Photo-mixotrophic bioreactor systems complement the heterotrophic systems but are unlikely to be the dominant reactor due to land and energy requirements. The macrophytic bioreactor is targeted for polishing 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. Macrophyte bioreactors, particularly floating wetlands, are promising tertiary systems that should be viewed in conjunction with water sensitive design principles to overcome potential land availability limitations. The solids bioreactor is an emerging beneficiation technology for biotransformation of bio-slurries and the solid phases recovered during WWBR operation to generate products of value, including biosolids. Solids bioreactors have great potential but require more investigation, with key challenges being mass transfer and separation technologies.

Operating waste treatment facilities as net income-producing bioprocesses require a mindset change about investment, risk and associated returns. WWBRs require higher capital investment due to the additional process units and downstream processing required and have higher operating costs due to the greater control required during the process and greater number of operators with advanced skillsets. An identification of the relevant product range and comparison between conventional processing routes and those possible from the wastewater is required on a case by case basis, and an overview is given in this thesis. Waste may need to be re-classified to be used as an intermediate by-product or raw material, requiring legal considerations in terms of both the solid waste as per the National Environmental Management Act (NEMA) and liquid waste as per the National Water Act (NWA). The added complexity of reclassifying waste as raw material needs an acknowledgement of institutional challenges such as speaking across department silo's.

In this thesis, a model of these integrated unit operations was developed to generate material inventories across the system. This can be used to evaluate possible scenarios in an integrated context using a generic flowsheet as well as mass balances generated through the model. Three case studies were examined: municipal, abattoir and pulp and paper wastewater. Municipal wastewater was chosen

as it represents a complex, dilute, 'suboptimal' wastewater stream. Abattoir wastewater was chosen as an example of a complex, nutrient-concentrated stream that may be well suited to biological transformation. Pulp and paper wastewater was chosen as an example where the biorefinery concept is already well established, and is a low complexity, low nutrient, high carbon content stream.

In considering the above case studies, a number of key learnings resulted. The impact of solids removal was clear and in keeping with existing bioprocessing and wastewater treatment principles of decoupling the hydraulic and solids residence times. Low nitrogen and phosphorus content in the pulp and paper wastewater as compared to the other two case studies indicated the need to conduct integrative studies of the unit operations to determine the most appropriate unit operations across the system. The effect of improving the product conversion yields and product recovery yields were examined, and a surprising result is the amount of nutrients that remain in compliant effluent, due to the large volumes of liquid involved. This leads to the conclusion that while the WWBR is a valuable way to address resource recovery, separation at source and internal process efficiencies are critical to improve overall resource efficiency and environmental protection. With regards to municipal wastewater, which contributes by far the most in terms of volume and nutrients of wastewaters in South Africa from the perspective of reactor design for waste(water) beneficiation, considering the cleaner production principle of separation at source, along with the need to decouple the solid and hydraulic residence times, dry sanitation presents a clear argument for the best WWBR approach. This approach must acknowledge that the transport of the sanitation raw materials is more difficult if hydro-transportation is not available, and needs to ensure operator equity, health and safety, particularly in the handling of the sanitation raw materials.

This thesis was developed in conjunction with the Water Research Commission (WRC) project "Introducing the wastewater biorefinery concept: A scoping study of polyglutamic acid production from a Bacillus-rich mixed culture using municipal waste water" (Verster, et al., 2014) and Water Research Commission (WRC) K5/2380 project titled "Towards Wastewater Biorefineries: integrated bioreactor and process design for combined water treatment and resource productivity" (Harrison, et al., 2017). While the project focused on a global and national review on research on wastewater biorefineries and wastewater as a resource, this thesis explores in greater depth the requirements of each of the reactor units and their integration.

COMMUNICATING THIS WORK

i. Academic communication

i. Water Research Commission reports

This work was conceptualised in the Water Research Commission (WRC) project K5/2000 titled "Introducing Wastewater Biorefineries", which commenced in 2010 and was published in 2014. The report was co-authored by Ziningi Madonsela, Sanet Minnaar, Brett Cohen and Sue Harrison.

Building on this, the thesis was developed in conjunction with the WRC project K5/2380 titled "Towards Wastewater Biorefineries: integrated bioreactor and process design for combined water treatment and resource productivity", concluded in 2016 and co-authored by Lesley Mostert, Madelyn Johnstone-Robertson, Tayana Raper, Sharon Rademeyer, Shilpa Rumjeet and Sue Harrison.

WRC reports are freely available online at http://wrc.org.za/

ii. Conference presentations

First International Water Association Resource Recovery (IWARR) conference 29 Aug – 2 Sept 2015, Ghent, Belgium; poster presentation. Organised by the University of Ghent and the International Water Association. http://iwarr2015.org/

Renewable Resources and Biorefineries (RRB10) conference 4-6 June 2014, Valladolid, Spain. Oral presentation: "Wastewater biorefineries: Recovering value while producing cleaner water". http://www.rrbconference.com/rrb-10-program

Renewable Resources and Biorefineries (RRB7) conference 8 - 10 June 2011, Bruges, Belgium. Oral presentation: "Producing poly-glutamic acid from wastewater, using bacillus - considerations when moving from bioprocess to environmental engineering". To my knowledge, this was the first public presentation using the term 'wastewater biorefinery'. http://www.rrbconference.com/rrb7-2011

Renewable Resources and Biorefineries (RRB2) conference 6 – 8 September 2006, York, UK. Poster presentation. http://www.rrbconference.com/rrb2-2006

Oral presentation at the African Utility Week, Expo Centre, Johannesburg, South Africa – 21-23 May 2012, "Building ecosystems not empires" https://www.esi-africa.com/african-utility-week-water-2/

Young Water Professionals (YWP) conference, 3-5 July 2011, Pretoria, South Africa, poster: "Producing poly-glutamic acid from wastewater, using Bacillus – making a financially viable business case with social and industrial benefit"

iii. Water Research Commission Steering Committees

Knowledge contributed to the following projects through membership of their steering committees

K5/2096//3: Exploring knowledge on natural processes for novel approaches to constructed wetlands design and performance for wastewater using biomimicry

K5/2123//3: Performance and efficacy of integrated algae ponding systems in wastewater treatment for water reuse and cost recovery through biomass valorisation

iv. Book Chapter

Wastewater biorefineries: integrating water treatment and value recovery (2017) Pott, R.W.M., Johnstone-Robertson, M., Verster, B., Rumjeet, S., Nkadimeng, L., Raper, T., Rademeyer, S., and Harrison, S.T.L. The Nexus: Energy, Environment and Climate Change. Springer. http://www.springer.com/us/book/9783319636115

ii. Public-minded communication

Transdisciplinary engagement involves much more than moving across disciplines in the academic environment to find new knowledge. It is also an approach to integrate and highlight the values and ethics behind the research. It involves stakeholder engagement across industry and participation with the general public over many years, in a cooperative, fun, non-judgemental way, to understand and integrate different worldviews, resolve complex challenges, and achieve what appears to be mutually exclusive outcomes – the included middle (Max-Neef, 2005; Polk, 2014). Public interaction through public talks, interviews and discussions contribute to achieving this, and helps to uncover knowledge not specifically confined to a specific discipline or institution – e.g. lived experience, that can then be systematically analysed through formal research to establish facts and reach new conclusions. These interactions assist to cross the divide between the public and science. This section lists some examples of such interactions.

Links indicate transcriptions of presentations.

i. Talks and interviews

Evening talk at the SA Geography teachers' conference, 24 September 2014. "Permaculture, water and the landscape: the connectedness of things", Rickety bridge, Franschhoek. http://indiebio.co.za/rickety-bridge

Evening talk at Greendrinks "Tame is not sustainable" 18 September 2011, St. Josephine's Mill, Newlands, Cape Town. http://indiebio.co.za/tame-is-not-sustainable

Radio: RSG interview on getting value from water (in Afrikaans): Onderhoud (Interview): afvalwater herwin en waardevolle produkte (podcast) with Middag op RSG 1 — Ettienne Ludick and Sue Pyler, 24 November 2015 https://iono.fm/e/230479

The Mediamatic Foundation, 12 June 2014, Amsterdam, the Netherlands http://www.mediamatic.net/369210/en/algae-eating-robots-food-from-waste-water-amp with the transcribed talk: http://www.mediamatic.net/372331/en/quot-water-is-a-cycle-like-everything-in-nature

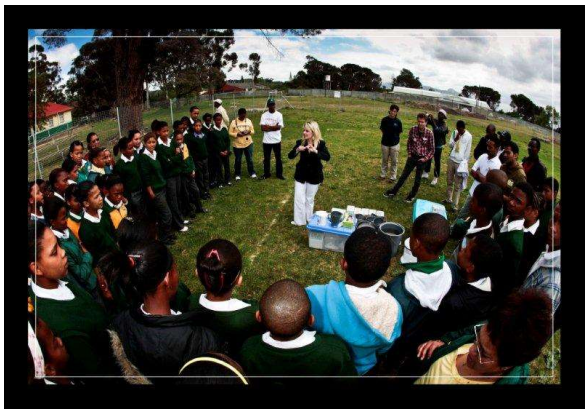
ii. Articles and online media

Public Understanding of Biotechnology interview, a SAASTA initiative: http://www.pub.ac.za/biotech-in-business-wastewater-is-a-resource-not-a-problem/

Forbes Africa, August 2012 edition "Poop scoop, how to make money from waste" written by Sumitra Nydoo : http://indiebio.co.za/forbes

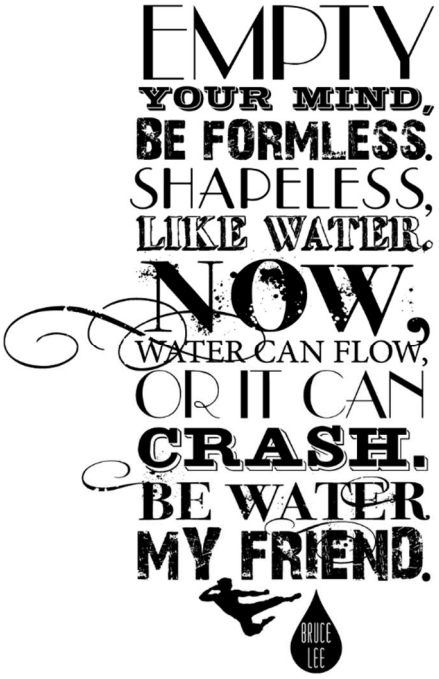
iii. Initiatives and competitions organised as part of this thesis approach

Waterbus Schools Awareness and Education event, 8 October 2010. A trip to look at the water system in the Bergriver catchment, and build connection between students and industry professionals (funded by the Water Institute of South Africa (WISA)).
www.supernews.co.za/protecting-water-resources-by-empowering-young-citizens/
http://indiebio.co.za/waterbus , http://www.ebe.uct.ac.za/usr/ebe/staff/dec2010.pdf



TEDxCapeTown, an independent TEDx event committed to ideas worth spreading, held on 16 April 2011 at Ratanga Junction, Cape Town. Themed 'Be Water My Friend', the day saw talks addressing the topic in a combination of metaphorical (going with the flow as a way to think and exist at peace in the world) and literal (water treatment and crises) interpretations. The theme comes from a Bruce Lee quote that speaks of the formlessness, adaptability and power of water.

Weblink with links to talks: http://www.ted.com/tedx/events/2234



The Moola for Amanzi Business Concept Competition was organised by the author and launched at the Small Wastewater Treatment conference held in East London in November 2010 and was a competition 'by young professionals, for young professionals'. Its aim was to build confidence and partnerships among young water entrepreneurs, help communicate their ideas in a way that makes business sense, and encourage the industry to think a little differently.

The competition, which was part of a bigger initiative – the Dutch-SA water partnership – aimed to generate high quality investment proposals addressing water and sanitation issues and build awareness in the public eye, the water sector and sectors outside conventional water-related industries, so that business can go hand in hand with access to clean and affordable water.

Anyone was eligible to enter – students, SME's, informal settlement communities, municipalities. Big ideas, small ideas, technological ideas, social solutions, IT solutions, non-profit ideas: any idea in water was welcome. The prize money totalled Euro 15 000 (sponsored by the Dutch government), and some support to implement the winning idea was on offer. The final was held at the UN World Water Day, in Cape Town, March 2011, and attended by the (then) Chair for the United Nations Secretary-General's Advisory Board on Water and Sanitation (UNSGAB), His Royal Highness Willem Alexander Prince of Orange and the Deputy Minister of Department of Water Affairs.

Coverage for Moola for Amanzi in WISA magazine: Water & Sanitation Africa May-June 2011, in editorial (p3), President's comment (p7), article 'Innovation pays off' (p80-85) . http://indiebio.co.za/moola-for-amanzi

ACKNOWLEDGEMENTS

This PhD represents growing up at many levels and is a culmination of a lifelong (so far) struggle, driven by frustration. A struggle to figure out what I wanted, and once I knew that what I wanted was a biotech company, a struggle to figure out what that meant.

Thanks to the fat Maltese cross and the vet who gave me a vac job and operating room nausea in 2000 who saved me from veterinary service.

Thanks to Colin Kenyon and the ladies at the CSIR. When things unravel I always think back at those times and remember what a good lab must be like. Thanks to the people at the Water Institute of South Africa (WISA), whose discussions helped me shape my own approach, including my own little lab – called 'Dave's kitchen' after Dave Crombie who said if it can't work in your kitchen, don't bother out in the field (or in academia).

Thanks to the Commonwealth Scholarships, for funding my adventures to England where I met Sue Harrison. After all this time and all the research and networking, this is still the best place for me. Figures that you have to go travel all over to meet your destiny, which is back home.

My entire life has been an unintended study in the unconventional. It's ironic that this phase of my life concludes in such a comparatively conventional and rigorous thing as an engineering PhD. I was not happy in Cape Town, at UCT or in Engineering, but I have not found a place I rather need to be – and believe me, I looked. Now, that the worst is over and I have worked out a way to be both outside and within, I'm quite happy here. I guess I had to prove that if my crazy ideas were to work at all, they should work in such a constrained environment.

Thanks to the dude at the castle in 2006 for asking me how I feel about the inequality in South Africa. You coloured how I make my choices.

To the organisers of the second Renewable Resources and Biorefineries (RRB) conference held in York, 2006. I cut my Euro-trip, for which I saved an entire year for, in half to attend without knowing what to expect. This conference was the defining feature that shaped my career. Thanks in particular to Christian Stevens (Ghent).

To the people in the Chemical Engineering department, especially the many Friday afternoons spent at beerclub, who shaped my ideas, where we even hatched some of them, including team Rural Rocks: Doreen Nabaho, Mlu Mnguni, Allison Kasozi and Naadia van der Bergh.

Then, thanks to all the fish. Since that first wobbly back in 2008 when I was looking for myself and built you a pond, you taught me how to manage nutrients holistically. Well, not you, per se, you simply swam around doing what you do, but you expressed your pleasure at the health of the pond through shagfests, spawning jewels each generation successively more beautiful than the one before, and this pleased me.

Fiona and Rasputin, my dogs, who distract me from depression and the PhD in equal measure. I don't know if I should thank you for that or not. But sitting next to you writing, you are beautiful unique animals and you remind me to never lose my wildness. Oxo, you raised me and I miss you a lot.

I gratefully acknowledge funding from the National Research Foundation as well as the Centre for Bioprocess Engineering Research Centre. I acknowledge and greatly appreciate the funding contribution of the South African Water Research Commission (WRC) through projects WRC K5/2000 and WRC K5/2380, as well as the technical input of the steering committee to this project. This is however not the full story. In July 2009 (I think), hope, money and wits have worn through. A last ditch effort saw me writing a funding proposal to the WRC, with no experience to guide me. I wrote with only my Big Dream in mind. It was a crazy dream and one notable grumpy man already said it couldn't be done. My mentors were all out of town and the deadline was around the corner. I thought, this is it. If I don't get this project, then I quit. If I do, then someone in this universe, at least, believes in me. Project K5/2000 was underfunded (my fault) and ran overtime (also my fault), but it was enough to let me know that this big dream had at least some cause for closer investigation.

Thank you to Joe Macke and the workshop, my father Koos Verster, and pretty much the whole of Paarden Eiland (and beyond) who helpfully dealt with my weird descriptions of valves and pipes and thingies to make cheap plastic prototypes that actually worked, well.

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I would like to extend my deepest thanks and gratitude to the examiners for taking the time to read the thesis, and I appreciate immensely the quality and depth of the comments. The compliments are also cherished; it is an enormous validation to hear that others appreciate this work.

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To my favourite authors: Leonie Joubert, Rose George and Carol Gilligan. How I write and what I know, deeper than conscious thought, I learnt from you. The amazing UCT library where I first encountered these authors, particularly Fiona Jones who guided and ordered books with what seemed to me wild abandon, thank you.

To my mother, Beulah Verster, and my supervisor, Sue Harrison, thank you for your unwavering support. Often financially, sometimes professionally, but always, patiently, as strong women. You are frustrating at times, but always spectacular role models. Add to these, strong mentors Lesley Mostert, Allison Lewis and Dee Bradshaw.

Almost lastly, to those who crushed me, negated me, frustrated me. I pulled through not because of you but in spite of you. Don't go give yourself airs that your poor form helped me grow in any way. It didn't. It near killed me. To the countless people whose insight made the sun shine a bit brighter that day, I live for you, for those little nuggets of commitment that makes ecosystems work. And to the many people I cared for and who let me down: I will always be fond of you, you taught me so very much, but with this thesis I leave you behind.

Lastly, my gratitude to Graham, for the calm.

While I stood here, in the open, lost in myself,

I must have looked a long time

Down the corn rows, beyond grass,

The small house,

White walls, animals lumbering toward the barn.

I look down now. It is all changed.

Whatever it was I lost, whatever I wept for

Was a wild, gentle thing, the small dark eyes

Loving me in secret.

It is here. At the touch of my hand,

The air fills with delicate creatures

From the other world.

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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
DHET	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
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
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
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	Minimal Medium E
MPO	Milk Producers Organisation, South Africa
MXC	Microbial Electrochemical Cells
NatSurv	National Industrial Water and Wastewater Survey
nl	not listed
NOB	Nitrite Oxidizing Bacteria
NREU	Non-renewable energy usage
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	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
SEV	Specific Effluent Volume
SIIT	Sirindhorn International Institute of Technology
SOL	Soluble Organic Loading
SSF	(Bio)Solid Substrate Fermentation
SSF	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	Tshwane 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
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
Drop-ins	bio-based products chemically identical to their petrochemical counterparts
Economy of scale	reduction in cost per unit produced directly resulting 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

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
Proto-wastewater biorefinery	An initiative or process that uses waste materials to produce products, but does not take into account aspects like adequate cost recovery, logistics and distribution, and issues of scale.
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
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 producing clean or ‘fit for purpose’ water as the non-negotiable product (see Section 2.3)

1 INTRODUCTION

There is much excitement about resource recovery from wastewater (IWA Resource Recovery Cluster, 2015), but what does this actually mean? How do we move beyond a few successful cases? Can we learn from these to create a new industry or platform?

1.1 Rationale for this thesis

Effective wastewater treatment is a major challenge globally (Moe & Rheingans, 2006) and in South Africa (CSIR SA, 2010)). This is the result of multiple interrelating factors, including greater water consumption as a result of both population growth and increased wealth per capita, despite greater water-use efficiencies in many cases (World Business Council for Sustainable Development, 2005), urbanisation, stricter environmental regulations, generally poor resource productivity leading to multiple by- and waste products without clear treatment plans, leading to a greater variety of substances discharged into receiving waters, and declining environmental buffers to absorb nutrients exiting treatment works, as well as, in the context of domestic municipal wastewater, unpredictable and more severe storm events causing flooding and polluted stormwater discharges into current sewer infrastructure due to climate change, and ageing infrastructure (United Nations, n.d.; Armitage, et al., 2014). This thesis concerns the topic of wastewater biorefineries (WWBR), in which wastewater is not seen as a waste stream to be cleaned but as a valuable material flow to be converted into bioproducts, while still meeting discharge limits at the end, using both industrial wastewaters and domestic municipal water as case studies.

The emerging Bioeconomy promises a more sustainable use of renewable materials, but has a significant water footprint, and the constraints of water use may significantly influence the bioeconomy (Rosegrant, et al., 2013). In existing biorefineries, wastewater treatment remains a challenge (Bohlmann, 2006). This thesis complements biorefineries by providing a beneficiation avenue for the wastewater.

In addition to water scarcity, nitrogen and phosphorus supplies are of concern. Nitrogen is not in shortage but the method to produce combined nitrogen for agriculture is energy intensive, nitrous gases are high-impact greenhouse gases (Galloway, et al., 2008), and diffuse pollution of nitrogen contributes to eutrophication. Phosphorus reserves are approaching depletion and is a significant contributor to eutrophication (Cordell & White, 2014). Wastewater is a potential source of all these nutrients, and water. WWBR aims to recover these nutrients as well as additional products.

In bioproduction, some products are very difficult to produce in the conventional stirred tank reactor configuration, prompting investigations into alternate forms of bioproduction, revisiting biofilm reactors (Rosche, et al., 2009; Qureshi, et al., 2005), novel cell-retention based systems (Fraser & Endres, 2013) and increasing interest in solid substrate fermentation (Mitchell, et al., 2010). WWBR can provide a unique niche to complement these reactor configurations.

Radical advances in wastewater treatment allow a productive approach. Examples include the development of the aerobic granular sludge (AGS) process with rapid settling times, that enables production of polyhydroxyalkanoates (PHA) at levels potentially competitive with sterile systems (Fernández-Dacosta, et al., 2015). This technology and the approach that gave rise to it provides the space to raise the question of the feasibility of adapting this approach to create a platform of bioproduction in highly dilute, complex environments like wastewater. Table 1-1 highlights the differences that characterise these approaches, with the process objective being the main bridging point.

Table 1-1: Historic differences between environmental and industrial biotechnology (adapted from Kleerebezem and van Loosdrecht, 2007)

	Environmental biotechnology	Industrial biotechnology
History	Wastewater treatment	Product formation
Basis	Catabolism	Anabolism
Biomass	Mixed culture (sludge)	Specific strains of microorganisms
Process type	Continuous	Batch or fed-batch
Process models	Lumped black box models	Lumped black box models, omics-based metabolic network models
Process objectives	**Minimise effluent substrate concentrations**	**Maximise productivity of product**
Substrates	Mixed substrates (waste)	Pure and well-defined substrates, complex substrates as byproducts (dependent on value of product)
Process establishment	Ecological selection by process operation	Specific microorganisms and, frequently, genetic engineering

Megatrends like population growth, migration, climate change, industrialisation, densification, urbanisation makes it critical to rethink wastewater management. The complex challenges around wastewater necessitates shifting the paradigm from engineering a treatment solution with disposal (end of pipe) to refining value products while dealing with health and environmental requirements. This approach could demand a disruptive rethink of what parts of the waste is useful, how to separate and transport to a biorefining centre with appropriate unit processes. In this study a more sustainable and incremental change approach is used by adding discrete unit processes to an existing centralised facility with mixed waste that has lower energy requirement and is more ecologically sustainable. A key need exists to both ensure the maintenance of our water resources, through both ensuring availability for use (quantity) and preventing their degradation through pollution (quality) and the maximizing 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 resource productivity is the motivator of 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.)). It is expected that a wastewater biorefinery (WWBR) contributes significant environmental benefit through its operation through the effective and financially sustainable treatment of wastewater, as well as contributing to the closing of nutrient and energy cycles.

1.2 Opportunities driving new approaches to wastewater

The opportunity for a new approach is clear when one considers that typical municipal wastewater contains in the region of nine-fold the chemical energy required for its treatment (Shizas & Bagley, 2004). This energy is in a diffuse form, which results in treatment works commonly using a significant fraction of the municipal energy to treat the water with no combined products, rather than employing biological systems that are adapted to biotransform the dilute, varied sources of energy and nutrients to higher value 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, ethanol production and microbial fuel cells, could contribute towards energy products. This study could be extended to consider all potential products.

The aim of this thesis is to outline and examine a relatively new thinking at the intersection between traditional bioprocessing and wastewater treatment, to utilise waste streams as a valuable raw material or substrate for conversion into 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 “complex waste biorefinery”. 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.

This new thinking is supported by recommendations from policy advisors. 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:

1. increase the availability of water
2. improve the governance, planning and management of supply and delivery
3. enable water and sanitation services to operate as a sustainable “business”
4. increase the efficiency and productivity of water use

In this Water RDI report, several factors were highlighted where the WWBR concept can contribute directly, including addressing the 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 and improved financial sustainability of the water system. Further, the concept can incentivise improvement in other factors listed, including 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.

1.3 The design of the thesis

This thesis places 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.

Biological systems and reactor configurations that may be appropriate are discussed. Several examples of potential products from the wastewater biorefinery are presented. A generic wastewater biorefinery flowsheet constructed with the four reactor groupings and separator steps relevant to each, and an associated material balance model, have been compiled. The function of the first reactor unit, the heterotrophic microbial bioreactor, is to remove a high proportion of the organic carbon, along with the production of commodity product. The second reactor unit, the photo-mixotrophic reactor scavenges high proportions of nutrients, particularly nitrogen and phosphorus while producing algal product. The third unit, the macrophytic bioreactor is targeted for polishing 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 to generate products of value, including biosolids.

The flowsheet and mass balance model are then used to explore hypothetical wastewater biorefinery flowsheets compiled for processing of three types of South African wastewaters: poultry abattoir, papermill and domestic municipal wastewater. The compilation of these findings allows the potential value of the wastewater biorefinery in South Africa to be considered. Further it allows critical components of the wastewater biorefinery to be targeted for maximum impact on improved performance.

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, this thesis considers 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.

1.3.1 Thesis objectives

This thesis considers the critical factors needed from an engineering point of view, to enable the concept of the wastewater biorefinery. The objectives of the thesis are:

1. To categorise potential wastewater feedstocks in terms of volume, concentration and complexity in order to evaluate the feasibility of their use in specific scenarios from a SA perspective as an example (Chapter 3)
2. To categorise critical product requirements that determine the potential product range possible from a WWBR (Chapter 4)
3. To evaluate the critical reactor requirements for successful WWBR operation (Chapters 5 to 9)
4. To create an integrated generic flowsheet model to explore the interaction between different reactor units at a high level. (Chapter 10)
5. To demonstrate the applicability of the WWBR through using the model with its calibration from the literature (Chapter 11)

1.3.2 Thesis structure

The thesis is divided into three broad sections: introducing the concept within the framework of available wastewaters, evaluating the bioreactors required to enable this concept, and illustrating this concept as an integrated process using generic flowsheet compilation together with a material balance model.

The first section critically introduces the concept of the wastewater biorefinery, first through an evaluation of the need for a different approach to waste treatment (Chapter 1), followed by current relevant work in the area. Challenges encountered by early attempts to beneficiate wastewaters in both academic and industrial research, and special interest groups are discussed in Chapter 2. The potential of wastewater as a raw material input concludes setting the stage (Chapter 3).

The evaluation required when considering products from a wastewater biorefinery is considered in Chapter 4. Adequate reactor design is critical to enable the wastewater biorefinery. In Chapter 5, the requirements for reactor design in an integrated generic model system is evaluated through the introduction of the flowsheet, followed by a detailed discussion of the four reactor unit trains in this model, in Chapters 6 to 9. These chapters consist of four parts: A general overview of suitable reactor types, products possible for these reactor systems in the wastewater context, likely bioreactor factor values found in literature, to be used in the integrated model, which is then detailed in mass balances. Chapter 10 considers the aspects involved in combining the reactor trains to form an integrated system.

Once the reactor considerations are qualitatively explored, the material balance model is used to integrate and evaluate these mathematically in the third section. Chapter 11 demonstrates the model with a single unit process, producing PHA from wastewater, followed by a demonstration of an integrated process, with three case studies using different wastewaters; domestic municipal, poultry abattoir and paper mill wastewater. The chapter concludes with an interrogation of the domestic municipal case study with recommendations on pressure points where future work should be directed as a priority. The thesis concludes with presentation of its overall contribution and integration of specific finding and their potential impact as well as recommendations in Chapter 12.

2 WASTEWATER BIOREFINERY REVIEW

Generating value from waste as a 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 a circular economy (Verster, et al., 2014). In this section, examples of global projects utilising wastewater are summarised. These reviews lead to questions around integrating the bioprocess and remediatory components of the biorefinery, which need to be addressed to determine the possible application of the wastewater biorefinery (WWBR), their potential and their design.

2.1 The biorefinery 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 (Kamm, et al., 2006). Co-products can also be food or feed. These objectives necessitate the integration of a range of different methods and technologies (Verster, et al., 2014). 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 (Polprasert, 2007). It is, however, potentially more economically sustainable to produce High Value-Low-Volume (HVLV) products from this biomass and its associated side-streams (Verster, et al., 2014) and use residual fractions for conversion to biogas or other energy-carriers (Wolkers, et al., 2011).

2.1.1 Biorefinery outlook and future trends relevant to the WWBR

Biorefineries are commonly presented as a “new paradigm” for using renewable resources to produce energy and chemicals, for example bio-based equivalents of petro-chemical based plastics like bioPET, bioethanol production particularly in Brazil (UN-Energy, 2011), biodiesel production in the European Union (European Biofuels Technology Platform, n.d.; European Biodiesel Board, 2014), and poly-lactic acid or starch-based biopolymers complementing, improving, and in some cases replacing plastics (Rehm, 2009). In reality, the (biomass) biorefinery concept represents more of a push towards diversification of products from agro-industrial systems. This is likely particularly aimed at compensating for the low level of added value in agri-business, via applications in non-food uses (Nieddu & Vivien, 2013). As an example, the paper industry has been experiencing market saturation with the emergence of excess production capacity, which is leading it to explore other products from fibre than only paper-related (Stuart, 2006). The biorefinery approach should thus not blindly be considered as a genuine forecast of what is most desirable from a systems perspective.

The wastewater biorefinery has a significantly different paradigm, while clean – fit for purpose – water is still a dominant objective. In well-functioning institutional systems the economic value of the water justifies it being the only product, and the potential for conflicting economic objectives (and potentially political or other agendas) initially lead to much resistance in the need for the ‘biorefinery’ component in wastewater in conversations with industry leaders (Ekama, et al., 2011). In the larger context, additional economic benefit can be achieved which transcends the need to shift which parameters

needs to be regulated to ensure environmental and human health. Looking further, the closing of nutrient and energy cycles, and particularly as it pertains to nitrogen and phosphorus, demand an alternative approach to wastewater.

As the concept of the circular economy gains traction, the idea of industrial ecology is revisited, which aims to design systems where no wastewater is produced, and industries should certainly try to limit waste production, recycle water within the plant as far as possible and keep waste streams separate to allow more concentrated and less complex waste treatment. Where this cannot be achieved, waste reduction should still be targeted. At the same time it needs to be acknowledged that the most effective industrial eco-parks emerged spontaneously, at times with sub-optimal wastewater streams (Desrochers & Suatet, 2008).

Many examples on using waste materials to produce products are listed in the popular literature (Pauli, 2010), but these often do not take into account issues of market needs, adequate cost recovery, logistics and distribution, and issues of scale (Blottnitz, n.d.). The 'living machines' concept (http://www.livingmachines.com/), a well-marketed example of biomimicry, can be seen as an example of a proto-wastewater biorefinery. On closer investigation, however, there is no well understood material inventory available that can inform the mass balance, and thus, economic potential of the system (Todd, et al., 2003). Without additional investment to increase system robustness, these systems are only cost competitive at small scales (United States Environmental Protection Agency, 2002). Rather than discredit these initiatives, there is a need to contribute to the toolsets available that may help investigate the feasibility of the wastewater biorefinery concept underpinning these projects, to overcome these limitations at the design stage.

While using a WWBR to produce energy may be useful as an economic contributor or as a resource during intermittent energy supply, the decision about directing the chemical potential towards energy rather than commodity products needs to take into account what chemical potential is required for recovering nutrients. This ability may be limited after the energy required for biological nutrient recovery is directed elsewhere but recovering nutrients from the wastewater remains the first priority of a WWBR. Secondly, the complex (bio)chemistry prevalent in the wastewater environment is not directly related to the specialty chemistry employed in beneficiation of plant-based sugars and oils (Clark, 2017). The WWBR complements these biorefinery concepts through adding another route of potential development, with its own most suitable products.

2.2 The wastewater biorefinery

2.2.1 Positioning the wastewater biorefinery

The wastewater biorefinery considers effluent from industrial and mining sources as well as domestic wastewater as the incoming resource. These may include effluent streams from the biomass biorefinery. This makes the WWBR conceptually different, but complementary to, biomass biorefineries. Owing to the substantial differences in feedstock and hence reaction requirements, simply extending the approach to biomass biorefineries to include the wastewater biorefinery is not appropriate. The industries that evolved towards biomass biorefineries, mainly agri-business, do not have existing, effective know-how for dealing with wastewater. The wastewater biorefinery needs to be developed from first principles, considering the current understanding of wastewater treatment as well as bioprocess engineering, coupled with an understanding of the industry and market needs.

In addition, while the biorefinery concept, and the emerging bioeconomy seeks to maintain access to renewable feedstocks to produce products and hopes to influence stakeholders to support this view (Nieddu & Vivien, 2013), the wastewater treatment industry is mainly concerned with reducing risk and ensuring public and environmental health. It should not be surprising that the emergence of the wastewater biorefinery could create significant tension as these "patrimonies" interact.

WWBR should aim to complement conventional biorefineries by providing additional resources to enable this value-addition. 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. 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.).

A WWBR operates in the wastewater arena; however, it is designed to generate products of sufficient value from the waste nutrients simultaneously for economic viability while 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. In keeping with cleaner production principles, particularly in-process recycling and process efficiency improvements, the WWBR can be more effective when wastewaters are considered where they are produced, and assimilated into the operations of either the wastewater producers or the wastewater treatment, rather than attempting to work with a final complex mixture of unknown origins far downstream (Verster, et al., 2014; Barclay & Buckley, 2011).

The brewing industry is a popular choice for resource recovery concepts and represents a prototype 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 (Cohen, 2006). 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 (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 chrome of 79.2% and 86.5%, respectively (Zhang, et al., 2007).

By-product streams and streams that form the effluents or waste products of other processes 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 dilute 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, predictable or concentrated 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.

The consideration of wastewaters for biorefining has been a recent development in the literature, being tabled for the first time only in around 2007/8 (Werker, 2008; Mooibroek, et al., 2007) which correlates with relevant research emerging at the University of Cape Town starting around that time as well. The thesis focuses on domestic wastewater more, because of the great volumes produced. This thesis aims to be generally applicable and hence required consideration of a variety of wastewaters.

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 investigating single technologies in isolation, but with an understanding that they could be integrated into a WWBR context, where the products from wastewater

and sanitation are directed towards a combination of biogas, compost or biofertiliser (Harrison, et al., 2017).

The unit processes typically found in a functional WWTW using biological nutrient removal can be 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 of products in demand, depending on the characteristics of the incoming waste stream(s), surrounding market needs and similar factors. At this point in the work the focus is on “maximise the overall product outputs” and the scales of production required to be financially viable is not considered yet.

2.2.2 European facilities creating value from wastewater

In European countries such as the 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 Harrison et al. (2017). Researchers in the Netherlands and Denmark are international leaders in the field, as highlighted in Table 2-1.

Table 2-1: Companies in Europe that produce value from wastewater (adapted from (Harrison, et al., 2017))

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, biogas -Fertilisers distributed to local farmers -Final product used as an additional soil nutrient	Industrial	150 000 tons of fertilisers were produced in 2010
Denmark [3]	Krüger A/S, Billund Vand A/S, Billund Municipality, and consortium	Billund BioRefinery (BBR)	Domestic, industrial and agricultural wastewater	Biogas, organic fertiliser, bioplastic	Demo Plant	

[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] Billund BioRefinery, 2014. *Billund BioRefinery.* [Online], Available at: http://www.billundbiorefinery.dk/en/, [Accessed 2014 October 2014].

2.2.3 The need to consider multiple process units in the WWBR

To optimise multiple objectives simultaneously multiple unit operations need to be included in the WWBR. This requires the maximising of conversion to product and maximising of quality of product water to be separated. This is well understood in wastewater treatment, which 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 the product(s) 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, minimise system burdens and to ensure compliance with respect to water quality. This approach is visualised with a simplified, generalised flow sheet shown in Figure 2-1. This potential WWBR process flowsheet illustrates bacterial, algal, (together filling the primary treatment function) macrophyte (assisting with polishing) and solids reactors (to deal with the settled fractions) and these are discussed further in the thesis, generally in Chapter 5 and with specific focus in Chapters 6 to 9.

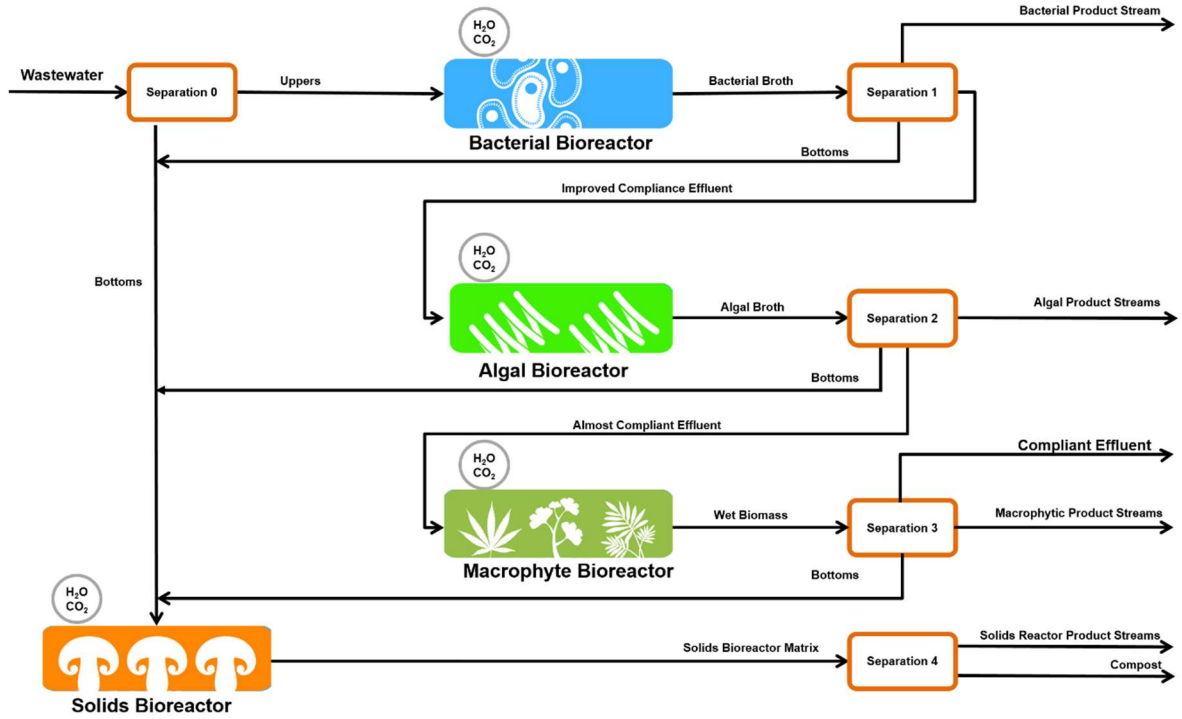


Figure 2-1: A simplified flowsheet of the wastewater biorefinery concept

A significant difference between wastewater treatment bioreactors and conventional bioprocessing is that the former are defined by function and typically catalysed by mixed and dynamic cultures, while the latter rely on defined and maintained microbial populations. For the sake of simplicity, the reactors in the generic WWBR flowsheet are called bacterial, algal, macrophyte (plants) and solids bioreactors. In reality, these reactors may have many and mixed biological catalysts present. Due to the reactor design, certain groups of organisms are selected for and enriched, based on functionality and tolerance to the reactor environment rather than a specific species. For example, in the “bacterial” bioreactor, the greater depth reduces light ingress, excluding photosynthetic organisms as a dominant group. The relatively high stirring rates may exclude larger organisms. Thus, this bioreactor may be better described as a “heterotrophic microbial bioreactor" and could be dominated by bacteria, yeast or filamentous fungi. This reactor functions as the primary mechanism to remove organic carbon and nutrients. Microbial nutrient removal is well understood and can be operated intensively.

The algal bioreactor is designed for nitrogen and phosphorus removal, and care is taken for effective light ingress to allow greater energy contribution from photosynthetic activity for this purpose. Through reactor design, operation and regular maintenance, larger organisms are again excluded. This bioreactor may be better described as a “photo-mixotrophic microbial bioreactor".

The macrophyte bioreactor is defined by the use of plants, but it is acknowledged that macrophytes may not be the only biocatalysts effecting nutrient removal, for example bacteria associated with the root zones of the plants and larger animals in turn feeding on them.

It is expected that the solids bioreactor’s dominant microbial group may be fungal organisms. This does depend on the water activity and reactor operation, and thus the reactor is called by its main defining characteristic, that of very high solids content.

2.2.4 The wider perspective

From a wider perspective, several factors need to be in place for the 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 synergistic 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 2-2, with emphasis on not only the technology and process tools, outlined in this thesis, but also the need for communication across disciplines, industries, and the public, in future work.

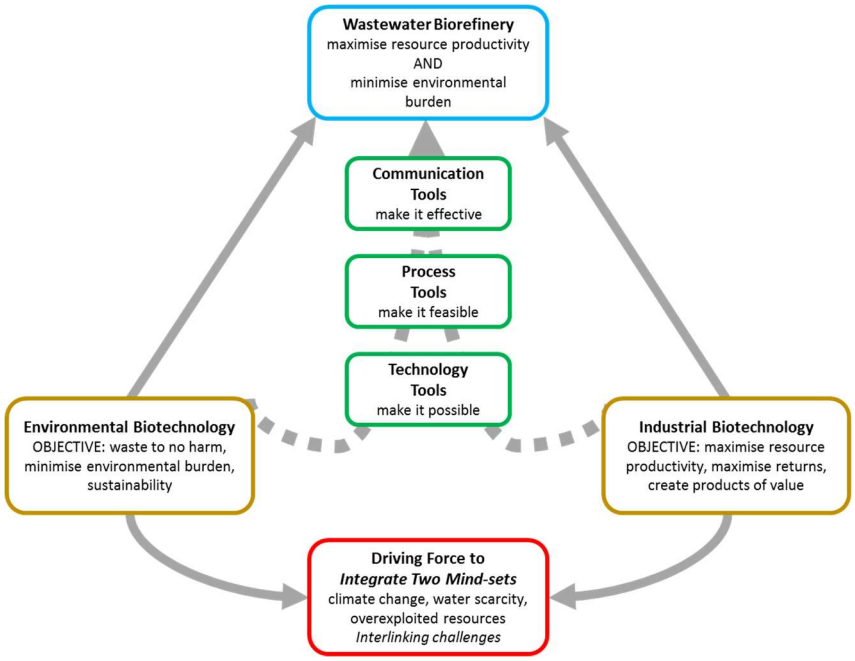


Figure 2-2: Integration of industrial and environmental technologies for emerging WWBRs

2.3 The wastewater biorefinery concept positioned in South Africa

To assess the suitability of WWBR in South Africa, several critical requirements need to be addressed. An overview of these considerations can be found in the related WRC project report (Harrison, et al., 2017) with discussion on relevant factors here.

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 and represents a prototype of the WWBR concept. The increasing electricity prices and energy insecurity make it an attractive investment.

There is a lack of available information from industry on wastewater streams and their handling, which 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, difficulty in the required collaboration of companies with different core business and levels of maturity, and 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 lower value market than what is possible, 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 disrupt established partnerships. This contrasts to complex wastewaters that do not have an existing outlet, for example municipal wastewater and abattoir wastewaters, which represents a greater challenge, but also a greater potential benefit to be gained from WWBR.

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

- Is a new plant required or an existing plant or part thereof to be retro-fitted?
- Is technology being bought in or internal technology to 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?
- Is the WWBR a robust solution?

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 (Harrison, et al., 2017). Currently they are considered the only valuable products, which limits the perceived potential of WWBR.

2.3.1 Municipal wastewater treatment works in South Africa as existing prototype wastewater biorefinery

In the South African context, Pitman & Boyd (1999) worked with local government wastewater departments to structure tariffs to encourage discharge of industrial effluents having a high readily biodegradable concentration (which would assist the BNR process) into the sewerage system. Effluents having high concentrations of heavy metals (which would degrade the reuse value of sludge by-products) imposed higher tariffs. This approach illustrates that the differentiation of resources within wastewater is possible. This example is also 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 (Harrison, et al., 2017).

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 had 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 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 (Franks, et al., n.d.)

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 an industrial park with suitable wastes. 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 (Harrison, et al., 2017).

2.3.2 The effect of economics on the WWBR

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 element of the declining water quality is largely due to poorly treated wastewater effluent which does not meet regulatory standards. 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).

There are attempts to address these challenges through improved enforcement. In the face of shortage of personpower in enforcement agencies, there has been a move towards incentivising towards better voluntary compliance through, for example, the Green Drop Report (DWS SA, 2014). 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.

Capital expenditure may be higher in a new development, rather than a retrofit of an existing plant, but the design of a new plant may allow more efficient operation with reduced operating costs. With many WWTW in dire need of upgrading it may make most sense to retrofit existing plants in the short term, with new WWBR built in the context of industrial ecoparks only (Leeuwen, et al., 2003). A discussion on the economic considerations can be found in Harrison, et al. (2017).

Due to the large amount of dilute liquid, it is not feasible to heat the bioreactors to the extent that conventional bioprocessing may require, but using bioenergy created through digestion of residual organics may improve the reactor kinetics significantly, improving process economics.

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; Harding, et al., 2007).

Operating cost related differences between WWTW and WWBR include improved analysis and process control, more and more skilled operators, and increased maintenance to the point where maintenance is considered part of the plant's normal operation.

The economics of a WWBR may be influenced by the value of the clean water. Economic studies may assume offset of wastewater treatment (Fernández-Dacosta, et al., 2015), but in the context of South Africa, or countries with poor regulatory enforcement of environmental laws, the default scenario assumed here is one of no treatment, rather than conventional treatment. The value of the clean water is therefore, at least in part, predicated on the governmental policies and regulations with respect to effluent discharge standards as mentioned in Section 2.3.3 as well as by the geographic location determining the value of re-use (which leads to lower water consumption) or from mitigation of standards' transgression (Winpenny, et al., 2010).

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.

2.3.3 The effect of wastewater policy on the WWBR

To establish a good understanding of water effluent criteria, the wastewater treatment standards of South Africa must be considered. The General Authorisation Standards for treated effluent is listed in Table 2-2**Error! Reference source not found.**. 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; DWA SA, 2009).

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. Therefore, it is 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 (Kings, 2014; Harrison, et al., 2017; Palmer, 2009); however, clear cooperation with regulatory requirements is requisite.

Table 2-2: General Authorisation Standards for treated effluent (DWA SA, 2009)

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
Turbidity (NTU)	0.1 - 10	< 0.1
Taste (FTN)	1 - 10	< 1
Ammonia (ionised and un-ionised) as Nitrogen (mg/l)	6	2
Chlorine as Free Chlorine	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 of grease (mg/l)	2.5	0
Sulphate as SO_4	200 - 600	< 200
Suspended Solids (mg/l)	25	10
Zinc as Zn	3 - 10	< 3
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
Dissolved organic carbon as C (mg/l)	5 - 20	< 5
Total trihalomethanes (µg/l)	100 - 300	< 100
Phenols (µg/l)	5 - 70	< 5

General limits refer to the standard compliance requirement, whereas special limits apply for more ecologically sensitive receiving waters.
*after removal of algae

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 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 was due to be commissioned in March 2016 to be operated by Veolia for ten years (Bizcommunity, 2015; Western Cape Business News, 2015). This partnership highlights that the early opportunity may rest within the private sector to private sector partnerships.

2.4 Closing remarks on the wastewater biorefinery review

In this chapter the wastewater biorefinery idea was introduced and contextualised with current international practice around resource recovery from wastewaters. The potential for value from wastewater in a circular economy context was considered as an additional, complementary route of incentive through economic potential along with the reality of the current economic (dis)incentives, potential regulatory constraints and resistance to changing away from current ways of operation. The great need that exists to approach municipal wastewater was highlighted. This chapter contributes to the changing perception of wastewaters to that of a resource that is justified to invest in.

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

In this chapter, three selected wastewater resources within South Africa are reviewed within the context of the total national wastewater burden or resource. Papermill wastewater is considered as an example of an industry that is already looking to expand its product offering and reduce its water footprint and represents a low complexity wastewater. Poultry abattoir wastewater and domestic municipal wastewater are examples of high complexity wastewaters without an existing outlet other than conventional treatment. A comprehensive review of a broader selection of South African wastewaters across a broad spectrum of industries can be found in Harrison, et al. (2017). Supplementary data, sources and calculations are presented in Appendix A.

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. These need to be translated into carbon, nitrogen and phosphorus compositions, to allow assessment of suitability for use as feedstock for wastewater biorefineries. Where possible, known complexities have been mentioned.

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 substrate in any bioprocess is an important determinant for the process. Understanding what is in wastewater and how it changes over time, and between industries, is an important factor in considering bioproduction, and understanding its complexity is part of the different way of thinking about WWBR.

3.1.1 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. They used a first order desktop analysis of South African wastewaters. It was found that 3,200 to 9,000 MWh of energy had the potential to be recovered, albeit in a diffuse form, using data collected in 2007. 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.

The WWBR emphasises recovery and re-use of all elements of the wastewater. This thesis focuses on the elements typically reported on for effluent compliance to illustrate the concept: carbon, nitrogen and phosphorus. Other products, like energy and salt recovery, may also be feasible but is not included here. For WWBR purposes, information on the complete composition of the waste stream is desirable, including variability and complexity. This is more than what is typically reported. Logistical information is also important, including the volumes available, the distribution and the localities.

3.1.2 Categorising wastewater streams for wastewater biorefineries

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 5 million litres every day (CoCT, 2010), with metropolitan plants ten times larger. These wastewaters 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.

Volume

The volume classification must be considered from both an individual plant perspective and in terms of national production. Many wastewater sources, like abattoirs or municipal wastewater have relatively few large industrialised plants with large wastewater flows, with many small plants whose wastewater are discharged to evaporation dams or discharged to the sewer and thus inadequately accounted for. 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. On the other hand, the operations producing the wastewater may not have the funds necessary to invest in adequate waste treatment infrastructure. 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 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 3-1 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 for municipal wastewater, poultry abattoir and papermill wastewater in Section 0, with more wastewaters considered in Harrison et al. (2017).

Concentration

The concentration of dissolved solutes in the wastewater influences their beneficiation potential for products other than clean water. In this thesis high concentrations are classified as 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 here as the lower end of the concentration range. All wastewaters are likely to have varying concentration over time.

Figure 3-2 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 undervalues COD-poor, nutrient rich waters

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. This includes the temporal aspect, changing with the time of day and season. 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 recalcitrant 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. Main components are considered to be either higher in concentration, or have a significant effect on the process, like inhibitors.

The complexity of wastewater for the purpose of this thesis is classified 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

3.1.3 A matrix representing wastewaters as feedstock

Figure 3-1 introduces a matrix for qualitative representation of feedstock qualities according to the variables suggested for categorisation in Section 3.1.2. In

Figure 3-2 this matrix is used for an initial, subjective and comparative categorisation of a broad spectrum of wastewaters in South Africa. The graphic is a conceptual grouping of the wastewaters, introducing the idea of grouping based on these three parameters. Currently there is no real way to determine the complexity quantitatively. This work introduced the need to consider complexity in addition to the concentration and volume. Examples are detailed below.

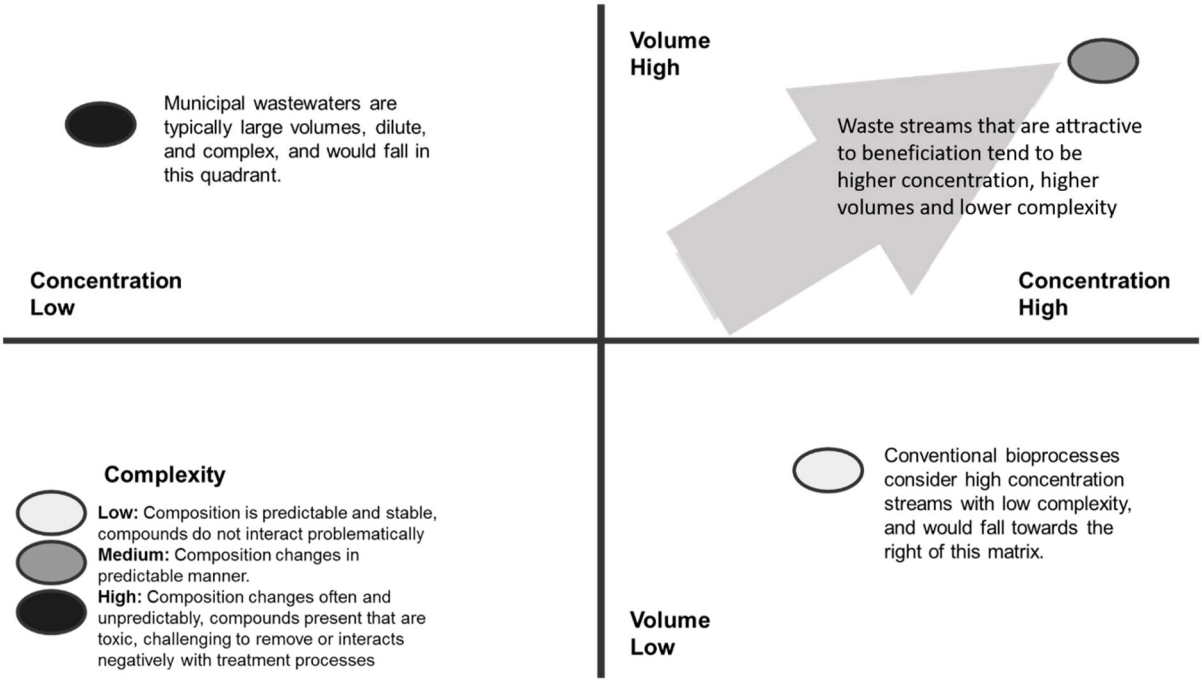


Figure 3-1: Matrix for qualitative representation of feedstock qualities of volume, concentration and complexity

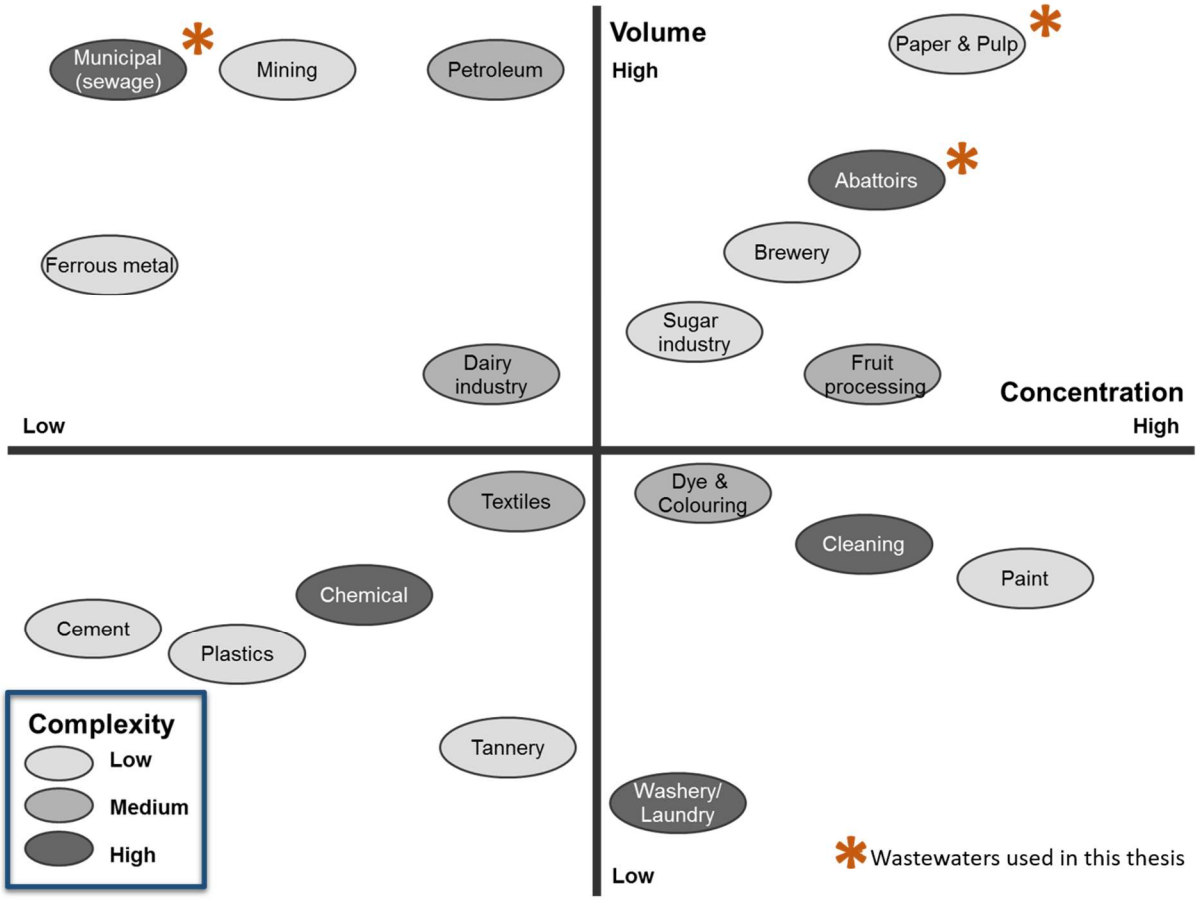
Brewery wastewater is an example of low complexity. The wastewater is well characterised because the preceding process is well understood and controlled from a biological perspective. The components do not interact negatively with each other and can be treated by few unit processes.

The textile industry is an example of medium complexity. The dye processes change between batches, and the presence of high salt and often 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.

Abattoirs have high concentration wastewaters which 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 municipal wastewater across sites, the associated treatment required and the product potential. Further intermittent disposal aggravates variability.

A recent overview (Harrison, et al., 2017) contains a quantitative presentation of data collected on different wastewater streams from various industries in South Africa, with 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.



Overall, the influent wastewater can also be classified in terms of potential products:

Figure 3-2: Matrix illustrating grouping of wastewater in terms of volume, concentration and complexity (adapted from Harrison et al (2017))

Very complex, diffuse wastewater from which niche products can be produced, which likely may not be related to the producers of the waste (e.g. biosurfactants from domestic municipal wastewater)

Defined wastewater, but most feasible products fall outside of the market focus of the industry player producing the water (e.g. biopolymers from confectionary wastewater)

Defined wastewater, with potential for conversion into a product used within the process or market focus of the industrial player (e.g. textile industry, recovery of catalysts)

This classification is most useful when considering specific case studies.

3.2 Re-visioning wastewater in South Africa

3.2.1 Sources of data on South African wastewaters

Overview studies include that of Burton et al. (2009) and Cloete et al. (2010), with the study by Harrison, et al. (2017), to which this thesis contributed, forming the current most comprehensive review to date. Industry specific surveys conducted through the Water Research Commission (WRC) include the "NatSurv" reports (WRC SA, 2015b). Further information was obtained through personal communications with staff at the WRC. Municipal wastewater data was obtained through personal communications with the City of Cape Town Water and Sanitation Department, as well as through the Green Drop Report (DWS SA, 2014). Other information was 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 were 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.

The Green Drop initiative of the Department of Water and Sanitation has reported the performance of municipal, public and private WWTW. 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 WWTW nationally and gives an indication of the sizes of these WWTWs (DWS SA, 2014).

3.2.2 Assessing wastewater as feedstock

Approximations of wastewater produced in South Africa is reviewed extensively in Harrison, et al. (2017) who include the annual production volumes, concentration of carbon, nitrogen and phosphorus as well as indications of handling issues and complexities, where available.

The composition data is often reported in COD, NO_3^- or NO_2^- or NH_4^+ or TKN or TN and PO_4^{3-}. Most volumes are given on an annual basis. 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 (= 1 000m^3/day). To standardise to concentrations of C, N and P, the following conversions from the COD, TKN/ ammonia/ nitrate/ nitrites and PO_4^{3-}, found in literature, were used (details in Appendix A.1):

Concentration of C (mg/ℓ) = COD/3 (mg/ℓ)

Concentration of N (mg/ℓ) = (14/62) x NO_3^--N (mg/ℓ) plus (14/46) x NO_2^--N (mg/ℓ) plus (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)

3.2.3 Identifying the valorisation potential of South African wastewater

The annual effluent production volumes from the main industries in South Africa, as well as their potential C, N, P contributions, are summarised in Table 3-1. This is a summary of the data presented

in Harrison, et al. (2017), with papermill, poultry abattoir and municipal wastewater discussed and characterised in more detail here (Section 0).

Table 3-1: Annual effluent production and their potential C, N and P contribution from several South African industries (detailed data and references of data sources are provided in Appendix A.2.1 (Harrison, et al., 2017) Shaded rows indicate the streams considered in this thesis.

Industry Sector	ML effluent per year	Estimated tonne C / year	Estimated tonne N / year	Estimated tonne P / year	Comment
Municipal	1 825 000	4 650 000	118 000	28 000	
Abattoir (poultry)	5 400	71 000	900	300	Blood, skin, fat, viscera, faeces, significant solid waste
Abattoir (red meat)	8 200	140 000	100	nl	Blood, skin, fat, viscera, faeces, significant solid waste
Brewing	8 300	100 000	400	250	
Canning	1 000	12 000	nl	nl	
Cleaning and Cosmetics	300	5 000	10	5	
Dairy	87 000	3 900 000	30 000	3 500	Fats, protein, faeces, grit
Distillery (alcoholic beverages)	400	100	430	nl	
Dyeing and Colouring	700	2 200	nl	nl	Alkaline pH, toxic organic residues, high NaCl concentration (1590 mg/l)
Edible oil	1 400	540 000	40	3 500	Pollutants such as fats, oils and grease, sodium, sulphates and phosphates
Fishery	2 000	30 000	60	nl	Flesh, scales, blood
Laundry	200	600	0.07	2	solvents, surfactants
Petroleum	80 000	1 800 000	3 700	100	Oil and grease, phenols
Pulp and Paper	340 000	970 000	3 00	450	AOX, dioxin, chlorinated organics
Soft drinks	4 000	74 000	nl	nl	
Sugar	400	2 200	nl	nl	Fibres, sand
Textiles	30 000	454 000	15	200	Azo dyes
Winery	2 500	50 000	300	130	Polyphenols, inorganics such as sodium and potassium

nl not listed

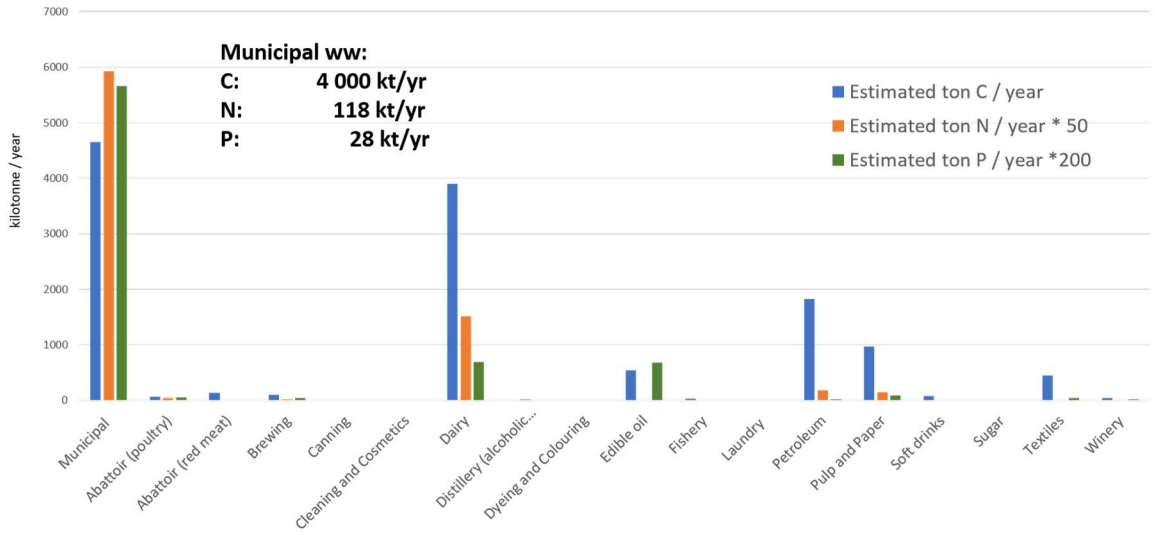


Figure 3-3: Visual comparison of annual effluent production and their potential C, N and P contribution from several South African industries

3.3 Characterisation of Three Wastewaters in South Africa

For the implementation of WWBR, the specific site or regional information is more important than the national values. The benefit that can be expected from WWBRs differs for different scales of industry. Large, standardised plants gain from economy of scale and must comply with industry water-use standards. The wastes from the smaller industries are also valuable and have high potential in the wastewater biorefinery space as they may be more flexible in addressing a local and/or niche industry market need.

For this thesis three representative wastewaters were considered: papermill wastewater as a large, centralised plant, poultry abattoir as an example of a complex, higher concentration with both small- and large- scale operations, and municipal wastewater as an example of a complex dilute 'problem' area.

3.3.1 Municipal wastewater

Municipal wastewater usually includes considerable amounts of discharged industrial effluent. In the Green Drop report (2014), the status of municipal WWT in South Africa from a total of 152 municipalities and 824 plants was assessed and is shown in Appendix A.3. The total amount of wastewater 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 entering general-purpose WWTWs is comparable to the estimate of 7 600 Mℓ/day obtained by Burton et al (2009). The volume, concentration and complexity data for the South African municipal WWTW is shown in Table 3-2.

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 ranges 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 167 - 400 mg-C/ℓ, 30-100 mg-N/ℓ and 6-25 mg-P/ℓ. The pH ranges between 7 and 8 and the total suspended solids (TSS) between 250 and 600 mg/ℓ (Appendix A.3).

Table 3-2: Volume, concentration and complexity data for the South African municipal WWT industry

effluent volume in South Africa	**total estimated effluent volume in South Africa per year**	*ML/year*	1 825 000
	Days of operation	*days*	365
	total estimated effluent volume in South Africa per day	*ML/day*	5 000
cross-reference	to worksheet with primary data and calculations.	Appendix A.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*	300
	estimated average nitrogen content	*mg/L*	65
	estimated average phosphorus content	*mg/L*	15
	pH		7-8
	conductivity	*mS/m*	70-120

3.3.2 Pulp and paper industry

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.

According to Hagelqvist (2013) an estimated 400 million tonnes of paper and paperboard were 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 phosphorus and nitrogen in terms of use as substrate for microorganisms, hence supplementation of these components may be needed in biological treatment (Harrison, et al., 2017).

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 pulp and 2 262 000 tonnes paper (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 A.4.1) and Cloete et al. (2010) reported 111 971 Mℓ/year (0.11 million Mℓ/year) which indicates some water efficiency already in place. According to 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 (230 – 400 mg C/ℓ) (Cloete, et al., 2010) while Burton et al. (2009) reported an average of 700 mg/ℓ COD (230 mg C/ℓ) (Appendix A.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, 2001) listed in Table 2-2**Error! Reference source not found.**. 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 6000 mg/ℓ. Table 3-3 illustrates the volume, concentration and complexity data for the South African pulp and paper industry (Harrison, et al., 2017).

The pulp and paper sector utilise 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, including chloroform, chlorate, phenols, catechols, guaiacols, furans, dioxins, syringols, vanillins (IWA, 2009). These compounds are formed from reactions between residual lignin from wood fibres and chlorine/chlorine compounds used for bleaching. Coloured compounds and adsorbable organic halogens (AOX) pose serious threats to aquatic organisms if released from pulp and paper mills into the environment (IWA, 2009).

Table 3-3: Volume, concentration and complexity data for the South African pulp and paper industry (summarised from Appendix A.4.1) ((Harrison, et al., 2017), used with permission)

effluent volume in South Africa	**total estimated effluent volume in South Africa**	*ML/year*	111 611
	Days of operation	*days*	365
	total estimated effluent volume in South Africa	*ML/day*	305
cross-reference	to worksheet with primary data and calculations.	Appendix A.4.1 Pulp and Paper industry (Section 3.3.2)	
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*	300
	estimated average nitrogen content	*mg/L*	9
	estimated average phosphorus content	*mg/L*	1
	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

3.3.3 Poultry abattoirs

The animal-based food subsector uses large quantities of water because of the stringent cleanliness requirements. Poultry abattoir wastewater contains high-complexity organics, fats and oils and considerable solids content, with a nutrient rich composition, and is contaminated with fat, viscera,

blood, feathers and faeces (Harrison, et al., 2017). 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%) (Molapo, 2009). Rendering is a heating process for meat industry waste products through which fats are separated from both water and protein residues to produce edible lards and dried protein residues. Commonly rendering includes the production of a range of products of meat and 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 (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 for between 80 and 85% of the waste load (Bremner & Johnston, 1996). The slaughtering and operational status of these plants (26 abattoirs) is detailed in Appendix A.4.2 along with the composition of poultry abattoir effluent characteristics found in literature and the volume of wastewater generated (Molapo, 2009). These are summarised into Table 3-4. .

Table 3-4: Volume, concentration and complexity data for the South African poultry abattoir industry (summarised from Appendix section A.4.2) ((Harrison, et al., 2017), used with permission)

effluent volume in South Africa	**total estimated effluent volume in South Africa per year**	*ML/year*	5400
	Days of operation	*days*	365
	total estimated effluent volume in South Africa per day	*ML/day*	15
cross-reference	to worksheet with primary data and calculations.		Appendix A.4.2
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*	1500
	estimated average nitrogen content	*mg/L*	175
	estimated average phosphorus content	*mg/L*	57
	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. Production of fungal products, integrated with energy recovery in the form of biodiesel, may be particularly well suited (Harrison, et al., 2017). 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 was constructed and commissioned in March 2017 for concomitant biogas production for electricity

generation for the RCL facilities and remediation of wastewater to reduce the COD load by 80% (RCL Foods, 2016).

3.4 Closing remarks on wastewater biorefinery feedstock

Wastewaters have inherent value and potential for nutrient and energy recovery. The wide range of concentration, volume and complexity, with a variety of predictability presents its own challenges. The characteristics of wastewaters may pose a challenge to conventional chemical and physical treatment approaches, but with the advances in bioprocessing understanding, presents a unique opportunity not only for valuable biotransformation, but also improved waste management. An initial examination of the potential of what high value products can be derived from wastewaters is discussed in Chapter 11, focusing on domestic municipal, poultry abattoir and papermill wastewater, acknowledging that detailed feasibility studies are outside of the scope of this work.

4 CATEGORISING WASTEWATER BIOREFINERY PRODUCTS

The ideal biorefinery product candidates are required to meet two main requirements: a market demand and a suitability to production within the constraints of the WWBR. This can limit the choice of product. Ideally, the market demand is for use on the plant itself, and addresses the economics, policy considerations and the market acceptance or required quality standards ('pull' or demand). The bioprocess or technology 'push' or supply responds to non-sterile production, mixed culture production, value of crude product etc.

Products may be favoured that play a role in the functioning of the treatment works or in the industry producing the wastewater, thereby catering to their 'internal market needs'. 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).

4.1 Constraints of the wastewater biorefinery bioprocess on potential products

The key to the concept of the WWBR is the production of multiple value-added products, simultaneously with improvement in water quality as well as production of commodity products to scavenge the residual nutrients or contaminants to yield an appropriate water quality. 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. Wastewaters are often or traditionally 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. Therefore it is beneficial to select culture conditions and a product that contributes a selective advantage to the microbial community of interest (De Bruin, et al., 2004; Winpenny, et al., 2010; Verster, et al., 2014), and bioreactor designs that facilitate process robustness. WWBRs, therefore, are most suitable for products that fulfil a defined role in the microbial ecology allowing natural selection for the groups of microorganisms that produce this product (Mooij, et al., 2015). The individual microorganism species is NOT the important determinant and cannot simply be inoculated as in conventional bioproduction.

The conditions or characteristics of the waste stream can be used to direct bioproduct selection. If the waste stream contains high salt concentrations, for example, polymers that protect the organism against osmotic stress may be prevalent and could be selected. Feast-famine regimes can select for carbohydrates to store energy or for forms of nitrogen or phosphorus storage (De Kreuk, et al., 2005). Waters with high oil content may promote surfactant production.

Separation from the same phase for product recovery is too costly in terms of capital investment, chemicals and energy in a dilute environment. 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. The required DSP has a major influence on the appropriateness of product selection, as discussed in Chapters 6 to 9 specific to each bioreactor type.

The regulations and the required level of purity depend on the product, and its position in the value chain, i.e. if it is for final use like biosurfactants or an intermediate feedstock to a subsequent process (Chen & Zhang, 2015; Ghatak, 2011), for example, a platform chemical. Generally speaking, the higher the required purity of a product, the higher the cost of DSP. In addition, the wastewater environment forms a health barrier, actual or imagined dependent on the waste considered, to the direct use of products for human consumption or applications (Dolnicar, et al., 2011; Asano & Cotruvo, 2011)..

4.2 Categorising potential products

Bio-based products can be described 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 range 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 4-1 presents an overview of common bio-based products and their corresponding characteristics. Owing to the large organic resource contained in wastewater and the potential that higher value products have to enhance the overall economics of the WWBE, commodity products have been considered as the most relevant products of wastewater biorefineries.

Table 4-1: An overview of common bio-based products and their corresponding characteristics (excluding food, energy and fuel products) (adapted from European Commission, 2009)

Product type	**Characteristics or functionalities**	**Product Category (Section** Error! Reference source not found.**)**
Chemical and chemical building blocks Various chemicals made from renewable raw materials, organic acids, diols, alcohols, VFAs	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	1.1
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	1.1
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)	1.2
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)	1.2
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	1.2
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)	1.2
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)	3

The products from wastewater currently typically considered feasible, following the conventional approach, are energy in the form of biogas, phosphate in the form of struvite, cellulose recovery, polyhydroxyalkanoate (PHA) production and alginic acid production (de Fooij, 2015). These products are for the most part considered in isolation of their potential markets.

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

Category one products: bioproducts derived from microbial bioreactors

Category two products: biofuels and bioenergy

Category three products: processed biomass (fertiliser, animal feed, fibre, compost)

Category four products: acceptable quality water: fit-for-use, or compliant for discharge

4.2.1 Category one products: bioproducts

This category is of the highest potential economic value, but traditionally not considered as part of a resource recovery strategy. These bioproducts can further be classed into two subcategories. 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 subcategory include organic acids and volatile fatty acids (VFAs)(Pandey, et al., 2010). The practical approach for the production of metabolites 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 subcategory includes function-based products that use complex macromolecules with minimal modification and purification. Examples of these are industrial enzymes, bioflocculants and biosurfactants or soil conditioner additives (e.g. hydrogels). Products like pigments and alginate can occur in both categories

4.2.2 Category two products: bioenergy and biofuels

This category of products relates to the established need to recover energy from wastes. 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, energy production for use on site will always be an important factor in the WWBR (Sheik, et al., 2014). Due to the lower expected total amounts of production, because of the lower, diffuse chemical energy present in the wastewater which is directed to first level products, and potential difficulty in transport of low-density energy carriers, energy production on the WWBR will almost exclusively be for use on site and the immediate surroundings.

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.

4.2.3 Category three products: processed biomass

To fulfil the “zero waste” and “zero harm” potential of the WWBR, the process needs to go beyond these two levels especially in the arena of the macrophyte and solids bioprocesses. These two processes typically produce products such as cellulosic fibre and compost but does not exclude possible biomass-for-energy products (level two) and bioproducts (level one). Sludges for fertiliser and associated operations may also be handled in this category. The third level products are largely non-specialised commodity products and the stabilised biosolids from municipal treatment works for land application fall in this category. This thesis does not explore the production of this level of product in detail, but does consider it in the process flowsheet analysis.

4.2.4 Category four products: 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.

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. The following chapters examine the potential of various bio-based products available through each bioreactor unit train, focussing particularly on bioproducts likely to be associated with the functioning of the bacterial bioreactor, positioned to deplete the organic loading of the wastewater.

4.3 Closing remarks on potential products from the WWBR

The WWBR is established to maximize resource productivity across the entire system 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 and/or environmentally. Dependent on the composition of the feed stream to the WWBR, the process train used may have different groups of products associated with them. This means that potential products must be carefully assessed and a selection made from the most viable alternatives, specific to each case. The specific products require evaluation 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 produced from wastewater must also be considered. 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. Considering the wide range of products at all levels possible within the WWBR constraints, selection of products becomes a function of the particular feedstock stream and potential market.

5 THE WASTEWATER BIOREFINERY CONCEPT: FROM GENERAL REQUIREMENTS FOR BIOREACTOR DESIGN IN THE WWBR CONTEXT TO FRAMING THE WWBR PROCESS

Owing to the typically dilute nature of wastewater streams, their variability, the impracticalities of sterilisation, the need to handle large effluent volumes, and the requirement to produce compliant effluent, 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 required to serve the dual purpose of bioproduction and water production (see Section 2.2.3) – bacterial (representing heterotrophic microbial bioprocesses like bacteria, yeast, fungal and archea), algal (representing photo-mixotrophic bioprocesses), macrophytic (representing plants that can grow in high water content environments) and solids (representing low water content environments) - must be assessed with the constraints of the WWBR in mind. This chapter gives a generic overview of the constraints for a hypothetical bioreactor, with the chapters following detailing the requirements for each reactor group used in the generic flowsheet, noting that the solids bioreactor is a special case due to the high solids content. Downstream processing is a critical component of bioproduction, and is discussed after the reactor units, in Chapter 10. The requirements for coupling the units together is also discussed in that Chapter.

5.1 Challenges for bioproduction from wastewater informing bioreactor selection requirements

Current wastewater bioreactors are well designed to achieve nutrient removal from the wastewater with the explicit focus on the delivery of clean water as a product. From a bioprocess engineering perspective, using wastewater streams as raw material presents unique challenges in terms of both product formation and recovery. Traditional bioproduct-focused bioreactor optimisation aims to maximise productivity through working at ideal substrate concentrations. Reducing the bioreactor volume to reduce the energy invested per unit product is one such strategy (Harding, et al., 2007). Achieving a high biomass concentration which results in lower downstream processing cost per unit product is another (Richardson, 2011). Using wastewater as raw material requires new thinking as it combines wastewater treatment and bioprocess approaches. Intentionally innovative bioreactor design contributes to, and is essential for, the option 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 be suited to the utilization of organics from large stream flows and to serve an ecological function for the microorganism to drive its competitive advantage (Kleerebezem & van Loosdrecht, 2007; Mooij, et al., 2015) while meeting commodity market needs. Bioreactor design needs to enhance this ecological niche to produce the desired product.

The implementation of the WWBR concept benefits from adhering to key principles in the selection for each unit operation of the system. Bioreactor selection is a crucial element of this. The key principles include:

- Decoupling hydraulic residence time and biomass residence time through biomass retention or biomass recycling.
- Biomass retention enables higher biomass concentration which can contribute to faster volumetric rates for processing of larger volumes.
- Application of non-sterile bioproduction systems where the biological system is selected for by the chosen reactor system and how it is operated, creating an ecological niche for the selected product.

- Ensuring adequate nutrient provision to the cells without excessive energy requirement for mass transfer, and without compromising the ability to recover the product.
- Designing 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. Ideally the product must be in a different phase to the water processed, or easily recovered by adsorption or rapid contacting. Biomass retention may contribute to this product recovery, especially where the product is biomass associated.
- The utilization of a multicomponent system allowing the integrated optimization 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 (Verster, et al., 2014). The principles for integrated optimisation, including product recovery and product formation operations, should also be explored. The sub-sections below provide further insight into overcoming key challenges of wastewater as the WWBR feedstock, using this framework.

5.1.1 Large volumes of wastewater

Very low concentration of valuable product

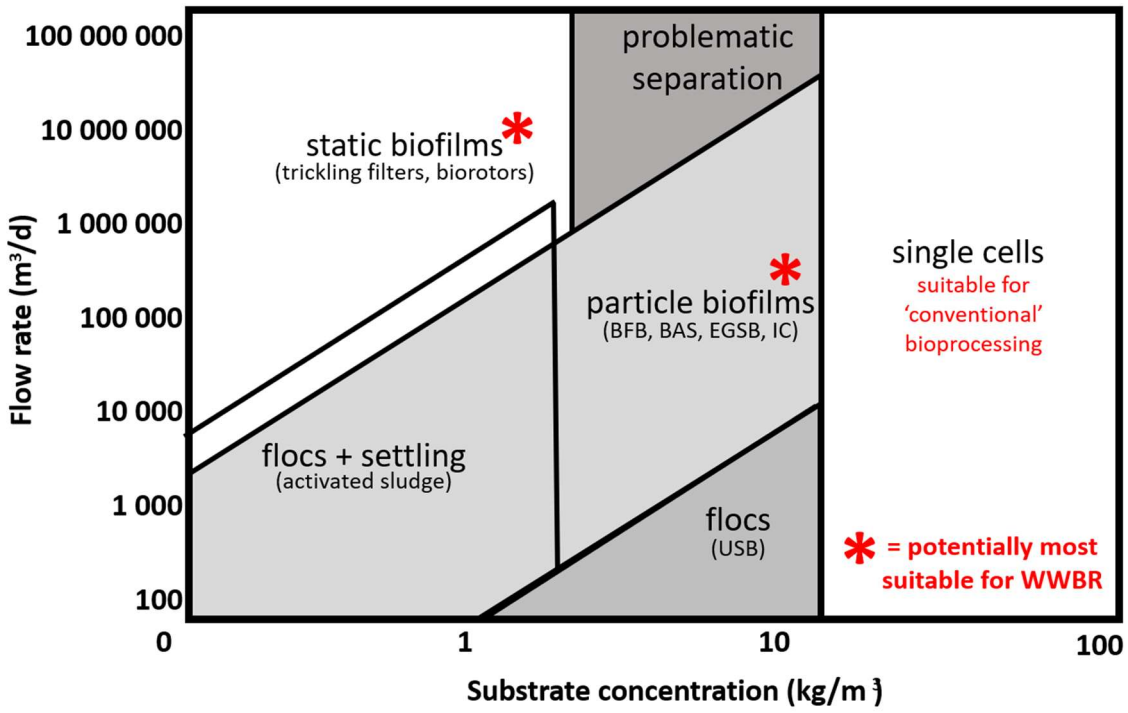
One significant challenge of bioprocesses is the dilute nature of the medium, with both substrates and products present at very low concentration, typically less than 5% of the total dissolved solids. When using waste streams like municipal wastewater which can be a thousand-fold more dilute, this aspect is even more challenging. Pre-concentration is an option but needs to consider the trade-offs for additional chemicals, energy and infrastructure. Reactor design that increases the apparent biomass concentration, allowing a reduction in residence time and hence enhances process intensity is a better option in most cases. Imposing cleaner production principles and saving water upstream would de facto lead to concentration of the streams. 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 (Section 5.1.2), these requirements can be better managed as biomass retention leads to increased conversion rates, more intense processes and hence smaller reactors. With respect to the product recovery, for cost and energy efficient downstream processes, localising product in an accessible location with high apparent concentration is preferred over recovering the product from the bulk medium.

Aeration

Oxygen is sparingly soluble in water. In the typical high-volume low-concentration aerobic bioprocesses energy for aeration is the biggest burden in terms of economics and sustainability (Harding, 2009). In wastewater treatment, aeration can contribute up to 70% of the operating costs (Tchobanoglous, et al., 2003). Oxygen supply often controls stoichiometric limitation, and frequently also governs the reaction rate (Bailey & Ollis, 1986). Dissolved oxygen supply in biofilms presents a special challenge due to the additional barrier to mass transfer that the thickness of the biofilm layer poses to oxygen diffusing through to the deeper biomass. For this reason the current work in value from wastewater favours anaerobic production (Kleerebezem, et al., 2015).

5.1.2 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, the microorganisms tend towards suspended growth, as shown in



(Nicolella, et al., 2000), Biomass retention at these concentrations can still augment, or intensify the process. As conversion is limited in dilute streams by the amount of biomass present, biomass retention allows the necessary increase in biomass concentration (Nicolella, et al., 2000) to ensure sufficient conversion rates. This may be applied to the retention of an inoculated or a naturally-occurring mixed microbial community. Biomass retention also facilitates the effective decoupling of the hydraulic and biomass (or solid) retention time which may be used to improve bioreactor volumetric conversion capacity.

A majority of WWTW employ the use of activated sludge with the resultant flocs requiring large settling ponds. In contrast, the two approaches that are most promising for WWBR bioreactor design, are to generate conditions suitable for static biofilms with slightly higher flowrates than found in the activated sludge process, and particle biofilms occurring at slightly higher substrate concentrations than in the

activated sludge process, as both these approaches may result in higher conversion efficiencies than found in activated sludge, and may make product recovery easier.

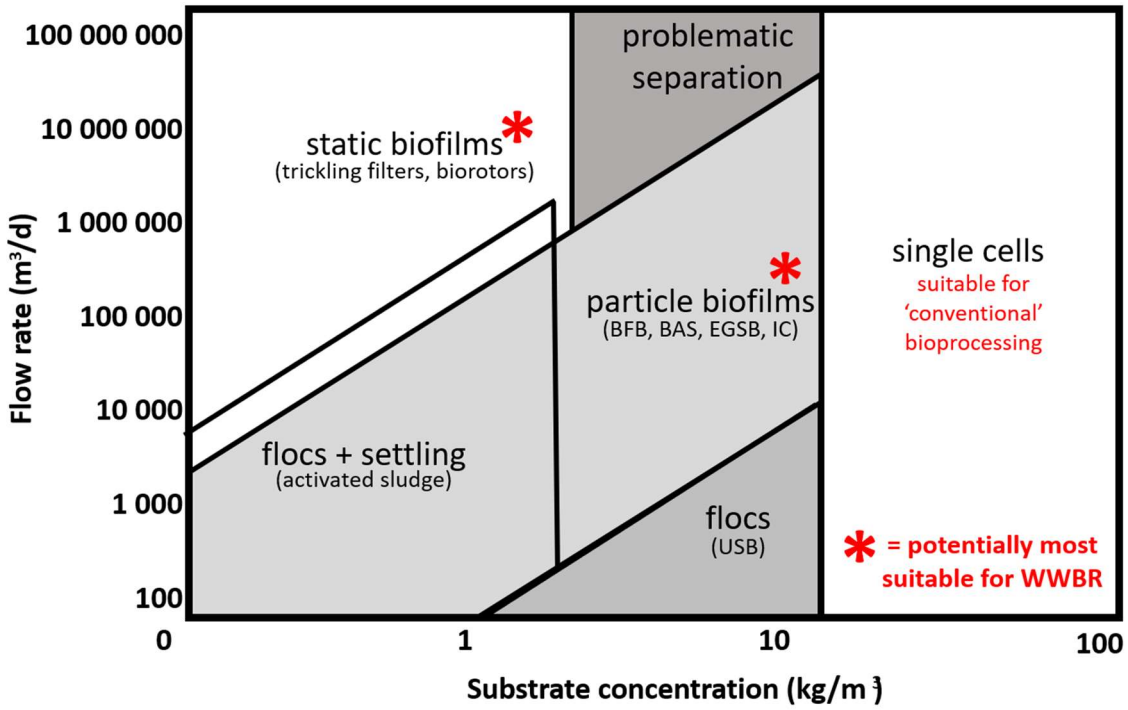


Figure 5-1 Concentration-flowrate phase diagram for application of microbial floc and biofilm bioreactors (adapted from (Nicolella, et al., 2000)) illustrating the most promising areas for WWBR reactor design

Biomass retention can be established by using biomass recycle loops, immobilisation through biofilm formation, granulation of biomass, 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. When membranes are used to retain biomass there is also potential to recover water as in the case of reverse osmosis or ultrafiltration. These membrane filters may require less maintenance if the biomass is retained in some way to not get in contact with the filtration media, for example through combining cell immobilisation with filtering or by including a settling stage prior to filtering.

Biomass retention is used to increase the 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. 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.

5.1.3 Design for downstream processing

Many processes currently use standard bioreactor setups and optimize the downstream processing (DSP) after production, but bioreactor design has scope to facilitate DSP and can have a greater impact on overall process optimization (Richardson, 2011). The entire process needs integrated optimization, cognisant of the performance at the level of unit operation, process operation and systems operation (including aspects outside of the process), from both an economic and environmental point of view. In the dilute systems typical of wastewater, 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 prioritised.

To recover the product, it needs to be in a different phase without excessive addition of chemicals like alum, commonly used for flocculation. For this reason, gaseous products like biogas has been favoured to date, and phosphorus recovery is gaining traction because precipitation of struvite can be induced as part of the bioprocess. Biomass associated product like biopolymers stored in intracellular vacuoles or attached as part of the extracellular polymeric substances (EPS) can be recovered through the biomass retention used for the reactor operation IF the bioreactor is designed to enable this. Design for cell retention and product recovery can therefore be used in combination for improved productivity and facilitation of DSP.

If the product is soluble and excreted in the bulk medium, like ethanol, separation of the biomass from the liquid first requires recovery and concentration, prior to purification. This may involve steps such as distillation, precipitation, adsorption, ultra-filtration and chromatography. The product and whatever additives have been used to recover it still needs to be separated from the bulk liquid to purify the water. Therefore the need for addition of chemical reagents such as precipitation agents is not a preferred route for the recovery of products from dilute suspension and this may disqualify some soluble products excreted into the bulk broth from being produced in a WWBR.

While biomass retention is important, it serves different functions depending on where the product is located, and whether the biomass itself is recovered or not. This plays a role in bioreactor selection. **Error! Reference source not found.** is an initial guideline for wastewater biorefinery bioreactor selection that considers DSP.

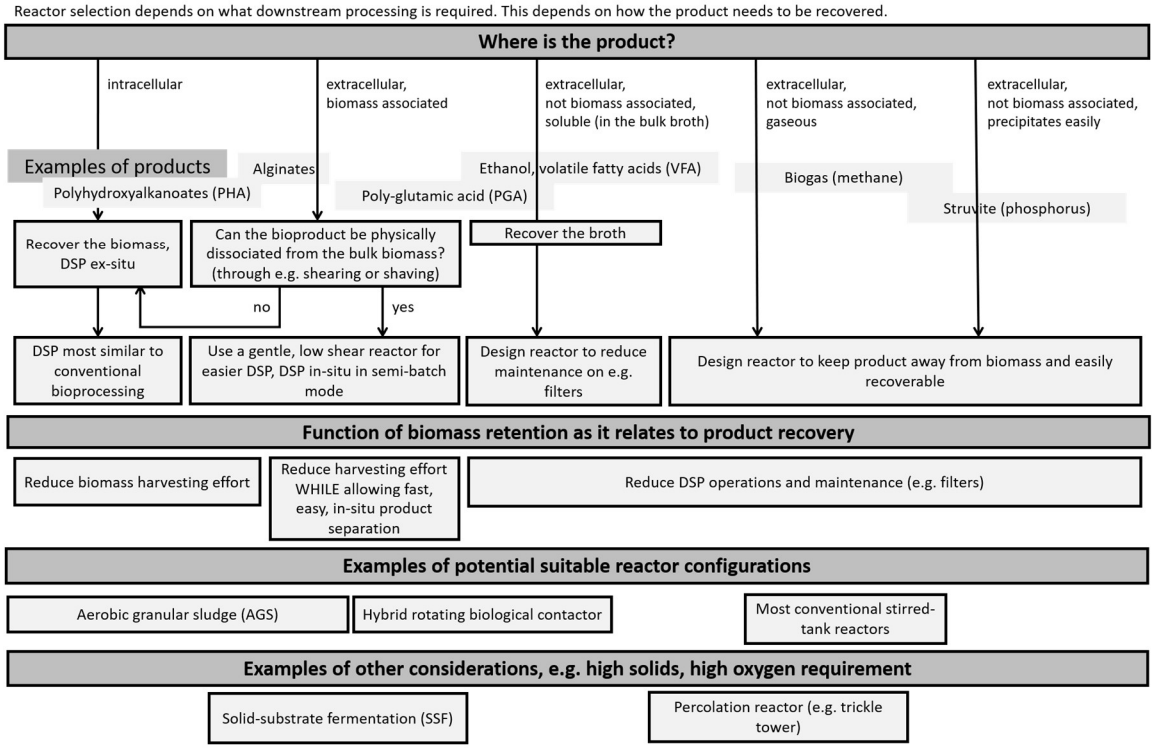


Figure 5-2: Suggested guideline for wastewater biorefinery bioreactor selection using bacterial reactors as example

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5.1.4 Fit for purpose water

Fit for purpose water needs to be comply with authorisation standards as outlined in Section 2.3.3 if discharged, and if reused must meet the quality needed for the reuse. Conventional bioprocessing requires a homogenous, highly controlled environment, but wastewaters tend to be more complex, heterogeneous and variable. As the water is destined for re-use, whether industrial, potable or contributing to ecosystem services in the waterways, 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, based either on the energy requirements or the chemical requirements. 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). To enable product formation, the desired producers must be enriched in this environment through its design. Further, the associated members of the microbial community may exist synergistically to offer process robustness.

Product recovery can assist in reducing pathogen loading directly. One example is biogas production through thermophilic bioprocesses, which increases the temperature to kill pathogens. Reverse osmosis, currently used to filter out pathogens and contaminants to produce potable water, can also serve as a concentration step, where the brine production is a concentration step as part of product recovery. (Stephenson, et al., 2000).

5.1.5 Biocontrol

This chapter outlines strategies for improving the presence of the desired product and enabling a competitive advantage of the desired microbial groupings over contaminating species, but predation of the desired microorganisms by other life-forms remain a challenge. This aspect has been considered in depth in the algal bioprocesses, where for example shear and turbulent flow select for bigger algal cells that cannot be eaten by predators (Kazamia, et al., 2012).

Food web control through predators that keep herbivore grazer numbers down is a biocontrol strategy used in crop cultivation which have been adapted to algal bioproduction. For example, fish predation may be a feasible method to reduce microalgal biomass losses to large-bodied herbivores such as *Daphnia*. Studies have used the mosquitofish, *Gambusia affinis* to achieve this (Kazamia, et al., 2012). However, aiming to produce fish for both a major economic product as well as biocontrol agents in the (e.g. algal bioreactor of the) WWBR would not be advised, as that would create direct tension between operational choices aiming to produce (algal) product or fish.

Directing predation away from the species of interest is also possible. Smith & Crews (2014) concur that the species not initially selected for, which could be considered as ‘weeds’, may have a number of potentially important benefits with regards to (1) total biomass production; (2) crop protection against grazing losses; and (3) crop protection against disease losses.

Managing an ecosystem becomes exponentially more difficult with an increase in size. This may apply to mixed-culture reactors as well. The number of resident plants and animal species scales positively with their habitat size. These Species–Area Relationships (SARs) have been reported for phytoplankton, protozoa, and zooplankton in aquatic ecosystems worldwide (Smith & Crews, 2014). This aspect may be an argument for smaller and/or modular WWBR plants.

5.2 Developing a set of evaluation criteria for WWBR bioreactor design or selection

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. In Section 5.1 this was discussed mainly with respect to the bacterial and algal reactors. 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. These challenges have been converted into a set of requirements that the chosen bioreactor needs to comply with, listed in Table 5-1, and this set of evaluation criteria is applied to the four reactor units in the following chapters.

Table 5-1: Wastewater biorefinery bioreactor design requirements

	#	Challenge that wastewater poses	Design requirement
Design Priority	1	Wastewater as feedstock: large volume, dilute concentration	Decoupling of hydraulic and solid retention times
	2	Wastewater as feedstock: continuous but variable inflow of wastewater	Continuous or semi-continuous process
	3	Dilute medium: cost of downstream processing for product recovery	Product formation in different phase
	4	Complex, variable medium: biomass retention and multiple constituents complicate product recovery	Facilitation of product recovery
Operational Priority	5	Wastewater remediation: need to use the entire wastewater flow for bioproduction	Think big! Commodity rather than niche
	6	Complexity and volume of feedstock: energy for sterilization unfeasible, need robust biocatalysts	Selecting for/enriching microbial community under non-sterile operation, including biocontrol considerations
	7	Complex, variable feedstock: cannot maintain a monoculture	Ecological selection to maintain desired cultures and give advantage to product
	8	Wastewater remediation: non-negotiable production of ecologically compliant effluent	Production of water fit for use or release into environment

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 four categories that have been labelled as “Operational Priority” refer 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.

5.3 Approach to Flow Sheet Development for Biorefineries

The WWBR is defined as a bioproduction system that integrates multiple unit operations to ensure compliant water as well as the ability to produce a bioproduct or bioproducts. Four groupings of unit operations were considered in this project because they each contribute a specific role 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 reactor represented by the algal bioreactor is helpful to scavenge high proportions of nutrients, particularly nitrogen and phosphorus. The algal bioreactor is also known to produce commodity products. The macrophytic bioreactor is targeted for polishing 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 to generate products of value, including biosolids.

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 to a product of value. 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, discussed in detail in Chapters 6 to 9, and illustrated in the validation study in Chapter 11. Chapter 10 provides the links between the unit trains and discusses the downstream processing options.

5.3.1 An overview flowsheet for WWBRs

An overview flowsheet for a generalised wastewater biorefinery is presented in Figure 5 3, with its accompanying lists of unit operations and process streams presented in Table 5-2 and Table 5-3. 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 bioreactor (1), algal bioreactor (2) and macrophyte bioreactor (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 final effluent completely compliant water-as-product (Z). Each bioreactor 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 bioreactor (4), which is likely to be a fungal reactor. The solids bioreactor 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 bioreactor also has carbon dioxide (photosynthesis and respiration) and water (precipitation and evaporation) flows, either forming a net inflow or a net outflow. The generic flow diagram allows provision for a biomass recycle (C4) in the bacterial bioreactor and a feedstock bypass (D2) to the algal bioreactor 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 in Chapters 6 to 9.

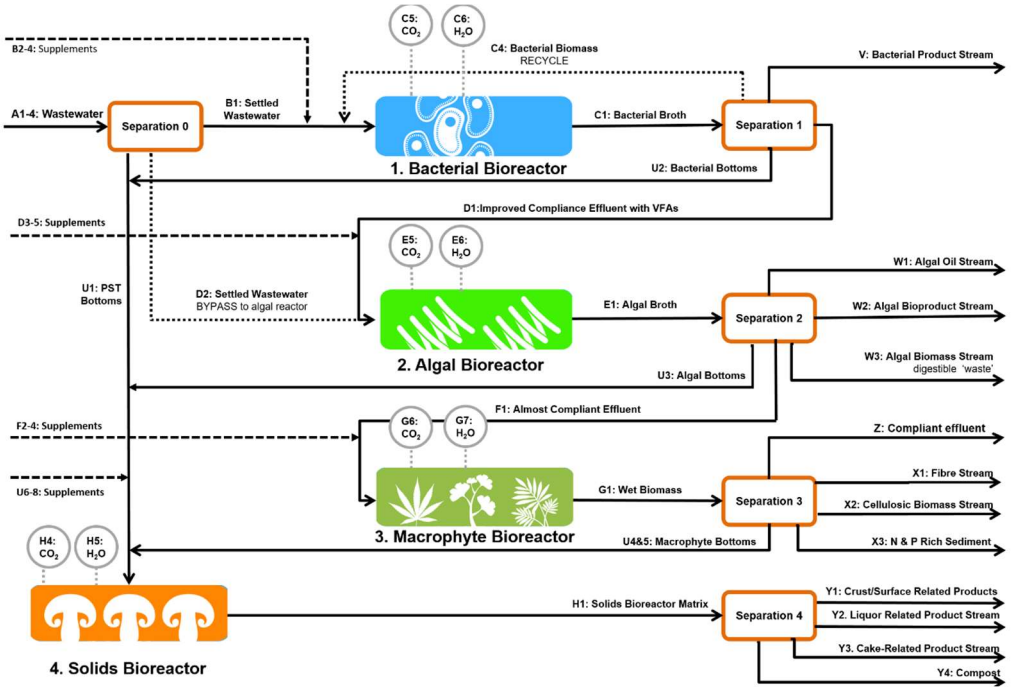

Figure 5-3: Generic wastewater biorefinery overview flowsheet (see Table 5-2 and Table 5-3)

Table 5-2: Overview of operations for generic wastewater biorefinery (see Figure 5-3)

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)
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 5-3 Overview of streams for generic wastewater biorefinery

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

Stream number	Stream description	Relation to process units	Relation to other streams (equations refer to mass balance, not volume)
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
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)

5.3.2 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 in Chapters 6 to 9. In the overall 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 (to account for occurrences such as biomass build-up during retention) and averaged to the per day basis. In this model, a year was used. In particular, aspects of the macrophyte bioreactor train 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 operation (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. 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 5-4: Mass balance equations for 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

5.4 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. 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, or its residue following product extraction, 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. The potential for energy production from “residual” streams within the WWBR should be included (Ghatak, 2011).

5.5 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 thesis focussed on material balances only to describe material flows, and only considers the nutrients of interest for compliant effluent – carbon, nitrogen and phosphorus. Further work to incorporate sulfur, metals and oxygen is recommended.

5.5.1 The approach to the mass balances

For each biorefinery case, a lead commercial product is selected based on the characteristics of the wastewater and the demands of the surrounding market. Reactor trains are selected based on the characteristics of the wastewater, which informs initial product selection. The lead product is selected based on suitability to manufacture from the particular wastewater, and market analysis of the local needs and demand for products. Secondary products are selected to further process the water by removing further contaminants / resources. Further to this, production of water as a product with a quality compliant with specifications is a prerequisite. A selection of case studies illustrating this approach are presented in Chapter 11.

5.5.2 General symbol conventions

C-inflow: The amount of carbon in the inflowing water streams in kg/day, available to be converted into biomass, product or CO_2. 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.
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 phosphorus (kg/m^3) in stream

$N_{S(STREAM)}$ = Total constituent in specified stream, concentration * flowrate (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 phosphorus in specified stream (kg/day)
$N_{W(STREAM)}$ = Total water in specified stream (kg/day)

$X_{React,S(STREAM)}$ = Biomass mass flow rate from specified reactor in specified stream (kg/day) (X = biomass, $_{React}$ = Bacterial, Algal, Macrophyte, Solids, $_S$ = $_{C,N,P}$)
$X_{React,C(STREAM)}$ = Carbon mass flow rate in Biomass component of specified stream (kg/day)
$X_{React,N(STREAM)}$ = Nitrogen mass flow rate in Biomass component of specified stream (kg/day)
$X_{React,P(STREAM)}$ = Phosphorus mass flow rate in Biomass component of specified stream (kg/day)

$P_{i,S(STREAM)}$ = Product i mass flow rate 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 mass flow rate in Product i component of specified stream (kg/day)
$P_{i,N(STREAM)}$ = Nitrogen mass flow rate in Product i component of specified stream (kg/day)
$P_{i,P(STREAM)}$ = Phosphorus mass flow rate in Product i component of specified stream (kg/day)

$S_{S(STREAM)}$ = Unconverted substrate component in specified stream (kg/day) (S = substrate inflow component, $_S$ = $_{C,N,P}$). This component exits the bioreactor, becoming the influent substrate for the next bioreactor. Inflow component may consist of unconverted substrate, biomass or product, entering the specified reactor unit, and available to biological conversion.
$S_{C(STREAM)}$ = Carbon mass flow rate in unconverted inflow component of specified stream (kg/day)
$S_{N(STREAM)}$ = Nitrogen mass flow rate in unconverted inflow component of specified stream (kg/day)
$S_{P(STREAM)}$ = Phosphorus mass flow rate in unconverted inflow component of specified stream (kg/day)

In any given stream, N = S + X + P e.g. the exiting stream mass flow rate (kg/day) is the sum of the residual unconverted component from the unconverted substrate entering the reactor (kg S/day), biomass component (kg X/day) and the product component(s) (kg P/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 Roels (1983). 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 Phosphorus 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 (Roels, 1983). This set of values ($F_{P/C,Xreact}$, $F_{P/C,INreact}$, $F_{P/C,PXi}$) link the Carbon and Phosphorus balances.

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

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

The reactor conversion values used to describe a generic bioreactor (Bioreactor 1) are set out in Table 5-5. 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 is taken for the N and P balances.

Table 5-5: 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)	$Y_{C,IN,unconverted/IN} = 1 - (Y_{C,X/IN} + Y_{C,Xi/IN} + Y_{C,CO2/IN})$

5.5.4 Nitrogen and phosphorus mass balances

The material balance for each reactor train is set up based on a mass balance on the element expected to be the limiting nutrient – carbon for the bacterial and solids bioreactor, nitrogen for the algal bioreactor and phosphorus for the macrophyte bioreactor. For each reactor, the yield based on the chosen element is determined for the conversion from the inflow organic components to biomass and products. The remaining elements' material balances are estimated from conversion factors of relative mass fractions normalised to the base element 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.

5.5.5 Assumptions for mass balances in separation steps

In the integrated generic flowsheet for WWBRs (Figure 5-3), each separation is represented as a lumped operation i.e. as a single step. In the detailed generic flowsheets for each bioreactor train (Chapters 6 to 9), 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 5-6: Overview of separation steps for removal of solids in the integrated generic WWBR

Unit number	Separation description	Relevant parameters	Solids Content symbol
0	Primary Settling	Slurry solids content in "Solids to Bottoms" U1	$SC_{A1\text{-}4,U1}$
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 is optimised for the main product, so that residual biomass, secondary products and unconverted inflow goes to the "bottoms" or waste stream 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 or recycle streams or which are directed to the solids reactor are always the secondary streams. The ratio for both streams sums up to 1.

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

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

5.6 Closing remarks on reactor requirements in the WWBR

In this chapter, key features of the wastewater biorefinery flowsheet mass balances were considered, working with stoichiometric element analysis. The generic flowsheet was introduced, showing how the four bioreactor unit trains fit together. In the following chapters, this is approached in detail for each of the unit trains.

6 THE BACTERIAL BIOREACTOR UNIT TRAIN IN THE CONTEXT OF THE WASTEWATER BIOREFINERY

In traditional WWTW, a heterotrophic microbial bioreactor cultivating mainly bacteria, but which can also include archaea, yeast or submerged culture fungi, is mainly used to treat complex high COD. 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 the first major reactor for organic carbon removal in the WWBR layout, as outlined in Section 2.2.3.

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. The potential for recycle of biomass after product recovery depends on the product produced and the nature of its recovery process.

For the particular needs of the WWBR, the criteria used to select the most appropriate bioreactor type differs from WWTW or conventional biotech applications. This evaluation process is captured in Chapter 5, and include microbial selection (niche environment), microbial retention and product recovery. Selected turnkey bacterial bioreactors are evaluated for the WWBR in the next section, and discussed in more detail in Harrison, et al. (2017). There may be key modifications needed to tailor the design to microbial selection and concomitant bioproduction. Application of the reactor evaluation to the bacterial bioreactor is presented in Section 6.1.

The bacterial bioreactor is selected to produce a high-level value-added product. However, if it is optimised for productivity, depletion of all nutrients is unlikely. This bioreactor 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, for example, a mixotrophic 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.

This chapter describes the requirements of the heterotrophic microbial bioreactor in the context of the WWBR. It provides an overview of specific products possible from this reactor system and provides a framework mass balance to provide early stage feasibility analysis.

6.1 Evaluating reactors against the selection requirements

The chief purpose of the bacterial bioreactor includes converting the organic carbon to value add product, with selective recovery from the large volume effluent. The most commonly used reactor type for WWT is the activated sludge bioreactor. Activated sludge reactors 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. It is one the most common forms of wastewater treatment technologies used in South African municipalities (Grady, et al., 2011; DWA SA, 2009; van der Merwe & Quilling, 2012).

The most promising reactors for the WWBR are aerobic granular sludge, moving bed biofilm and rotating biological contactors. Aerobic granular sludge (AGS) reactors makes use of dense aggregates of biomass with a much higher settling rate than the conventional activated sludge flocs (Adav, et al., 2008). 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. The Moving Bed Biofilm Reactor (MBBR) process is based on biofilm carrier particles. The biofilm is fixed to carrier particles that is thoroughly mixed and retained within a bioreactor. Carrier particle circulation within the bioreactor is provided by the aeration system or by mixers for anaerobic conditions (Grady, et al., 2011). Rotating Biological Contactors (RBCs) contain disks on which microorganisms attach to form biofilms. The disks

are attached to a shaft and rotate in the liquid (wastewater). The shaft and disks are oriented perpendicularly to the direction of the influent (Grady, et al., 2011).

Table 6-1 presents an interpretation of the selection criteria developed in Section 5.2 through the three most promising reactors for use in a WWBR (Harrison, et al., 2017), as well as the most commonly used reactor type for WWT in South Africa, the activated sludge system.

Table 6-1: Bioreactor Design Requirements in order of priority

	#	Requirement	Aerobic Granular Sludge	Moving Bed Biofilm Reactor	Rotating Biological Contactor	Activated Sludge (Stirred tank)
Design Priority	1	Decouples hydraulic and solid retention times	Yes, very well	Yes	Yes	No, with some attempts through a recycling loop
	2	Continuous or semi-continuous (cannot store flows)	Yes, with the incorporation of surge tanks and multiple units	Yes, very well	Yes	Yes, very well
	3	Product formation in different phase	Yes, very well	Yes	Yes	No
	4	Bioreactor design facilitates the recovery of the product	Yes, very well	Yes	Yes	No
Operational Priority	5	Think big! Commodity rather than niche	Yes	Yes	Yes	Possibly
	6	Influences microbial community, non-sterile	Yes, very well	Yes	Yes	Yes, to an extent
	7	Gives advantage to product: creates ecological niche	Yes, very well	Yes	Yes	No
	8	Water released into environment eventually	Yes, may need slightly more polishing than other reactors	Yes, with polishing	Yes, with polishing	Yes, with polishing

As expected, the reactor types selected all release compliant effluent. With respect to enabling product recovery, the activated sludge system fails. Activated sludge along with biological nutrient removal is the most used reactor system in South Africa but is the least suitable to the WWBR. Being a big stirred tank, however, it is fairly straightforward to retrofit these systems to upgrade to aerobic granular sludge (AGS) processes or moving bed biofilm reactors (MBBR). Rotating biological contactors have seen significant operational improvements since they were first commercialised (Verster, et al., 2014) and is a promising bioreactor for smaller WWBR concepts, for example in rural areas.

6.2 Potential bacterial products from wastewater

Various options for microbially produced bioproducts using predominantly the organic, carbon-rich components of the wastewater are assessed in this section. As indicated in Chapter 4.1, products that

contribute a selective advantage to the microbial community holds greatest potential. Final product selection depends on the characteristics of the wastewater, along with analysis of market demands.

Anaerobic bioproduction is not directly considered for the first stage of the WWBR, as this limits the production to the lower value biofuel and bioenergy products, with loss of the molecular complexity of the biological components (Clark, 2017). While the technology is well developed, widespread and well utilised (Mata-Alvarez, et al., 2000; Inglesby, et al., 2015), if the main investment decision is towards anaerobic products, there is an opportunity cost to consider other products that may be more economically attractive. Anaerobic processes and energy production is a viable route but needs to be considered in the context of co-producing higher value products, while also focusing on water quality as key design criteria where anaerobic digestion is applied.

Products in the fine chemicals category are particularly valuable as low volume high value products which can cross-subsidise the operations of the WWBR as a whole. This category includes antioxidants, antimicrobial agents, and industrial enzymes. These are likely only to be feasible if produced intracellularly, or closely associated with the biomass, and where the biomass can easily be recovered. Products excreted into the main (dilute) volume of liquid are very difficult to recover and may only be feasible if a technology like reverse osmosis is employed for final reuse of the water, which may enable product recovery from the brine. Organic acids in the form of short-chain VFAs fall in this category. Further, products that are required to meet high purity standards are typically not suitable for production from wastewaters, as the cost of the final purification may prove uncompetitive when compared to conventional bioproduction using pure substrate. Function-based commodity chemicals like bioflocculants (Buthelezi, et al., 2010), metal chelators (Jackson, et al., 2009) and biosurfactants (Tripathy, et al., 2018) have the same requirement for recovery, but they may have less stringent purification requirements. Biopolymers like polyhydroxyalkanaoates (PHA) alginate and polyglumatic acid (PGA) also need to be cell associated to ease recovery, but it may be possible to recover these products by exploiting their chemical and/or physical characteristics to aid recovery from the bulk liquid. Here significant research in adapting recovery and DSP technology from other industries working with dilute raw materials is needed, for example flotation used in the mining industry (Cilliers, et al., 1994)

Whole cell recovery for niche application is an option, for example in the pursuit of novel microorganisms from wastewater (Bramucci & Nagarajan, 2000). Microorganisms from environments that impose a selective pressure, like the phenol-rich wastewaters of oil refineries, are of particular interest.

Of the main five products currently typically considered feasible from wastewater - alginic acid, phosphorus, biogas, cellulose and polyhydroxyalkanoates (PHA) (de Fooij, 2015), polyhydroxyalkanoate (PHA) production and alginic acid production are considered as examples of fine chemicals which are bacterial in origin. The ‘top 5’ products are already present in wastewater, with a promising market potential. In this thesis the approach is expanded to include novel products that may not be present in high amounts naturally. With this approach in mind, products containing polyglutamic acid (PGA) are included in this selection as a product of interest.

6.2.1 Polyhydroxyalkanoates (PHA)

PHA’s biological function as a storage polymer gives the organisms producing an ecological advantage to survive famine periods (Morgan-Sagastume, et al., 2014). PHAs are economically relevant as a group of bio-based and biodegradable polymers that have a wide variety of physical and chemical properties resembling petroleum plastics and with chemical modification can complement petroleum plastics in various applications (Chen 2009). PHAs have been studied extensively due to their close resemblance to conventional plastics (Loo & Sudesh, 2007). The cost of organic carbon is a key contribution to the cost of PHA, making production from cost-effective waste carbon attractive, provided that good productivites and yields can be maintained (Rumjeet, 2016). Table 6-2 shows various applications of PHAs.

Table 6-2: Applications of PHAs in various industries, potentially suitable to wastewater origins (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 copolymerization 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
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

Nutrient imbalanced growth conditions 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 carbon or energy source (Lee, 1996). Wastewaters with high COD content and low nutrient content is suitable for PHA production, examples include VFA mixtures (acetate, propionate), food waste, olive and palm oil mill effluents, sugarcane molasses, diary effluents, paper mill effluents, fruit and tomato cannery effluents and brewery effluents (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). PHAs can be readily produced from activated sludge biomass using volatile fatty acids (VFAs) as carbon substrates (Johnson, 2010). 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 by 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.

6.2.2 Polyglutamic acid (PGA) containing function-based bioproducts

Polyglutamic acid (PGA), an extracellular biopolymer, is produced by many *Bacillus* species as protection in response to stresses like high osmotic pressure, the presence of metals, and/or as a protective capsule against potential predators (Goto & Kunioka, 1992). It is a biodegradable anionic substance that consists of D- and L-glutamic acid monomers held together by γ-amide linkages between the carboxylic groups.I It is often glycosilated and forms part of the extracellular polymeric substances surrounding the microbial cell (Madonsela, 2013).

This water soluble, non-toxic polyamino acid has potential for a diverse set of industrial applications and extensive functionality through further chemical modification of the side chain hydroxyl groups (Shih & Wu, 2009). In its pure form, it has been successfully used in the food and medical industries. 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 flocculent as shown in the treatment of vinasse from tequila production (Carvajal-Zarrabal, et al., 2011) and soil conditioner (Shih & Wu, 2009). When produced from wastewater it is expected to be more viable in less pure form, to avoid the expensive purification steps, and more appropriate for applications that avoid the acceptance issues of products produced from waste. Thus the food and medical industries are excluded. Table 6-3 lists applications for PGA produced from waste.

Table 6-3: Applications of PGA in various industries, suitable to production from wastewater

Applications	Function	Reference
Biopolymer flocculant	PGA supplemented with cations show a high flocculating activity.	(Bajaj & Singhal, 2011) (Carvajal-Zarrabal, et al., 2011)
Heavy metal removal	Up to 46 and 74% of heavy metal removal efficiencies were achieved. Major heavy metal removal mechanisms were (1) γ-PGA-promoted dissolution and (2) complexation of heavy metal with free carboxyl groups in γ-PGA, which resulted in heavy metal desorption from soils. Metal species on soils were redistributed after washing, and soils were remediated without destruction of soil structures and productivity..	(Yang, et al., 2017)
Textile 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)
Biodegradable plastic	PGA has a nylon-like backbone and is structurally similar to polyacrylic acid. The occurrence of multiple carboxyl residues in PGA likely plays a role in its relative unsuitability for the development of bio-nylon plastics and thus, establishment of an efficient PGA-reforming strategy is of great importance. Two strategies for PGA reforming include esterification and polymer γ-irradiation techniques. It is desired that PGA is transformed into plastics by strong but reversible binding with certain common (and preferably safe) chemicals.	(Kubota, et al., 1995; Ashiuchi, 2013)
Bio-control agent	γ-PGA is not only important to the motile and plant root colonization ability of BsE1, but also essential to the biological control performed by BsE1 against Fusarium root rot.e	(Wang, et al., 2017)
Soil conditioner	γ-PGA greatly strengthened the plant nutrient uptake capacity through enhancing both root biomass and activity. γ-PGA affected carbon (C) and N metabolism in plant which was evidenced with increased soluble sugar contents and decreased nitrate and free amino acids contents.	(Zhang, et al., 2017)
Water retaining agent	The data showed that the PGA hydrogels had gelation times, water contents and mechanical properties that were tunable by adjusting the precursor composition.	(Fan, et al., 2017)

The *Bacillus* species is a well-known robust workhorse that is used in many industrial applications such as production of heterologous proteins, enzymes, antibiotics, nucleotides, biosurfactants, biofuels and biopolymers (Meissner, et al., 2015). They produce PGA under starvation as a glutamate source (Ogunleye, et al., 2014) as well as for protection under harsh conditions (McLean, et al., 1990).The industrial production of PGA is traditionally by running the bioprocess in a classic continuous stirred tank reactor (CSTR) with a steady supply of nitrogen source (Bending, et al., 2014). In response to the identification of PGA as a potential product for production in the WWBR in this study, a *Bacillus* species producing a PGA-containing biopolymer was isolated from the activated sludge obtained from Mitchells Plain WWTW, Cape Town, South Africa (Madonsela, 2013), illustrating both that *Bacillus* species is present and that PGA production is possible in domestic municipal wastewater, thus confirming its great promise for the WWBR concept. The size of these polymers differ from organism to organism and is also dependent on the nutrients available in the cultivation medium (Bajaj & Singhal, 2009).

Due to its potential for wastewater treatment and the wide range of other possible uses, producing this polymer in the wastewater biorefinery will be beneficial. The polymer's protective function towards the bacteria producing it (Ogunleye, et al., 2014) makes it likely that its production from wastewater by a

mixed microbial consortium could be successful due to its ability to create a niche environment for self-promotion.

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 the WRC project K5/2000 (Verster, et al., 2014) this thesis contributed to, and extended in the Master's dissertation of Madonsela (2013).

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 Harrison, et al. (2017) and in the MSc(Eng) dissertation of Raper (in preparation).

6.2.3 Alginate

Alginates are a family of polysaccharides, produced by seaweed and some bacteria as a storage polymer and protective coating (Urtuvia, et al., 2017) with industrial utility as hydrogels at mild pH and temperature conditions. Potential exists for "value-added" alginates, through derivatization of the polysaccharide backbone as reviewed in Pawar & Edgar (2012). Alginates have recently been produced from wastewater (Lin, et al., 2015; van der Hoek, et al., 2015) and their use in wastewater treatment is reviewed in Sudha et al (2014). Similar to PGA, the uses of alginate produced from wastes is restricted to applications that do not require high purity, and non-food, non-medical applications. Other than the application in wastewater treatment, alginates produced from wastewater sources can find application in the cement industry. Curing is the process of controlling the rate and extent of moisture loss from the surface of cement-based materials. It is the final stage in the production of cement-based materials and it is the essential part for achieving continuous hydration of cement, while avoiding cracking due to drying shrinkage. Adding sodium alginate as a curing compound for concrete contributed beneficially to an improved curing structure: a less porous microstructure and an improved durable cement-based material was achieved that was prepared for longer service life (Zlopasa, et al., 2014).

This example represents the archetype of the wastewater biorefinery concept. It looks at the holistic development of more than one industry, where both industries may need to invest effort to adapt their processes to suit the other, but to eventual benefit for both parties. It also shows the importance of involving industries that do not have significant existing links to the industrial water cycle.

6.3 Bacterial bioreactor factors for mass balances

The model used in this thesis to explore the potential of WWBR is based on stoichiometric mass balances. It does not consider bacterial growth rates or specific product formation rates, which vary widely and would require site and situation specific analysis.

6.3.1 Bacterial biomass yields and composition

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 before PHA accumulation (i.e. no PHA present in the biomass) (Fernández-Dacosta, et al., 2015; Tamis, et al., 2014). The bacterial biomass composition used in this model is for aerobic growth, $CH_{1.8}O_{0.5}N_{0.2}P_{0.01}$ which can also be written as $C_{100}H_{180}O_{50}N_{20}P$

(Roels, 1983) as illustrated in Table 6-4. Using these values give a value of 0.16 g C / g biomass.g-substrate-COD. The substrate in the Tamis et al (2014) was fully converted to VFAs and for simplification the VFA was assumed to be propionic acid, with a ratio of 0.486 g C / g propionic acid. This gives a ratio of 0.34 g biomass-C/g substrate-C for the elemental mass balance, where g-substrate-C is equivalent to the total organic carbon (TOC) reported in wastewater treatment. The bacterial biomass concentrations obtained during PGA production, reported in Harrison, et al. (2017), 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. These are values from early experiments without full substrate utilisation. Further work in the labs that this thesis originates from have shown improved yields (Raper, 2018) The conservative value of 0.164 g-biomass-C/g-substrate-C is used in Section 11.2. 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.

Table 6-4: Conversion of composition to mass percent for bacterial biomass

Element	**Composition: Normalised to C** (Roels, 1983) (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	1.00	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	8.00	0.32
Total	N/A	N/A	24.6	1.00

6.3.2 Bacterial bioproduct yields and compositions

The production of bacterial bioproducts is usually reported in terms of volumetric concentrations in the form g-product/ℓ-broth or productivity in g-product/ℓ-broth.hour. 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 elemental 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 – intracellular product requires disruption of the cell - 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 reactor 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; the resultant values for yields are acceptable for order of magnitude estimates typical of these early stage feasibility analyses.

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 g soluble COD/L + 0.8 g solid COD/L is described. Tamis et al. (2014) performed the experiment, while a techno-economic study was performed using the data. 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. The Tamis et al. (2014) case study was used as the intracellular bacterial bioproduct for a demonstration of the model simulation for a simple bacterial bioreactor train presented in Section 11.1. The PHB yield was reported as a biomass-accumulation of 0.76 g PHB / g biomass (as VSS). Using the biomass composition of 0.48 g biomass-C / g biomass and the PHB composition of 0.56 g PHB-C / g PHB gives a conversion factor of 1.17 leading to a yield of 1.67 g PHB-C / g biomass-C. With a biomass yield of 0.34 g biomass-C/g substrate-C this translates to a yield of 0.57 g-PHB-C / g-substrate C. It is important to note that for intracellular products, the overall biomass yield is reduced to reflect the removal of the product, in this case PHB and needs to be calculated appropriately.

Extracellular bacterial bioproduct V1

Polyglutamic acid (PGA) (Section 6.2.2) 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 was extended to experimental studies relevant to wastewaters in the Masters dissertations of Madonsela (2013) and Raper (2017). 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 11.2 as the extracellular bioproduct in the demonstration of the model using an integrated system, with the conservative value of 0.123 g-C-product/g-C-substrate.

Reported concentrations for PGA production vary widely, from less than 1 g/ℓ-broth to more than 100 g/ℓ-broth (Madonsela, 2013). Typical substrate compositions reported to date follow a 'Medium E' recipe (Birrer, et al., 1994). A modified version of this medium (Harrison, et al., 2017) was used as shown in Table 6-5, with a maximum PGA concentration of 3.4 g/ℓ obtained at 37 °C compared to 6 g/ℓ at 30° (Harrison, et al., 2017), which translates to 0.123 and 0.216 g-C-product/g-C-substrate as shown in Table 6-5.

Table 6-5: 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	$C_6H_{12}O_6$	0.400	8.0	
Glycerol	1	$C_3H_8O_3$	0.390	0.4	
Citric acid	12	$C_6H_8O_7$	0.375	4.5	
Total g/ℓ	33			12.9	
Product-content					
Biomass produced (37°C, 30°C)	5.0, 4.4	$CH_{1.8}O_{0.5}N_{0.2}P_{0.025}$	0.480	2.4, 2.1	0.185, 0.164
PGA produced (37°C, 30°C)	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 0.400), 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 0.70 to 0.95.

In the Fernández-Dacosta et al. (2015) study, there was no significant COD loss in the conversion of incoming (complex) COD to VFA, supporting by their high yield factor. In conventional single unit bioreactor systems, as illustrated in Section 11.1 to validate the model developed in this thesis, 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. For aerobic growth respiration replaces VFA production.

6.3.3 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. The default value used by Henze et al. (2008) is 0.24/day. In the WWBR, endogenous respiration should be minimised to allow a greater product yield. In this model the conservative value of 0.33g C(CO_2) / g substrate is used, which reflect the theoretical stoichiometric yield based on aerobic metabolism using sugars through the Krebs cycle. Utilising fats would have better yield values committed to product and less to CO_2.

6.3.4 Summary of yield factors used for Bacterial Bioreactor

The values which will be used in Section 11.2 for the demonstration of simulated mass balance for the integrated system, using the extracellular product PGA as hypothetical example are presented in Table 6-6.

Table 6-6: Carbon-based yield factors for Bacterial Bioreactor

Conversion description	**Symbol of factor**	**Units**	**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)	$Y_{C,XBact/IN}$	g-biomass-C/g-substrate-C	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)	$Y_{C,V1/IN}$	g-productV1-C/g-substrate-C	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)	$Y_{C,VFA/IN}$	g-product VFA-C/g-substrate-C	0.7 to 0.95	0.7- $Y_{V1/IN}$ - $Y_{C,XBact/IN}$ - $Y_{C,CO2Bact/IN}$
Mass of carbon leaving as CO2 as a fraction of that present in influent stream to reactor (B)	$Y_{C,CO2Bact/IN}$	g-CO_2-C/g-substrate-C	0.24/day	0.33
Mass of carbon remaining unconverted as a fraction of that present in influent stream to reactor (B)	$Y_{C,INBact,unconverted/IN}$ = $1 – (Y_{C,XBact/IN} + Y_{C,V1/IN} + Y_{C,CO2Bact/IN})$	g-unconverted-C/g-substrate-C	remainder	remainder

6.4 Bacterial bioreactor unit train mass balances

In the generalised WWBR flowsheet, the bacterial bioreactor is placed as the first treatment and production step in the WWBR, because the bacterial bioreactions are generally the most intensively operated, resulting in the greatest productivity per land area and per unit wastewater. It is also the best

understood biological conversion system available, well developed to produce bioproducts with an established market.

Within the context of the overall WWBR flowsheet presented in Chapter 5.3, the flowsheet for the primary handling of the feedstock followed by the bacterial reactor train is presented in

Figure 6-1, with the accompanying unit descriptions and equations for the overall mass balance in Table 6-7 and the stream descriptions in

Table 6-8. The symbols used for bacterial bioreactor yields (Table 6-9**Error! Reference source not found.**) and separator and splitter factors (Table 6-10) 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 6-11**Error! Reference source not found.** to Table 6-17.

Figure 6-1: Bacterial bioreactor train detailed flowsheet

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

Unit	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/holding 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 unit(s)	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

Table 6-8: 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 6-9: 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 6-10: 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} $1 - r_{C4}$

6.4.1 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 (if required, recommended) and potentially to allow a bypass. The primary settling tank (0.1**Error! Reference source not found.**) receives the feedstock and the liquid component of settled wastewater (A) flows to the splitter (0.2**Error! Reference source not found.**) 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 (Chapter 7.4). 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 (Chapter 9.4).

Table 6-11: Mass balances 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 Liquid fraction	$N_{C(A1\text{-}A4)liq}$ = $Q_{(A1)liq}$* $C_{C(A1)liq}$ + $Q_{(A2)liq}$* $C_{C(A2)liq}$ + $Q_{(A3)liq}$* $C_{C(A3)liq}$ + $Q_{(A4)liq}$* $C_{C(A4)liq}$	$N_{C(A)liq} = N_{C(A1\text{-}A4)liq}$ * ($N_{W(A)}/N_{W(A1\text{-}A4)}$)	$N_{C(U1)liq} = N_{C(A1\text{-}A4)liq}$ * ($N_{W(U1)}/N_{W(A1\text{-}A4)}$)
Unconverted Carbon Solid fraction	$N_{C(A1\text{-}A4)sol}$ = $Q_{(A1)sol}$* $C_{C(A1)sol}$ + $Q_{(A2)sol}$* $C_{C(A2)sol}$ + $Q_{(A3)sol}$* $C_{C(A3)sol}$ + $Q_{(A4)sol}$* $C_{C(A4)sol}$	$N_{C(A)sol} = N_{C(A1\text{-}A4)sol}$ * (1-eff_{U1})	$N_{C(U1)sol} = N_{C(A1\text{-}A4)sol}$ * eff_{U1}
Totals	$N_{C(A1\text{-}A4)} = N_{C(A1\text{-}A4)liq} + N_{C(A1\text{-}A4)sol}$	$N_{C(A)} = N_{C(A)liq} + N_{C(A)sol}$	$N_{C(U1)} = N_{C(U1)liq} + N_{C(U1)sol}$
Checks: Total stream amounts: $(N_{C(A1\text{-}A4)}) - (N_{C(A)} + N_{C(U1)}) = 0$ 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	$N_{N(A1\text{-}A4)liq}$ = $Q_{(A1)liq}$* $C_{N(A1)liq}$ + $Q_{(A2)liq}$* $C_{N(A2)liq}$ + $Q_{(A3)liq}$* $C_{N(A3)liq}$ + $Q_{(A4)liq}$* $C_{N(A4)liq}$	$N_{N(A)liq} = N_{N(A1\text{-}A4)liq}$ * ($N_{W(A)}/N_{W(A1\text{-}A4)}$)	$N_{N(U1)liq} = N_{N(A1\text{-}A4)liq}$ * ($N_{W(U1)}/N_{W(A1\text{-}A4)}$)
Unconverted Nitrogen Solid Fraction	$N_{C(A1\text{-}A4)sol}$ = $Q_{(A1)sol}$* $C_{C(A1)sol}$ + $Q_{(A2)sol}$* $C_{C(A2)sol}$ + $Q_{(A3)sol}$* $C_{C(A3)sol}$ + $Q_{(A4)sol}$* $C_{C(A4)sol}$	$N_{N(A)sol} = N_{N(A1\text{-}A4)sol}$ * (1-eff_{U1})	$N_{N(U1)sol} = N_{N(A1\text{-}A4)sol}$ * eff_{U1}
Totals	$N_{N(A1\text{-}A4)} = N_{N(A1\text{-}A4)liq} + N_{N(A1\text{-}A4)sol}$	$N_{N(A)} = N_{N(A)liq} + N_{N(A)sol}$	$N_{N(U1)} = N_{N(U1)liq} + N_{N(U1)sol}$
Checks: Total stream amounts: $(N_{N(A1\text{-}A4)}) - (N_{N(A)} + N_{N(U1)}) = 0$ 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.			
Phosphorus Mass Balance: Unit 0.1: Separator: Primary Settling Tank			
Phosphorus Fraction	**A1,A2,A3,A4: Incoming Wastewater**	**A: Settled Wastewater**	**U1: PST Bottoms to Solids Bioreactor**
Unconverted Phosphorus Liquid Fraction	$N_{P(A1\text{-}A4)liq}$ = $Q_{(A1)liq}$ * $C_{P(A1)liq}$ + $Q_{(A2)liq}$* $C_{P(A2)liq}$ + $Q_{(A3)liq}$ * $C_{P(A3)liq}$ + $Q_{(A4)liq}$* $C_{P(A4)liq}$	$N_{P(A)liq} = N_{P(A1\text{-}A4)liq}$ * ($N_{W(A)}/N_{W(A1\text{-}A4)}$)	$N_{P(U1)liq} = N_{P(A1\text{-}A4)liq}$ * ($N_{W(U1)}/N_{W(A1\text{-}A4)}$)
Unconverted Phosphorus Solid Fraction	$N_{P(A1\text{-}A4)sol}$ = $Q_{(A1)sol}$ * $C_{P(A1)sol}$ + $Q_{(A2)sol}$* $C_{P(A2)sol}$ + $Q_{(A3)sol}$ * $C_{P(A3)sol}$ + $Q_{(A4)sol}$* $C_{P(A4)sol}$	$N_{P(A)sol} = N_{P(A1\text{-}A4)sol}$ * (1-eff_{U1})	$N_{P(U1)sol} = N_{P(A1\text{-}A4)sol}$ * eff_{U1}
Totals	$N_{P(A1\text{-}A4)} = N_{P(A1\text{-}A4)liq} + N_{P(A1\text{-}A4)sol}$	$N_{P(A)} = N_{P(A)liq} + N_{P(A)sol}$	$N_{P(U1)} = N_{P(U1)liq} + N_{P(U1)sol}$
Checks: Total stream amounts: $(N_{P(A1\text{-}A4)}) - (N_{P(A)} + N_{P(U1)}) = 0$ 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 6-12: Mass balance for Unit 0.2 Splitter: settled wastewater to bacterial bioreactor and bypass

Carbon, Nitrogen, Phosphorus 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 Phosphorus	$N_{P(A)}$	$N_{P(B1)} = N_{P(A)} * r_{B1}$	$N_{P(D2)} = N_{P(A)} * (1 - r_{B1})$
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: $(N_{C(A)}) – (N_{C(B1)} + N_{C(D2)}) = 0$ $(N_{N(A)}) – (N_{N(B1)} + N_{N(D2)})) = 0$ $(N_{P(A)}) – (N_{P(B1)} + N_{P(D2)}) = 0$ $(N_{W(A)}) – (N_{W(B1)} + N_{W(D2)}) = 0$			

6.4.2 Mass balances of mixing tank and bacterial bioreactor

The Bacterial Bioreactor Train begins with a mixing tank (1.0; Table 6-13) 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 6-14). Some bacterial reactors need an external mechanism for increasing the biomass residence time, and an optional biomass recycle stream (C4) is included. The bacterial respiration releases 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 6-13: Mass balance for Unit 1.0 Mixing Tank: bacterial bioreactor inflow

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

Table 6-14: 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 Culture**	**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	$S_{C(B)} = N_{C(B)} = N_{C(B1)} + N_{C(B2-4)}$	$S_{C(C1)} = N_{C(B)} * (1 - (Y_{XBacterial/C} + Y_{P,V1/C} + Y_{P,VFA/C} + Y_{CO2Bacterial/C}))$	$S_{C(C4)} = S_{C(C3)} * r_{C4}$		
Totals	$N_{C(B)} = S_{C(B)}$	$N_{C(C1)} = X_{C(C1)} + P_{V1,C(C1)} + P_{VFA,C(C1)} + S_{C(C1)}$	$N_{C(C4)} = X_{C(C4)} + P_{V1,C(C4)} + P_{VFA,C(C4)} + S_{C(C4)}$	$N_{C(C5)} = CO_{2Bacterial(C5)}$	
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 Culture**	**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	$S_{N(B)} = N_{N(B)} = N_{N(B1)} + N_{(B2-4)}$	$IN_{N(C1)} = IN_{N(B)} - X_{N(C1)} - P_{V1,N(C1)}$	$IN_{N(C4)} = IN_{N(C3)} * r_{C4}$		
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: $(N_{N(B)} + N_{N(C4)}) – (N_{N(C1)}) = 0$					
Phosphorus Mass Balance: Unit 1.1: Bacterial Bioreactor					
Phosphorus Fraction	**B: Inflow To Bacterial Bioreactor**	**C1: Bacterial Culture**	**C4: Bacterial Biomass RECYCLE**	**C5: CO2 Release = outflow**	**C6: H2O**
Biomass $X_{Bacterial}$		$X_{P(C1)} = X_{C(C1)} * f_{(XBact)P/C}$	$X_{N(C4)} = X_{C(C4)} * f_{(XBact)P/C}$		
Product P_{V1}		$P_{V1,P(C1)} = P_{V1,C(C1)} * f_{(V1)P/C}$	$P_{V1,N(C4)} = P_{V1,C(C4)} * f_{(V1)P/C}$		
Unconverted Phosphorus	$S_{P(B)} = N_{P(B)} = N_{P(B1)} + N_{P(B2-4)}$	$S_{P(C1)} = S_{P(B)} - X_{P(C1)} - P_{V1,P(C1)}$	$S_{P(C4)} = S_{P(C3)} * r_{C4}$		
Totals	$N_{P(B)} = S_{P(B)}$	$N_{P(C1)} = X_{P(C1)} + P_{V1,P(C1)} + S_{N(C1)}$	$N_{P(C4)} = X_{P(C4)} + P_{V1,P(C4)} + S_{P(C4)}$		
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	$N_{W(B)}$	$N_{W(C1)} = N_{W(B)} + N_{W(C4)} + N_{W(C6)}$	$N_{W(C4)}$		$N_{W(C6)} = (N_{W(B)} + N_{W(C4)})*(F_{rain} - F_{evap})$
$(N_{W(B)} + N_{W(C4)} + N_{W(C6)}) – (N_{W(C1)}) = 0$					

6.4.3 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 6-15) is operated to remove all biomass and product, sending the changed-composition water stream (D1) to the algal reactor train as the main influent (Chapter 7). 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 6-15: 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 Culture outflow**	**C2: Biomass & Product**	**D1: Improved Compliance Effluent**
Biomass $X_{Bacterial}$	$X_{C(C1)}$ = $((N_{C(B2)} + N_{C(B4-6)}) * Y_{XBacterial/C}) + X_{C(C4)}$	$X_{C(C2)} = X_{C(C1)} * eff_{C2}$	$X_{C(D1)} = X_{C(C1)} * (1 - eff_{C2})$
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	$S_{C(C1)}$ = $N_{C(B)} * (1- (Y_{XBacterial/C} + Y_{P,V1/C} + Y_{P,VFA/C} + Y_{CO2Bacterial/C}))$	$S_{C(C2)} = S_{C(C1)} * (N_{W(C2)}/N_{W(C1)})$	$S_{C(D1)} = S_{C(C1)} * (N_{W(D1)}/N_{W(C1)})$
Totals	$N_{C(C1)}$ = $X_{C(C1)} + P_{V1,C(C1)} + P_{VFA,C(C1)} + S_{C(C1)}$	$N_{C(C2)}$ = $X_{C(C2)} + P_{V1,C(C2)} + P_{VFA,C(C2)} + S_{C(C2)}$	$N_{C(D1)}$ = $X_{C(D1)} + P_{V1,C(D1)} + P_{VFA,C(D1)} + S_{C(D1)}$
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 Culture 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	$S_{N(C1)}$ = $(N_{N(B)}) – (X_{N(C1)} + P_{V1,N(C1)} + P_{VFA,N(C1)})$	$S_{N(C2)} = S_{N(C1)} * (N_{W(C2)}/N_{W(C1)})$	$S_{N(D1)} = S_{N(C1)} * (N_{W(D1)}/N_{W(C1)})$
Totals	$N_{N(C1)}$ = $X_{N(C1)} + P_{V1,N(C1)} + P_{VFA,N(C1)} + S_{N(C1)}$	$N_{N(C2)}$ = $X_{N(C2)} + P_{V1,N(C2)} + P_{VFA,N(C2)} + S_{N(C2)}$	$N_{N(D1)}$ = $X_{N(D1)} + P_{V1,N(D1)} + P_{VFA,N(D1)} + S_{N(D1)}$
Checks: Total stream amounts: $(N_{N(C1)}) – (N_{N(D1)} + N_{N(C2)}) = 0$			
Phosphorus Mass Balance: Unit 1.2: Separator			
Phosphorus Fraction	**C1: Bacterial Culture 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 Phosphorus	$S_{P(C1)}$ = $(N_{P(B)}) – (X_{P(C1)} + P_{V1,P(C1)} + P_{VFA,P(C1)})$	$S_{P(C2)} = S_{P(C1)} * (N_{W(C2)}/N_{W(C1)})$	$S_{P(D1)} = S_{P(C1)} * (N_{W(D1)}/N_{W(C1)})$
Totals	$N_{P(C1)}$ = $X_{P(C1)} + P_{V1,P(C1)} + P_{VFA,P(C1)} + S_{P(C1)}$	$N_{P(C2)}$ = $X_{P(C2)} + P_{V1,P(C2)} + P_{VFA,P(C2)} + S_{P(C2)}$	$N_{P(D1)}$ = $X_{P(D1)} + P_{V1,P(D1)} + P_{VFA,P(D1)} + S_{P(D1)}$
Checks: Total stream amounts: $(N_{P(C1)}) – (N_{P(D1)} + N_{P(C2)}) = 0$			

Water Mass Balance: Unit 1.2: Separator			
	C1: Bacterial Culture outflow	**C2: Biomass & Product**	**D1: Improved Compliance Effluent**
Total Water	$N_{W(C1)} = N_{W(B2)} + N_{W(B4-6)} + N_{W(C4)} - N_{W(C5)}$	$N_{W(C2)} = (N_{C(C2)}/C_{comp,bact}) * ((1-SC_{C2})/SC_{C2})$	$N_{W(D1)} = N_{W(C1)} - N_{W(C2)}$
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.			

6.4.4 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 6-16) which is operated to select for a very pure product stream (V1) and sending the biomass slurry (C3) to a splitter (1.4; Table 6-17). 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.

Table 6-16: 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	$S_{C(C2)} = S_{C(C1)} * (N_{W(D1)}/N_{W(C1)})$	$IN_{C(C3)} = IN_{C(C2)} * (N_{W(C3)}/N_{W(C2)})$	$S_{C(V1)} = S_{C(C2)} * (N_{W(V1)}/N_{W(C2)})$
Totals	$N_{C(C2)} = X_{C(C2)} + P_{V1,C(C2)} + P_{VFA,C(C2)} + S_{C(C2)}$	$N_{C(C3)} = X_{C(C3)} + P_{V1,C(C3)} + P_{VFA,C(C3)} + S_{C(C3)}$	$N_{C(V1)} = X_{C(V1)} + P_{V1,C(V1)} + P_{VFA,C(V1)} + S_{C(V1)}$
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	$S_{N(C2)} = S_{N(C1)} * (N_{W(C2)}/N_{W(C1)})$	$S_{N(C3)} = S_{N(C2)} * (N_{W(C3)}/N_{W(C2)})$	$S_{N(V1)} = S_{N(C2)} * (N_{W(V1)}/N_{W(C2)})$
Totals	$N_{N(C2)} = X_{N(C2)} + P_{V1,N(C2)} + P_{VFA,N(C2)} + S_{N(C2)}$	$N_{N(C3)} = X_{N(C3)} + P_{V1,N(C3)} + P_{VFA,N(C3)} + S_{N(C3)}$	$N_{N(V1)} = X_{N(V1)} + P_{V1,N(V1)} + P_{VFA,N(V1)} + S_{N(V1)}$
Checks: Total stream amounts:			

$(N_{N(C2)}) – (N_{N(V1)} + N_{N(C3)}) = 0$			
Phosphorus Mass Balance: Unit 1.3: Separator			
Phosphorus 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 Phosphorus	$S_{P(C2)} = S_{P(C1)} * (1 - eff_{D1})$	$S_{P(C3)} = S_{P(C2)} * (N_{W(C3)}/N_{W(C2)})$	$S_{P(V1)} = S_{P(C2)} * (N_{W(V1)}/N_{W(C2)})$
Totals	$N_{P(C2)} = X_{P(C2)} + P_{V1,P(C2)} + P_{VFA,P(C2)} + S_{P(C2)}$	$N_{P(C3)} = X_{P(C3)} + P_{V1,P(C3)} + P_{VFA,P(C3)} + S_{P(C3)}$	$N_{P(V1)} = X_{P(V1)} + P_{V1,P(V1)} + P_{VFA,P(V1)} + S_{P(V1)}$
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 6-17: Mass balance for Unit 1.4 Splitter: bacterial biomass to recycle and bottoms

Carbon, Nitrogen, Phosphorus 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 Phosphorus	$N_{P(C3)}$	$N_{P(C4)} = N_{P(C3)} * r_{C4}$	$N_{P(U2)} = N_{P(C3)} * (1 - r_{C4})$
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: $(N_{C(C3)}) – (N_{C(C4)} + N_{C(U2)}) = 0$ $(N_{N(C3)}) – (N_{N(C4)} + N_{N(U2)}) = 0$ $(N_{P(C3)}) – (N_{P(C4)} + N_{P(U2)}) = 0$ $(N_{W(C3)}) – (N_{W(C4)} + N_{W(U2)}) = 0$			

6.5 Closing remarks on the heterotrophic microbial bioreactor, represented by the bacterial bioreactor

The heterotrophic microbial bioreactor's main function is to reduce the COD load significantly. It reduces the nutrient load as well, but only as far as required to produce an economically relevant product. Through the representative bacterial bioreactor, this is the most well characterised unit operation with the highest current economic potential for the WWBR. Its operation allows more effective sizing of subsequent reactors, specifically a better conditioned stream for the algal reactor, discussed next in Chapter 7, and a smaller reactor requirement for the wetlands, or macrophyte bioreactor discussed in Chapter 8.

7 THE ALGAL BIOREACTOR UNIT TRAIN IN THE CONTEXT OF THE WASTEWATER BIOREFINERY

The main purpose of the photo-mixotrophic bioreactor, represented by the algal bioreactor but that can include photosynthetic bacteria, is to scavenge nitrogen and phosphorus while producing valuable product. Here, the main biocatalyst is represented by algae. While all algae can grow photoautotrophically (needing CO_2), many species are mixotrophic, being able to grow on organic carbon as well. 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. The algal species are not important as in conventional bioprocesses, instead, the products they produce are of direct interest. These products are selected for through applying ecological pressure to exploit the functional ecology these products contribute to.

Mixotrophic algal systems may be useful to scavenge residual organic carbon while simultaneously carrying out nitrogen and phosphorus 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). To select a product group of choice, factors like the nitrogen and/or phosphate content need to be controlled to influence the ecological pressure towards this product and the species producing it. To reduce bacterial contamination, the carbon content of the feed stream to the algal streams should be limited through optimisation of the bacterial reactor. CO_2 addition has been shown to enhance algal productivity as well as reducing nitrogen loss through ammonia volatilisation (Park, et al., 2011). At a WWBR facility, the CO_2 produced in the bacterial reactors could be re-used at the algal reactor to enhance productivity with a low increase in operating cost.

Most large scale algal production is currently done in raceways. Closed airlift algal reactors may be preferred for high value products due to the greater operational control possible (Jones, 2015). Literature on the use of microalgal 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 to 1m, mixed 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 are mainly used for low COD, high N and 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.

7.1 Evaluating the selection requirements

Table 7-1 evaluates the algal raceway reactor, the most commonly used algal bioreactor against the requirements for reactors used in the WWBR, given in Section 5.2, and includes the airlift bioreactor and a wave bioreactor for comparison.

Table 7-1: Algal bioreactor evaluation

	#	Requirement	High rate algal pond (HRAP)	Airlift bioreactor	Wave bioreactor
Design Priority	1	Decouples hydraulic and solid retention times	Not effectively	Dependent on morphology of algal cell	Dependent on morphology of algal cell
	2	Continuous or semi-continuous (cannot store flows)	Yes	Yes	Yes
	3	Product formation in different phase	Yes, but difficult to recover. Flocculation and sedimentation can be induced. Biofilm possible for macroalgae.	Yes, but difficult to recover. Flocculation and sedimentation can be induced.	Yes, but difficult to recover. Flocculation and sedimentation can be induced. Algal biofilm growth is possible.
	4	Bioreactor design facilitates the recovery of the product	Possible, difficult	Possible (e.g. flotation or filtration)	Possible (e.g. flotation, scraping)
Operational Priority	5	Think big! Commodity rather than niche	Yes, but high potential for high value niche products too	Yes, but high potential for high value niche products too	Yes, but high potential for high value niche products too
	6	Influences microbial community, non-sterile	Yes	Yes, very well	Yes, very well
	7	Gives advantage to product: creates ecological niche	Yes	Yes, very well	Yes, very well

The airlift reactor is generally not used in wastewater treatment due to its higher capital cost which makes it more suitable to pure culture operation. It has a higher energy requirement due to using air sparging to move large volumes of dilute water around (Jones & Harrison, 2014). Airlift bioreactors currently function with low transfer efficiencies and are limited in addressing the light requirement. In an analysis comparing several algal bioreactors, the wave bioreactor was the most energy efficient configuration, but still carries a high capital investment requirement (Jones, 2015).

HRAPs hold much promise for wastewater treatment in general, but they may be limited in WWBR application, because the hydraulic and solid retention times are not well enough decoupled, and the product is difficult to recover. The algal bioreactor does have promise as the main economic unit in a WWBR, but this would depend on very specific market requirements, and/or input streams low in carbon, but high in nitrogen and phosphate. The reactor should be designed to create a selective environment to favour the desired algal growth (Mooij, et al., 2015). 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 (Harrison, et al., 2017). Potential exists to expand algal systems to higher intensity closed photobioreactor systems for higher value products for application towards beneficiating smaller volume wastes.

7.2 Potential algal products from wastewater

In a WWBR approach, the nitrogen should be directed to product, or biomass, rather than lost to the atmosphere through denitrification. Algal product markets include use for bioenergy either on-site or externally, for animal and aquaculture feed additives, algal dyes and pigments, 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). Potential high-value products include phycocyanin and antioxidants like astaxanthin (Bumbak, et al., 2011). 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 and algal biomass for digestion, animal feed or fertiliser. Lipid producing algae could be used to convert waste materials into lipids for conversion into biodiesel as a mopping up of residual nutrients (Schenk, et al., 2008). Table 7-2 summarises the potential photomixotrophic product guide.

Table 7-2: Photomixotrophic (algal) product guide

Product	Wastewater characteristic that may assist in ecological selection	Process conditions that may assist in ecological selection	Product recovery considerations (especially with regards to extraction)
Phycobiliproteins e.g. phycocyanin pigment	High salt concentration to favour *Spirulina* species	May be feasible to produce from brine from membrane-processes	Intracellular, biomass associated
Carotenoids, astaxanthin, antioxidants	Stress conditions inherent in wastewater, for example high light and low nutrients	Stress conditions imposed by reactor operation	Intracellular, biomass associated
Lipids and fatty acids	Nitrogen starvation, silicon deficiency, phosphate limitations, high salinity and heavy metal stress have been found to increase lipid accumulation (Griffiths, et al., 2016).	Ecological selection pressure 'survival of the fattest' as outlined in Mooij et al (2015)	Intracellular, biomass associated. Extraction procedure is different for fatty acid production (for e.g. biodiesel production) (Louw, et al., 2016).
Phycocolloid polysaccharides, e.g. PHA, alginates, agarose, carrageenans	Not well known	May be well exploited through macroalgae (seaweeds), and could be viable in 'hanging cloth' reactors	Intracellular, biomass associated
Cyanophycin-related, N-storage polymers (multi-L-arginyl-poly (L-aspartic acid),	High nitrogen, salt-stress (both need to be present) (Page-Sharp, et al., 1998) Urine (Janssen, 2014)	Feast famine on nitrogen, may be possible with recycled streams.	Intracellular, biomass associated
Biomass	Any, with sufficient nutrients. Growing in alkaline, saline conditions may promote Spirulina which can be harvested more easily.	Process conditions intended to limit predation (Section 5.1.5)	Species exhibiting filamentous morphology may make harvesting easier. Growth factor recovery may be a co-product

7.3 Algal bioreactor factors for mass balances

The model used in this thesis to explore the potential of WWBR is based on stoichiometric mass balances. It does not consider algal growth rates or specific product formation rates, which vary widely and would require site and situation specific analysis.

7.3.1 Algal biomass yields and compositions

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 (micro)algal species (Mooij, et al., 2015). Algal biomass is typically not recycled, as increased biomass concentration is usually detrimental because of the resulting lower light availability.

Selecting a basis for the mass balance is complicated by the potential to select between photo-autotrophic, mixotrophic and heterotrophic algal populations which affects where the carbon is sourced from. Nitrogen was chosen as the basis to reflect the need to minimise the concentration of this nutrient into the effluent, while the macrophyte bioreactor is dedicated to Phosphorus (Chapter 8).

The model formula for algal biomass ($C_{106}H_{181}O_{46}N_{16}P$, also written as $CH_{1.71}O_{0.43}N_{0.11}P_{0.009}$) is based on Park et al (2011), with an N fraction of 0.0917 g N / g algal biomass (Appendix Table B-4). 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 microalgal concentration 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). At 10g/L the culture may become light limited. In a review of heterotrophic algal cultivation (Bumbak, et al., 2011), biomass yields on different substrates, but most commonly acetate and glucose ranged from 0.41 to 0.81 g CDW/g organic substrate.

As the algal bioreactor's main function is to scavenge nitrogen, algal biomass yield based on how effectively this is achieved is the basis for this reactor. A default value of 0.8 (80%) is used. Refinement of this number can be made with knowledge of Ks values which indicates an affinity, and hence scavenging potential of nitrogen containing compounds in the wastewater (Fuentes, et al., 2016).

7.3.2 Algal bioproducts factors

Algal high-value bioproducts W1

For this model, it is assumed that the high value products are biomass associated. The yield is therefore calculated as a biomass fraction. High value pigment yields from algae are reported in the range of 0.03 – 2.9 g/ℓ produced in a heterotrophic cultivation using 50 g/ℓ glucose with a biomass concentration of between 72 and 116 g/ℓ for phycocyanin and 51.8 g/ℓ for astaxanthin (Bumbak, et al., 2011). Using the conservative values gives a ratio between 0.0030 and 0.0038, and a mid-range of 0.0034 g phycocyanin / g algal biomass is used in the model. The elemental mass balance requires this ratio to be in terms of an element. Carbon was used as it is most likely to be present in the product. Phycocyanin ($C_{165}H_{185}O_{30}N_{20}$) has a C fraction of 0.68 g C / g phycocyanin, the C fraction for algae of 0.52 g C / g algal biomass, giving a ratio of 1.31 g C (phycocyanin) / g C (algal biomass). This can be understood as a correction factor: There is more carbon per gram phycocyanin than there is carbon per gram algae. The elemental yield requires this correction factor.

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% under nutrient replete conditions, 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 default value in this model uses the conservative value of 0.23 g lipid / g algal biomass. The elemental mass balance requires this ratio to be in terms of an element. Work from Lang et al (2011) shows C-16 fatty acids to be representative of algal lipids. The generic formula for a C-16 fatty acid $C_{16}H_{32}O_2$ was used, with a C fraction of 0.75, giving a ratio of 1.44 g C (C16 fatty acid) / g C (algal biomass).

Algal photosynthesis and respiration yields

Algal productivity varies widely between autotrophic, mixotrophic and heterotrophic growth, and the desired growth conditions are case specific. Mixo- or heterotrophic growth can give higher algal productivities (Park, et al., 2011), but the presence of dissolved carbon also increases the potential for bacterial contamination. At a WWBR facility, the CO_2 from the bacterial reactors could be reused at the algal reactor, 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, can give 3 to 10 times greater biomass

concentrations than autotrophic growth (Dhull, et al., 2014) but these findings may be highly dependent on the experimental conditions.

The model assumes mixotrophic growth to acknowledge the low amounts of carbon that may be entering from the bacterial reactor, but this can change for different incoming streams and bacterial reactor configurations. For the default configuration the CO_2 added is calculated as what is required to assimilate the nitrogen after the carbon in the influent stream is utilised, at a calculated ratio of 5.7 g C algal biomass / g N algal biomass. This is a conservative value that does not consider the higher ratio required for products like lipids.

7.3.3 Summary of yield factors used for Algal Bioreactor

The values which will be used in Section 11.2 for the simulated elemental mass balance for the algal bioreactor are presented in Table 7-3.

Table 7-3: Element-based yield factors for Algal Bioreactor

Conversion description	Symbol of factor	Units	Range of factor values in literature	Selected factor value for start-point
Mass of nitrogen in solution reporting to algal biomass as a fraction of that present in influent stream to reactor (D)	$Y_{N,XAlgal/IN}$	g N algal biomass / g N influent stream		0.80*
Yield of algal biomass that is product W1	$Y_{W1/,XAlgal}$	g product W1 / g algal biomass	0.00579 – 0.0266	0.0034
Ratio of gC in product W1 to gC in algal product for elemental mass balance	$f_{C,W1/C,XAlgal}$	g C (product W1) / g C (algal biomass)	calculated	1.3
Yield of algal biomass that is product W2	$Y_{W2/,XAlgal}$	g product W2 / g algal biomass	0.13 – 0.5	0.23
Ratio of gC in product W2 to gC in algal product for elemental mass balance	$f_{C,W2/C,XAlgal}$	g C (product W2) / g C (algal biomass)	calculated	1.4
Ratio of gC in algal biomass to gN in algal biomass	$f_{C,XAlgal/N,XAlgal}$	g C algal biomass / g N algal biomass	calculated	5.7
Mass of carbon entering as CO_2 as a function of the stoichiometric requirement	$X_{C,CO2Algal/IN}$	gC	n/a	$f_{C,XAlgal/N,XAlgal} * X_{N,IN} - N_{C(D)}$

7.4 Mass balance for the Algal bioreactor unit train

7.4.1 Overall mass balance of algal bioreactor

In the generalised WWBR flowsheet presented 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 7-1 displays the algal bioreactor train. Descriptions of units and related overall mass balance equations are presented in

Table 7-4**Error! Reference source not found.** and the streams enumerated in Table 7-5. The symbols used for algal bioreactor yields (Table 7-6) and separator and splitter factors (Table 7-7) are then given. Detailed equations for mass balances of the algal bioreactor is presented in Section 7.4.2 and for the separation steps in Section 7.4.3.

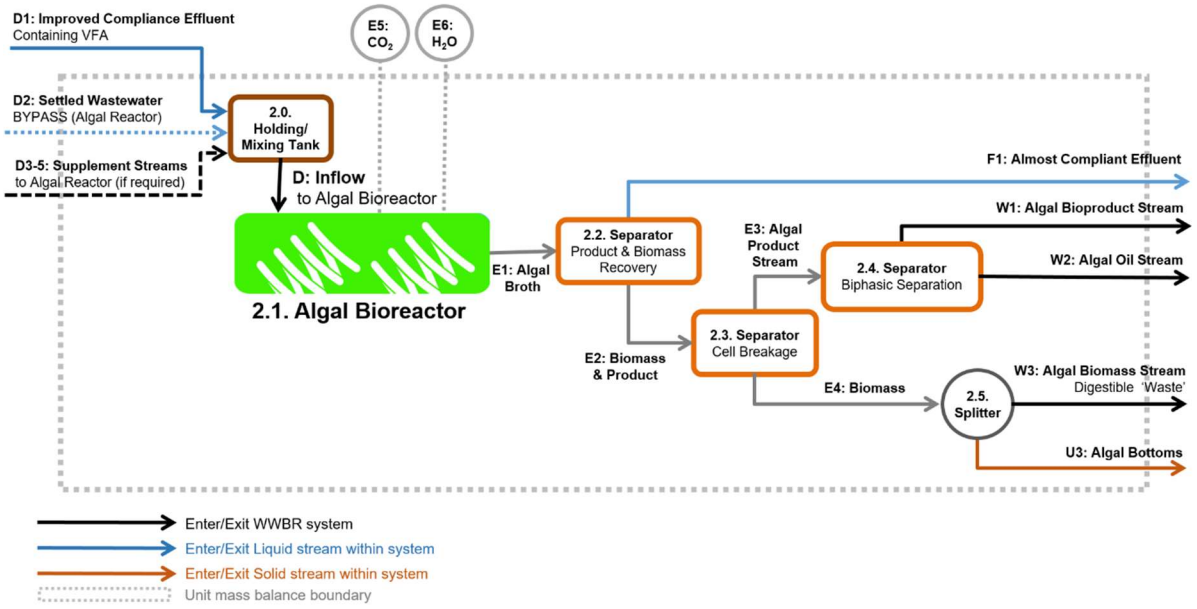

Figure 7-1: Algal bioreactor train detailed flowsheet

Table 7-4: 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	Separator	Separates product + algal biomass from improved effluent (to macrophyte bioreactor)	(E1) – (E2 + F1) = 0
2.3	Cell disruptor and Separator	Downstream processing: cell breakage and separation	(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 7-5: 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 considered for purposes of elemental mass balance
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 7-6: Algal bioreactor yields

Conversion description	Unit	Symbol of factor
Mass of nitrogen reporting to algal biomass as a fraction of that present in influent stream to reactor (D)	kgN(Algal Biomass)/kg N(Inflow Algal Bioreactor)	$Y_{,XAlgal/IN}$
Mass of carbon reporting to algal product W1 as a fraction of mass of carbon in algal biomass	g product W1 / g algal biomass kgC(Product W1)/kg C(Algal Biomass)	$Y_{C,W1/C,Algal}$
Mass of carbon reporting to algal product W2 as a fraction of that mass of carbon in algal biomass	kgC(Product W2)/kg C(Algal Biomass)	$Y_{C,W2/C,Algal/}$
Mass of carbon entering as CO_2 as a function of the stoichiometric requirement	kgC(CO_2 Algal Uptake))	$X_{C,CO2Algal/IN} = (f_{C,XAlgal/N,XAlgal} * X_{N,IN}) - X_{C,IN}$

Table 7-7: 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} $1 - r_{W3}$

7.4.2 Mass balances of algal bioreactor

The algal bioreactor train begins with a mixing tank (2.0, Table 7-8) which receives the inflow streams. The inflow is comprised of 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 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 serve the algal bioreactor adequately. 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, Table 7-9). Most algal reactions include photosynthesis, with a net transfer and assimilation of carbon dioxide from atmosphere (E5) to supplement carbon available in the inflow stream. Algal bioreactor designs also typically have a net inflow or outflow of water (E6) through precipitation and evaporation. Mass balances across these unit operations are presented in Table 7-8 and Table 7-9.

Table 7-8: Mass balance for Unit 2.0 Mixing Tank: Algal Bioreactor inflow

Carbon, Nitrogen, Phosphorus 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	$N_{C(D1)}$ = $X_{C(D1)} + P_{V1,C(D1)} + P_{VFA,C(D1)} + S_{C(D1)}$	$N_{C(D2)} = N_{C(A)} * (1 - r_{B1})$	$N_{C(D3-5)} = Q_{(D3)}* C_{C(D3)} + Q_{(D4)}* C_{C(D4)} + Q_{(D5)}* C_{C(D5)}$	$N_{C(D)} = N_{C(D1)} + N_{C(D2)} + N_{C(D3-5)}$
Total Nitrogen	$N_{N(D1)}$ = $X_{N(D1)} + P_{V1,N(D1)} + P_{VFA,N(D1)} + S_{N(D1)}$	$N_{N(D2)} = N_{N(A)} * (1 - r_{B1})$	$N_{N(B2-4)} = Q_{(B2)}* C_{N(B2)} + Q_{(B3)}* C_{N(B4)} + Q_{(B5)}* C_{N(B5)}$	$N_{N(D)} = N_{N\,(D1)} + N_{N(D2)} + N_{N(D3-5)}$
Total Phosphorus	$N_{P(D1)}$ = $X_{P(D1)} + P_{V1,P(D1)} + P_{VFA,P(D1)} + S_{P(D1)}$	$N_{P(D2)} = N_{P(A)} * (1 - r_{B1})$	$N_{P(B2-4)} = Q_{(B2)}* C_{P(B2)} + Q_{(B3)}* C_{P(B4)} + Q_{(B5)}* C_{P(B5)}$	$N_{P(D)} = N_{P(D1)} + N_{P(D2)} + N_{P(D3-5)}$
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 7-9: 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})		$X_{C(E1)} = X_{N(E1)} * f(X_{Alg})_{C/N}$		
Product P_{W1}		$P_{W1,C(E1)} = X_{C(E1)} * (f_{C,W1/C,XAlgal}) * Y_{W1/,XAlgal}$		
Product P_{W2}		$P_{W2,C(E1)} = X_{C(E1)} * (f_{C,W2/C,XAlgal}) * Y_{W2/,XAlgal}$		
Carbon Dioxide CO_{2Algal}			$CO_{2C,Algal(E5)} = (f_{C,XAlgal/N,XAlgal} * X_{N(E1)}) - N_{C(D)})$	
Unconverted Carbon	$S_{C(D)} = N_{C(D)} = N_{C(D1)} + N_{C(D2)} + N_{C(D3-5)}$	$S_{C(E1)} = 0$ (assumed)		
Totals	$N_{C(D)} = S_{C(D)}$	$N_{C(E1)} = X_{C(E1)} + P_{W1,C(E1)} + P_{W2,C(E1)}$	$N_{C(E5)} = CO_{2Algal(E5)}$	$N_{C(E6)} = 0$
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})		$X_{N(E1)} = N_{N(D)} * Y_{XAlgal/N}$		
Product P_{W1}		$P_{W1,N(E1)} = P_{W1,C(E1)} * f(W1)_{N/C}$		
Product P_{W2}		0		
Unconverted Nitrogen	$S_{N(D)} = N_{N(D)} = N_{N(D1)} + N_{N(D2)} + N_{N(D3-5)}$	$S_{N(E1)} = S_{N(D)} - X_{N(E1)} – P_{W1,N(E1)}$		
Totals	$N_{N(D)} = S_{N(D)}$	$N_{N(E1)} = X_{N(E1)} + P_{W1,N(E1)} + S_{N(C1)}$	$N_{N(E5)} = 0$	$N_{N(E6)} = 0$
Checks: Total stream amounts: $N_{N(D)} - N_{N(C1)} = 0$ Product W2 is Algal oil and contains no N or P.				

Phosphorus Mass Balance: Unit 2.1: Algal Bioreactor				
Phosphorus 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 Phosphorus	$S_{P(D)} = N_{P(D)} = N_{P(D1)} + N_{P(D2)} + N_{P(D3-5)}$	$IN_{P(E1)} = S_{P(D)} - X_{P(E1)} – P_{W1,P(E1)}$		
Totals	$N_{P(D)} = S_{P(D)}$	$N_{P(E1)} = X_{P(E1)} + P_{W1,P(E1)} + S_{P(E1)}$	$N_{P(E5)} = 0$	$N_{P(E6)} = 0$
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	$N_{W(D)}$	$N_{W(E1)} = N_{W(D)} + N_{W(E6)}$		$N_{W(E6)} = N_{W(D)} * (F_{precip} - F_{evap})$
$(N_{W(D)} + N_{W(E6)}) – (N_{W(E1)}) = 0$				

7.4.3 Mass balance for separation steps to harvest products from algal bioreactor outflow

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, Table 7-10) following the algal bioreactor where the now almost compliant effluent (F1) is separated, becoming the inflow for the macrophyte reactor train (Chapter 8). 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 liquid stream (E3) exiting this separator (2.3, Table 7-11) undergoes a further (biphasic) separation (2.4, Table 7-12), resulting in the algal bioproduct stream (W1), which is probably low-volume high-value and may require further downstream processing, and the algal oil product stream (W2), both leaving the biorefinery system. Finally, the residual biomass stream (E4) may be split (2.5, Table 7-13) into a stream leaving the system (W3) as a biomass product and an algal bottoms stream (U2) which is sent to the solids bioreactor train (Chapter 9). The mass balances across these units are detailed in Table 7-10 to Table 7-13.

Table 7-10: 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})	$X_{C(E1)} = N_{C(D)} * Y_{XAlgal/C}$	$X_{C(E2)} = X_{C(E1)} * eff_{E2}$	$X_{C(F1)} = X_{C(E1)} * (1 - eff_{E2})$
Product P_{W1}	$P_{W1,C(E1)} = N_{C(D)} * Y_{P,W1/C}$	$P_{W1,C(E2)} = P_{W1,C(E1)} * eff_{E2}$	$P_{W1,C(E1)} = P_{W1,C(E1)} * (1 - eff_{E2})$
Product P_{W2}	$P_{W2,C(E1)} = N_{C(D)} * Y_{P,W2/C}$	$P_{W2,C(E2)} = P_{W2,C(E1)} * eff_{E2}$	$P_{W2,C(E1)} = P_{W2,C(E1)} * (1 - eff_{E2})$
Unconverted Carbon	$S_{C(E1)} = N_{C(D)} * (1- (Y_{XAlgal/C} + Y_{P,W1/C} + Y_{P,W2/C} + Y_{CO2Algal/C}))$	$S_{C(E2)} = S_{C(E1)} * (N_{W(E2)}/N_{W(E1)})$	$S_{C(F1)} = S_{C(E1)} * (N_{W(F1)}/N_{W(E1)})$
Totals	$N_{C(E1)} = X_{C(E1)} + P_{W1,C(E1)} + P_{W2,C(E1)} + S_{C(E1)}$	$N_{C(E2)} = X_{C(E2)} + P_{W1,C(E2)} + P_{W2,C(E2)} + S_{C(E2)}$	$N_{C(F1)} = X_{C(F1)} + P_{W1,C(F1)} + P_{W2,C(F1)} + S_{C(F1)}$
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})	$X_{N(E1)} = X_{C(E1)} * f(X_{Alg})_{N/C}$	$X_{N(E2)} = X_{N(E1)} * eff_{E2}$	$X_{N(F1)} = X_{N(E1)} * (1 – eff_{E2})$
Product P_{W1}	$P_{W1,N(E1)} = P_{W1,C(E1)} * f(W1)_{N/C}$	$P_{W1,N(E2)} = P_{W1,N(E1)} * eff_{E2}$	$P_{W1,N(E1)} = P_{W1,N(E1)} * (1 – eff_{E2})$
Product P_{W2}	0	0	0
Unconverted Nitrogen	$S_{N(E1)} = S_{N(D)} - X_{N(E1)} – P_{W1,N(E1)}$	$S_{N(E2)} = S_{N(E1)} * (N_{W(E2)}/N_{W(E1)})$	$S_{N(F1)} = S_{N(E1)} * (N_{W(F1)}/N_{W(E1)})$
Totals	$N_{N(E1)} = X_{N(E1)} + P_{W1,N(E1)} + S_{N(C1)}$	$N_{N(E2)} = X_{N(E2)} + P_{W1,N(E2)} + S_{N(E2)}$	$N_{N(F1)} = X_{N(F1)} + P_{W1,N(F1)} + S_{N(F1)}$
Checks: Total stream amounts: $(N_{N(E1)}) – (N_{N(F1)} + N_{N(E2)}) = 0$			
Phosphorus Mass Balance: Unit 2.2: Separator			
Phosphorus Fraction	**E1: Algal Broth outflow**	**E2: Biomass & Product**	**F1: Almost Compliant Effluent**
Biomass X^{Algal} (including P_{W3})	$X_{P(E1)} = X_{C(E1)} * f_{(XAlg)P/C}$	$X_{P(E2)} = X_{P(E1)} * eff_{E2}$	$X_{P(F1)} = X_{P(E1)} * (1 – eff_{E2})$
Product P_{W1}	$P_{W1,P(E1)} = P_{W1,C(E1)} * f_{(W1)P/C}$	$P_{W1,P(E2)} = P_{W1,P(E1)} * eff_{E2}$	$P_{W1,P(E1)} = P_{W1,P(E1)} * (1 – eff_{E2})$
Product P_{W2}	0	0	0
Unconverted Phosphorus	$S_{P(E1)} = S_{P(D)} – X_{P(E1)} – P_{W1,P(E1)}$	$S_{P(E2)} = S_{P(E1)} * (N_{W(E2)}/N_{W(E1)})$	$S_{P(F1)} = S_{P(E1)} * (N_{W(F1)}/N_{W(E1)})$
Totals	$N_{P(E1)} = X_{P(E1)} + P_{W1,P(E1)} + S_{P(E1)}$	$N_{P(E2)} = X_{P(E2)} + P_{W1,P(E2)} + S_{P(E2)}$	$N_{P(F1)} = X_{P(F1)} + P_{W1,P(F1)} + S_{P(F1)}$
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 7-11: 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	$S_{C(E2)} = S_{C(E1)} * (N_{W(E2)}/N_{W(E1)})$	$IN_{C(E3)} = IN_{C(E2)} * (N_{W(E3)}/N_{W(E2)})$	$S_{C(E4)} = S_{C(E2)} * (N_{W(E4)}/N_{W(E2)})$
Totals	$N_{C(E2)} = X_{C(E2)} + P_{W1,C(E2)} + P_{W2,C(E2)} + S_{C(E2)}$	$N_{C(E3)} = X_{C(E3)} + P_{W1,C(E3)} + P_{W2,C(E3)} + S_{C(E3)}$	$N_{C(E4)} = X_{C(E4)} + P_{W1,C(E4)} + P_{W2,C(E4)} + S_{C(E4)}$
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	$S_{N(E2)} = S_{N(E1)} * (N_{W(E2)}/N_{W(E1)})$	$S_{N(E3)} = S_{N(E2)} * (N_{W(E3)}/N_{W(E2)})$	$S_{N(E4)} = S_{N(E2)} * (N_{W(E4)}/N_{W(E2)})$
Totals	$N_{N(E2)} = X_{N(E2)} + P_{W1,N(E2)} + S_{N(E2)}$	$N_{N(E3)} = X_{N(E3)} + P_{W1,N(E3)} + S_{N(E3)}$	$N_{N(E4)} = X_{N(E4)} + P_{W1,N(E4)} + S_{N(E4)}$
Checks: Total stream amounts: $(N_{N(E2)}) – (N_{N(E3)} + N_{N(E4)}) = 0$			
Phosphorus Mass Balance: Unit 2.3: Separator			
Phosphorus 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 Phosphorus	$S_{C(E2)} = S_{C(E1)} * (N_{W(E2)}/N_{W(E1)})$	$S_{P(E3)} = S_{P(E2)} * (N_{W(E3)}/N_{W(E2)})$	$S_{P(E4)} = S_{P(E2)} * (N_{W(E4)}/N_{W(E2)})$
Totals	$N_{C(E2)} = X_{C(E2)} + P_{W1,C(E2)} + S_{(E2)}$	$N_{P(E3)} = X_{P(E3)} + P_{W1,P(E3)} + S_{P(E3)}$	$N_{P(E4)} = X_{P(E4)} + P_{W1,P(E4)} + S_{P(E4)}$
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 7-12: 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	$S_{C(E3)} = S_{C(E2)} * (N_{W(E3)}/N_{W(E2)})$	$S_{C(W1)} = S_{C(E3)} * (N_{W(W1)}/N_{W(E3)})$	$S_{C(W2)} = S_{C(E3)} * (N_{W(W2)}/N_{W(E3)})$
Totals	$N_{C(E3)} = X_{C(E3)} + P_{W1,C(E3)} + P_{W2,C(E3)} + S_{C(E3)}$	$N_{C(W1)} = X_{C(W1)} + P_{W1,C(W1)} + P_{W2,C(W1)} + S_{C(W1)}$	$N_{C(W2)} = X_{C(W2)} + P_{W1,C(W2)} + P_{W2,C(W2)} + S_{C(W2)}$
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	$S_{N(E3)} = S_{N(E2)} * (N_{W(E3)}/N_{W(E2)})$	$S_{N(W1)} = S_{N(E3)} * (N_{W(W1)}/N_{W(E3)})$	$S_{N(W2)} = S_{N(E3)} * (N_{W(W2)}/N_{W(E3)})$
Totals	$N_{N(E3)} = X_{N(E3)} + P_{W1,N(E3)} + S_{N(E3)}$	$N_{N(W1)} = X_{N(W1)} + P_{W1,N(W1)} + S_{N(W1)}$	$N_{N(W2)} = X_{N(W2)} + P_{W1,N(W2)} + S_{N(W2)}$
Checks: Total stream amounts: $(N_{N(E3)}) – (N_{N(W1)} + N_{N(W2)}) = 0$			
Phosphorus Mass Balance: Unit 2.4: Separator			
Phosphorus 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 Phosphorus	$S_{P(E3)} = S_{P(E2)} * (N_{W(E3)}/N_{W(E2)})$	$S_{P(W1)} = S_{P(E3)} * (N_{W(W1)}/N_{W(E3)})$	$S_{P(W2)} = S_{P(E3)} * (N_{W(W2)}/N_{W(E3)})$
Totals	$N_{P(E3)} = X_{P(E3)} + P_{W1,P(E3)} + S_{P(E3)}$	$N_{P(W1)} = X_{P(W1)} + P_{W1,P(W1)} + S_{P(W1)}$	$N_{P(W2)} = X_{P(W2)} + P_{W1,P(W2)} + S_{P(W2)}$
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 7-13: Mass balance for Unit 2.5 Splitter: algal biomass to biomass product W3 and bottoms

Carbon, Nitrogen, Phosphorus and Water Mass Balance: Unit 2.5: Splitter			
Fraction	**E4: Biomass**	**W3: Algal Biomass Stream** "Digestable Waste"	**U3: Algal Bottoms**
Total Carbon	$N_{C(E4)} = X_{C(E4)} + P_{W1,C(E4)} + P_{W2,C(E4)} + S_{C(E4)}$	$N_{C(W3)} = N_{C(E4)} * r_{W3}$	$N_{C(U3)} = N_{C(E4)} * (1 - r_{W3})$
Total Nitrogen	$N_{N(E4)} = X_{N(E4)} + P_{W1,N(E4)} + S_{N(E4)}$	$N_{N(W3)} = N_{N(E4)} * r_{W3}$	$N_{N(U3)} = N_{N(E4)} * (1 - r_{W3})$
Total Phosphorus	$N_{P(E4)} = X_{P(E4)} + P_{W1,P(E4)} + S_{P(E4)}$	$N_{P(W3)} = N_{P(E4)} * r_{W3}$	$N_{P(U3)} = N_{P(E4)} * (1 - r_{W3})$
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: $(N_{C(E4)}) - (N_{C(W3)} + N_{C(U3)}) = 0$ $(N_{N(E4)}) - (N_{N(W3)} + N_{N(U3)}) = 0$ $(N_{P(E4)}) - (N_{P(W3)} + N_{P(U3)}) = 0$ $(N_{W(E4)}) - (N_{W(W3)} + N_{W(U3)}) = 0$			

7.5 Closing remarks on the algal bioreactor

Nitrogen was chosen as the basis to reflect the need to minimise the concentration of this nutrient into the effluent, while the macrophyte bioreactor is dedicated to Phosphorus (Chapter 8). The mass balancing illustrates this approach, and allows future interrogation via techno-economic analyses and environmental evaluation.

Phosphorus is the nutrient usually controlling freshwater lake eutrophication, while eutrophication in most coastal marine ecosystems is primarily controlled by nitrogen. Managing only nitrogen without also managing phosphorus inputs can lead to a situation where phosphorus becomes the nutrient controlling eutrophication (National Research Council, 2000), hence the incorporation of the macrophyte reactor to provide the polishing required. This is discussed next in Chapter 8.

8 THE MACROPHYTE BIOREACTOR UNIT TRAIN IN THE CONTEXT OF THE WASTEWATER BIOREFINERY

8.1 Defining the macrophyte bioreactor

The macrophyte reactor is positioned as a polishing step in the WWBR to ensure fit for purpose water quality, and likely not as the main commercially productive focus. The macrophyte reactor does not equate to a treatment wetland, which is defined as “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 on achieving effective macrophytic bioproduct removal (Fosso-Kankeu & Mulaba-Bafubiandi, 2014) and compliant, fit for purpose exiting water product simultaneously. This requires higher active maintenance and greater mechanical input to ensure high conversion and productivity than conventional treatment wetlands. It has more in common with an agricultural production system than a treatment wetland. Quantitative values are less well characterised for this reactor, with very little research to date on potential products and their recovery, working in parallel with the remediation function.

From the conventional constructed (planted in earth) wetland perspective, wetland classification is based on hydrology and type of macrophyte growth. There are three possible types of hydrology describing how the fluid and sediment interacts: open water-surface, horizontal subsurface flow and vertical subsurface flow. Macrophyte growth is usually classified as emergent, submerged, free-floating or floating-leaved. Hydrology and macrophyte growth are used in various combinations to achieve different reactor types (Vymazal, 2014). The treatment efficiencies and bioproduction potential of the different macrophyte bioreactor types lie in the same order of magnitude.

Floating Treatment Wetlands (FTWs) form another type of macrophyte bioreactor, first developed about 20 years ago in Japan (Dodkins & Mendzil, 2014b). There are several FTW in operation at small scales, using a variety of methods to bind the matrix and allow it to float, including bamboo, empty plastic bottles, netting, meshes etc. A commercial design, marketed by Floating Islands International (2016), makes use of post-consumer polymer fibres (Reinsel, n.d.), which from initial work supporting this thesis provides greater stability to the floating base, and provides a suitable environment for bacterial establishment that further contributes to residual nutrient removal and the polishing step while still allowing unimpeded fluid flow through the matrix. For this reason, it is possible for FTWs to be more efficient than conventional constructed wetlands (Dodkins & Mendzil, 2014b).

8.2 Evaluating the selection requirements

The greatest challenge with the macrophyte bioreactor is efficient harvesting and maintenance, on which all the types of constructed wetlands rate poorly. FTWs decouple the sediment-fluid interaction. Provided the holding tank or pond does not dry out and allow the roots to embed on the pond floor, the sludge removal potential of FTW is greatly enhanced. The depth of the bioreactor is also not constrained by the distance between the roots and emerged sections of the plants, which can enable smaller land footprint, but deeper bioreactor systems. 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. Finally, using a floating wetland system as the macrophyte bioreactor allows greater flexibility in the process design. Table 8-1 evaluates the FTW against the WWBR bioreactor requirements and compares this with constructed wetlands.

Table 8-1: Macrophyte bioreactor evaluation

	#	Requirement	Floating Treatment Wetlands	Constructed wetlands
Design Priority	1	Decouples hydraulic and solid retention times	Yes	Not effective solid (macrophyte) retention depends on land area, with constrained depth of fluid
	2	Continuous or semi-continuous (cannot store flows)	Yes	Yes
	3	Product formation in different phase	Yes	Separating product from sediment is problematic
	4	Bioreactor design facilitates the recovery of the product	Yes	No
Operational Priority	5	Think big! Commodity rather than niche	Yes, floating wetlands allow deeper tanks that can deal with larger flows	Very sensitive to land availability
	6	Influences microbial community, non-sterile	Possible	Possible
	7	Gives advantage to product: creates ecological niche	Possible, requires maintenance	Possible, channelling and short-circuiting are threats affecting the ecology as well as treatment efficiency

From this first evaluation, FTWs look very promising in the WWBR context. The design priority requirements are met without condition. The operational priority requirements need further research, but do not indicate serious flaws. In conventional treatment wetlands maintaining an ecologically diverse population is a challenge, with *Typha* and *Phragmytes* tending to dominate unmaintained wetlands. In the macrophyte bioreactor aimed at higher value production, maintenance similar to agricultural production would be required to maintain the plant of choice.

8.3 Potential macrophyte products from wastewater

High value bioproducts from macrophytes exist, such as crafted products like furniture, brushes or brooms, harvested products like cut flowers, fruits and seeds, and processed products like fibrous biomass, biomass for energy carriers, essential oils and natural colourants like indigo. Residual biomass can be used for lower value applications like bioenergy. Macrophyte products and residual byproducts can be used to supplement the organic material for the solids bioreactor in an integrated WWBR. While growing food on wetlands is possible (Kakuru, et al., 2013), wetlands used for wastewater treatment may be exposed to contaminants like heavy metals that bioaccumulate in the food and can be hazardous to (human) health.

Very high value products with very low yields – like the essential oils and colourants, may be feasible if the rest of the plant can still be used economically. As example, *Indigofera tinctoria* is reported to yield about 3 kg of indigo powder from 100 kg plant biomass; this contains 300 – 400 g of indigo (Bechtold & Mussak, 2010). At an estimated planting density of 220 000 plants per hectare (100 m^2) yielding 12-18 tons of green biomass or 4-6 tons of dry mass per hectare, this translates to a yield of 12 – 18 kg indigo per hectare, equating to up to 1.8 g per m^2 (Abdullaev & Ibragimov, 2009) per harvest. Indigo-producing plants are perennial and plant mass is harvested while leaving the rootstock intact. Two harvests per year is possible, giving a potential annual yield of 3.6 g indigo per square meter. South Africa has a variety of economically important indigenous plants that could be promising in this context, requiring further research (Wyk & Gericke, 2000). Yields of linseed and linseed oil are in the region of 1455 kg/ha and 483 kg/ha (at an average yield of 30%) respectively (Charlton & Ehrensing, 2001).

In this thesis, fibrous biomass was selected as it has the largest impact on the mass balance and a wide variety of potential uses that is acceptable for value-from-waste markets, like fibre for (geo)textiles, composites and the construction industry. Fibrous plants include flax, hemp, nettle, jute, kenaf, sisal,

coir, and cotton (Mussig, 2010). Hemp fibre shows great promise and has a large market globally but is currently not legal to grow in South Africa.

For the demonstration of the model, flax (*Linum usitatissimum*) has been chosen as it is well characterised. The stem of the plant is used for textile production and increasingly in building and structural applications. 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 which contributes to cleaning the water. Evidence suggests that removal of shoots does not negatively affect the roots (Dodkins & Mendzil, 2014b). For fibre production, the final plant density is about 2000 plants per square meter, and they are harvested before seed production. The main component used for the long fibres is called 'straw dry mass' and forms 25 – 30% yields of the stem ((Mussig, 2010).

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, but overall suitability, productivity, technical performance and market need for products from indigenous species remains limited. These factors are listed in Table 8-2.

Table 8-2: Plant requirements for use in WWBR macrophyte bioreactor

Importance	Requirement	Comment
Critical	High moisture tolerance	The plant roots must be able to withstand being submerged in water and may experience anoxic conditions. Treatment wetlands are typically low-oxygen waters (Kadlec & Wallace, 2009)
Critical	High capacity for uptake of nitrogen and other nutrients	The primary purpose of the plants is to remove nutrients.
Critical	Low sensitivity to wastewater constituents	Plants must be able to cope with, and preferably degrade or accumulate, recalcitrant chemicals, heavy metals and other contaminants. Existing knowledge about the mode of accumulation is preferred to determine the eventual fate of these contaminants after downstream processing.
Critical	Non-invasive	There is risk of plants escaping from even highly managed or controlled environments. This should be an acceptable risk.
Important	If an indigenous species can be used, then that takes strong preference.	Using indigenous species is best from a biodiversity point of view, but it can be difficult to find species that comply with the critical requirements. For example, in the Fynbos biome in the Cape, plants are adapted to survive in nutrient poor environments, and do not have a high nitrogen uptake capacity
Less important for macrophyte reactors than constructed wetlands	Minimum management requirements	Harvesting and management is part of the reactor system and is tolerable if it translates into higher productivity. Fast product growth and thus a frequent need for harvesting is good, for example, while excessive weeding is not.
Depends on treated water demand	High consumptive use or evapotranspiration demand	In conventional wetlands where the water load must be reduced to limit flooding risk, this is preferred, but where the water is desired as a product, as in the WWBR, then the evapotranspiration demand should be limited.

8.4 Macrophyte bioreactor factors for mass balances

8.4.1 Macrophyte biomass and bioproduct yields and compositions

The basis for the elemental mass balance around the macrophyte bioreactor was chosen to be phosphorus. This reflects the function of scavenging the last nutrients as a polishing step. A default value of 0.7 (70%) phosphorus uptake is used (Kadlec, 2016). Refinement of this number for specific cases can be made with knowledge of Ks values which indicates an affinity, and hence scavenging

potential of phosphorus in the wastewater. Care should be taken that incoming P concentration does not overwhelm the uptake capacity.

Plant fibre compositions are generally reported only in terms of structural polymers i.e. the polysaccharides cellulose and hemicelluloses and the aromatic polymer lignin, with little concern for the N and P content which is very low (Marques, et al., 2010). Flax contains 64% cellulose, 17% hemi-cellulose 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 g N/g biomass) was chosen, and the model assumes that this N content is met by the N uptake from the water, but that biological N fixing from the atmosphere could take place if N becomes limiting. For the P composition, the average value for grasses of 0.23% P (0.0023 gP/g biomass) (Harper, et al., 1933), is used, while noting that grasses are reported to be higher in N than flax (2.53%).

For simplicity of calculation, the remainder of the dry biomass is assumed to be of carbonaceous composition (1 – 0.00735 – 0.0023 gC/gbiomass). The elemental composition of cellulose and hemicellulose are similar, and for simplification of the elemental mass balance, cellulose was used to give a total C composition of flax of 0.441 g C / g macrophyte (Appendix Table B-4 and B-5). This gives a calculated representative molecular formula of $C_{494}H_{824}O_{412}N_7P$ or stated in terms of cellulose units $(C_6H_{10}O_5)_{82}N_7P$.

An important 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 stoichiometric model averages this to a daily rate and assumes that the climate is suitable for staggered planting throughout the year.

Product X1 is fibrous biomass used where good quality long fibres are required, for example (geo)textiles and furniture. The elemental composition is assumed to be the same as the whole plant. A yield of 25% (0.25 g Product X1 / g macrophyte biomass) is used as discussed in Section 8.3.

Product X2 and U4 are the waste, or poorer quality fibre, still assumed to be the same elemental composition as the whole plant. This fibrous biomass can be used for lower quality fibre products, like building materials and insulation (product X2), or organic material for solid substrate bioprocesses or composting (product U4 to the solids bioreactor).

8.4.2 Macrophyte photosynthesis and respiration factors

While the macrophyte bioreactor can use any water-dependent plant that can grow in watery conditions, including free-floating and floating leaved, emergent (not completely submerged) macrophytes are considered here due to the interaction with the matrix and its biome that is believed to also contribute to water quality (Dodkins & Mendzil, 2014b). Emergent macrophytes are photoautotrophic, meaning they obtain their CO_2 exclusively from the atmosphere. The CO_2-C contribution in the stoichiometric elemental mass balance modelled in this thesis is calculated based on what is needed to incorporate the phosphorus available into the biomass.

The default value of $C_{macrophyte}$ is 0.441 g-C/g-macrophyte biomass (Section: 8.4.1 above). Calculating from the representative molecular formula, the amount of C needed to accommodate 1 g P into the biomass is 192 g C / g P.

8.4.3 Sedimentation in the macrophyte bioreactor

In conventional treatment wetlands nutrient removal, particularly P, is mainly through settling into the sedimentation, and accounts for about 40 - 60% of the P (45 – 75 g/m^2/year (Dodkins & Mendzil, 2014b)). Total N removal through floating wetlands includes microbial denitrification processes as well and amounts to about 75%. The stoichiometric WWBR model does not make explicit allowance for sedimentation phenomena because it is likely that the preceding bacterial and algal biomass significantly reduce this sedimentation by effectively moving the biomass ‘sediment’ into the preceding bioreactors. Sedimentation is still an important component to bear in mind because dredging (at a

recommended rate of around once every 10 years) is still required for reactor maintenance, which is needed for sustained P removal (Dodkins & Mendzil, 2014b). The product streams X3 and U5 are to account for any residual material after harvesting to close the mass balance around the macrophyte bioreactor.

8.4.4 Summary of yield factors used for Macrophyte Bioreactor

Table 8-3: Element-based yield factors for Macrophyte Bioreactor

Conversion description	Symbol of factor	Units	Range of factor values in literature	Selected factor value for start-point
Mass of phosphorus in solution reporting to macrophyte biomass as a fraction of that present in influent stream to reactor (D)	$Y_{P,XMacrophyte/IN}$	g P macrophyte biomass / g P influent stream	0.05 – 0.87	0.70
Fraction of macrophyte biomass that is product X1	$Y_{X1/,XMacrophyte}$	g product X1 / g macrophyte biomass	0.25 – 0.30	0.25
Ratio of gC in product X1 to gC in macrophyte product for elemental mass balance	$f_{C,X1/C,XMacrophyte}$	g C (product X1) / g C (macrophyte biomass)	calculated	1
Fraction of macrophyte biomass that is product X2	$Y_{X2/,XMacrophyte}$	g product X2 / g macrophyte biomass	remainder	0.75
Ratio of gC in product X2 to gC in macrophyte product for elemental mass balance	$f_{C,X2/C,XMacrophyte}$	g C (product X2) / g C (macrophyte biomass)	calculated	1
Mass of carbon entering as CO_2	$Y_{C,CO2Macrophyte/IN}$	g C (CO_2) / g P (macrophyte biomass)	calculated	192

8.5 Macrophyte bioreactor unit train mass balances

8.5.1 Overall mass balance of 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 8-1 and the units with the corresponding overall mass balance equations (

Table 8-4**Error! Reference source not found.**) and stream descriptions (Table 8-5) follow. Macrophyte bioreactor yield symbols are presented in Table 8-3 , with the symbols for separator and splitter factors given in Table 8-6. The detailed mass balance equations for the macrophyte bioreactor train are discussed next in Section 8.5.2.

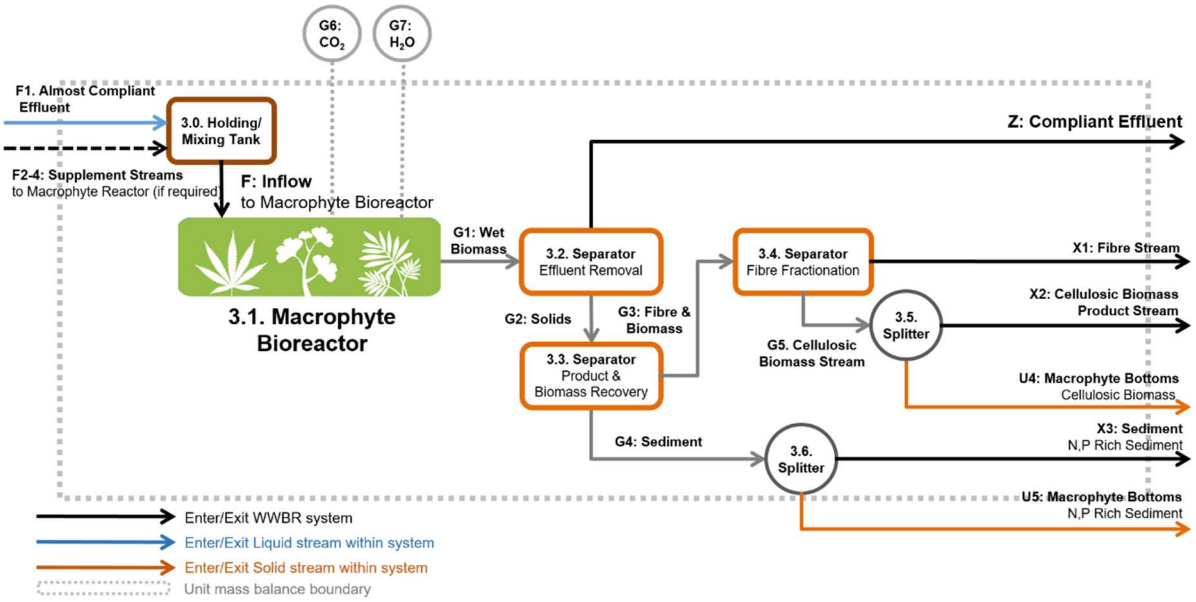

Figure 8-1: Detailed flowsheet of the macrophyte bioreactor train

Table 8-4: 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 involves separate steps, e.g. manual harvesting (seasonal), and sediment de-sludging (annual)	(G2) – (G3 + G4) = 0
3.4	Size Fractioning Separator	Macrophyte Biomass 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

Units 3.2 and 3.3 can be considered as virtual units as they do not operate continually and cannot be seen as distinct. Unit 3.2 is for harvesting the biomass but effectively is to provide a point of exit for the compliant effluent (Z). Unit 3.3 Is defined as a desludging operation.

Table 8-5: Streams in the 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)
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 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 8-6: 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} $1 - r_{X3}$

8.5.2 Mass balances of macrophyte bioreactor

The macrophyte bioreactor train may begin with a mixing tank (3.0,Table 8-7) 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 (Chapter 7); once combined with possible supplementary nutrients this forms the inflow (F) to the macrophyte bioreactor (3.1, Table 8-8). Macrophytes always have a net inflow of carbon dioxide from the atmosphere (G4) through photosynthesis which is considerably greater than respiration. The macrophyte bioreactors are usually exposed to the elements, hence, depending on the local climate, they have a net inflow or outflow of water (G7) from precipitation and evaporation. Evapotranspiration from the macrophytes are included in the water stream (G7).

Table 8-7: Mass balance for Unit 3.0 Mixing Tank: macrophyte bioreactor inflow

Carbon, Nitrogen, Phosphorus 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 Phosphorus	$N_{P(F1)}$	$N_{P(F2-4)} = Q_{(F2)} * C_{P(F2)} + Q_{(F3)} * C_{P(F4)} + Q_{(F5)} * C_{P(F5)}$	$N_{P(F)} = N_{P(F1)} + N_{P(F2-4)}$
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 8-8: 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**	**G7: H_2O**
Biomass $X_{Macrophyte}$		$X_{C(G1)} = X_{P(G1)} * f(X_{macrophyte})_{C/P}$		
Carbon Dioxide $CO_{2,Macrophyte}$			$X_{C(G4)} = (f_{C,XMac/P,XMac} * X_{P(F)}) - N_{C(F)}$	
Unconverted Carbon	$S_{C(F)} = N_{C(F)} = N_{C(F1)} + N_{C(F2-4)}$	$S_{C(G1)} = S_{C(F)} - X_{C(G1)}$		
Totals	$N_{C(F)} = S_{C(F)}$	$N_{C(G1)} = X_{C(G1)} + S_{C(G1)}$	$N_{C(G4)} = X_{C(G4)}$	$N_{C(G7)} = 0$
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**	**G7: H_2O**
Biomass $X_{Macrophyte}$		$X_{N(G1)} = X_{C(G1)} * f(X_{macrophyte})_{N/C}$		
Unconverted Nitrogen	$S_{N(F)} = N_{N(F)} = N_{N(F1)} + N_{(F2-4)}$	$S_{N(G1)} = S_{N(F)} - X_{N(G1)}$		
Totals	$N_{N(F)} = S_{N(F)}$	$N_{N(G1)} = X_{N(G1)} + S_{N(G1)}$	$N_{P(C5)} = 0$	$N_{P(C6)} = 0$
Checks: Total stream amounts: $N_{N(F)} - N_{N(G1)} = 0$				
Phosphorus Mass Balance: Unit 3.1: Macrophyte Bioreactor				
Phosphorus Fraction	**F: Inflow to Macrophyte Bioreactor**	**G1: Wet Biomass**	**G6: CO_2**	**G7: H_2O**
Biomass $X_{Macrophyte}$		$X_{P(G1)} = X_{C(G1)} * f(X_{Macrophyte})_{P/C}$		
Unconverted Phosphorus	$S_{P(F)} = N_{P(F)} = N_{P(F1)} + N_{P(F2-4)}$	$S_{P(G1)} = S_{P(F)} - X_{P(G1)}$		
Totals	$N_{P(F)} = S_{P(F)}$	$N_{P(G1)} = X_{P(G1)} + S_{N(G1)}$	$N_{P(G6)} = 0$	$N_{P(G7)} = 0$
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**	**G7: H_2O**
Total Water	$N_{W(F)}$	$N_{W(G1)} = N_{W(F)} + N_{W(G7)}$		$N_{W(G7)} = N_{W(F)} * (F_{precip} - F_{evap})$
$(N_{W(F)} + N_{W(G7)}) - (N_{W(G1)}) = 0$				

8.5.3 Mass balances for separation steps of macrophyte bioreactor

The products from the macrophyte bioreactor goes through several separation processes. The compliant effluent (Z), the key product of the biorefinery, flows through the bioreactor where it is either discharged into the environment or reused. The macrophytes are harvested as represented by the virtual separator unit (3.2, Table 8-9) producing a fibre and biomass stream (G3). The sediment from the reactor is periodically removed by desludging, represented by the virtual separator unit (3.3, Table 8-10) producing a nitrogen and phosphorus rich sediment stream (G4) which is not included in the model calculations. The fibre and biomass are separated in a further separation unit (3.4, Table 8-11),

with a fibre product stream (X1) and a cellulosic biomass stream (G5) 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 (Chapter 9). Likewise, the sediment may be split (3.5, Table 8-12) into a product stream (X3) and/or a sediment bottoms stream (U5) which could be combined with the solids bioreactor train (Chapter 9) inflow.

Table 8-9: 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 $X_{Macrophyte}$	$X_{C(G1)} = C_{,CO2Macrophyte(G6)}$	$X_{C(G2)} = X_{C(G1)} * eff_{G2}$	$X_{C(Z)} = X_{C(G1)} * (1 – eff_{G2})$
Unconverted Carbon	$S_{C(G1)} = S_{C(F)} - X_{C(G1)}$	$S_{C(G2)} = S_{C(G1)} * (N_{W(G2)}/N_{W(G1)})$	$S_{C(Z)} = S_{C(G1)} * (N_{W(Z)}/N_{W(G1)})$
Totals	$N_{C(G1)} = X_{C(G1)} + S_{C(G1)}$	$N_{C(G2)} = X_{C(G2)} + S_{C(G2)}$	$N_{C(Z)} = X_{C(Z)} + S_{C(Z)}$
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 $X_{Macrophyte}$	$X_{N(G1)} = X_{C(G1)} * f(X_{macrophyte})_{N/C}$	$X_{N(G2)} = X_{N(G1)} * eff_{G2}$	$X_{N(Z)} = X_{N(G1)} * (1 – eff_{G2})$
Unconverted Nitrogen	$S_{N(G1)} = S_{N(F)} - X_{N(G1)}$	$S_{N(G2)} = S_{N(G1)} * (N_{W(G2)}/N_{W(G1)})$	$S_{N(Z)} = S_{N(G1)} * (N_{W(Z)}/N_{W(G1)})$
Totals	$N_{N(G1)} = X_{N(G1)} + S_{N(G1)}$	$N_{N(G2)} = X_{N(G2)} + S_{N(G2)}$	$N_{N(Z)} = X_{N(G1)} + S_{N(G1)}$
Checks: Total stream amounts: $(N_{N(G1)}) – (N_{N(G2)} + N_{N(Z)}) = 0$			
Phosphorus Mass Balance: Unit 3.2: Separator			
Phosphorus Fraction	**G1: Wet Biomass**	**G2: Solids**	**Z: Compliant Effluent**
Biomass, including solids $X_{Macrophyte}$	$X_{P(G1)} = X_{C(G1)} * f(X_{Macrophyte})_{P/C}$	$X_{P(G2)} = X_{P(G1)} * eff_{G2}$	$X_{C(Z)} = X_{C(G1)} * (1 – eff_{G2})$
Unconverted Phosphorus	$S_{P(G1)} = S_{P(F)} - X_{P(G1)}$	$S_{P(G2)} = S_{P(G1)} * (N_{W(G2)}/N_{W(G1)})$	$S_{P(Z)} = S_{P(G1)} * (N_{W(Z)}/N_{W(G1)})$
Totals	$N_{P(G1)} = X_{P(G1)} + S_{N(G1)}$	$N_{P(G2)} = X_{P(G2)} + S_{P(G2)}$	$N_{P(Z)} = X_{P(G1)} + S_{P(G1)}$
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	$N_{W(G1)} = N_{W(F)} + N_{W(G7)}$	$N_{W(G2)} = (N_{C(G2)}/C_{comp,macrophyte}) * ((1\text{-}SC_{G2})/SC_{G2})$	$N_{W(Z)} = N_{W(G1)} - N_{W(G2)}$
Checks: Total stream amounts: $(N_{W(G1)}) – (N_{W(G2)} + N_{W(Z)}) = 0$			

Table 8-10: 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 $X_{Macrophyte}$	$X_{C(G2)} = X_{C(G1)} * eff_{G2}$	$X_{C(G3)} = X_{C(G2)} * eff_{G3}$	$X_{C(G4)} = X_{C(G2)} * (1 - eff_{G3})$
Unconverted Carbon	$S_{C(G2)} = S_{C(G1)} * (N_{W(G2)}/N_{W(G1)})$	$S_{C(G3)} = S_{C(G2)} * (N_{W(G3)}/N_{W(G2)})$	$S_{G(G4)} = S_{C(G2)} * (N_{W(G4)}/N_{W(G2)})$
Totals	$N_{C(G2)} = X_{C(G2)} + S_{C(G2)}$	$N_{C(G3)} = X_{C(G3)} + S_{C(G3)}$	$N_{C(G4)} = X_{C(G4)} + S_{C(G4)}$
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 $X_{Macrophyte}$	$X_{N(G2)} = X_{N(G1)} * eff_{G2}$	$X_{N(G3)} = X_{N(G2)} * eff_{G3}$	$X_{N(G4)} = X_{N(G2)} * (1 - eff_{G3})$
Unconverted Nitrogen	$S_{N(G2)} = S_{N(G1)} * (N_{W(G2)}/N_{W(G1)})$	$S_{N(G3)} = S_{N(G2)} * (N_{W(G3)}/N_{W(G2)})$	$S_{N(G4)} = S_{N(G2)} * (N_{W(G4)}/N_{W(G2)})$
Totals	$N_{N(G2)} = X_{N(G2)} + S_{N(G2)}$	$N_{N(G3)} = X_{N(G3)} + S_{N(G3)}$	$N_{N(G4)} = X_{N(G4)} + S_{N(G4)}$
Checks: Total stream amounts: $(N_{N(G2)}) – (N_{N(G3)} + N_{N(G4)}) = 0$			
Phosphorus Mass Balance: Unit 3.3: Separator			
Phosphorus Fraction	**G2: Solids**	**G3: Fibrous Biomass**	**G4: Sediment**
Biomass $X_{Macrophyte}$	$X_{P(G2)} = X_{P(G1)} * eff_{G2}$	$X_{P(G3)} = X_{P(G2)} * eff_{G3}$	$X_{P(G4)} = X_{P(G2)} * (1 - eff_{G3})$
Unconverted Phosphorus	$S_{P(G2)} = S_{P(G1)} * (N_{W(G2)}/N_{W(G1)})$	$S_{P(G3)} = S_{P(G2)} * (N_{W(G3)}/N_{W(G2)})$	$S_{P(G4)} = S_{P(G2)} * (N_{W(G4)}/N_{W(G2)})$
Totals	$N_{P(G2)} = X_{P(G2)} + S_{P(G2)}$	$N_{P(G3)} = X_{P(G3)} + S_{P(G3)}$	$N_{P(G4)} = X_{P(G4)} + S_{P(G4)}$
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: $(N_{W(G2)}) – (N_{W(G3)} + N_{W(G4)}) = 0$			

Table 8-11: 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	$S_{C(G3)} = S_{C(G2)} * (N_{W(G3)}/N_{W(G2)})$	$S_{C(G5)} = S_{C(G3)} * (N_{W(G5)}/N_{W(G3)})$	$S_{C(X1)} = S_{C(G3)} * (N_{W(X1)}/N_{W(G3)})$
Totals	$N_{C(G3)} = X_{C(G3)} + X_{C,S,Bact(G3)} + S_{C(G3)}$	$N_{C(G5)} = X_{C(G5)} + X_{C,S,Bact(G5)} + S_{C(G5)}$	$N_{C(X1)} = P_{X1,C(X1)} + S_{C(X1)}$
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	$S_{N(G3)} = S_{N(G2)} * (N_{W(G3)}/N_{W(G2)})$	$S_{N(G5)} = S_{N(G3)} * (N_{W(G5)}/N_{W(G3)})$	$S_{N(X1)} = S_{N(G3)} * (N_{W(X1)}/N_{W(G3)})$
Totals	$N_{N(G3)} = X_{N(G3)} + X_{N,S,Bact(G3)} + S_{N(G3)}$	$N_{N(G5)} = X_{N(G5)} + X_{N,S,Bact(G5)} + S_{N(G5)}$	$N_{N(X1)} = P_{X1,N(X1)} + S_{N(X1)}$
Phosphorus Mass Balance: Unit 3.4: Separator			
Phosphorus 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 Phosphorus	$S_{P(G3)} = S_{P(G2)} * (N_{W(G3)}/N_{W(G2)})$	$S_{P(G5)} = S_{P(G3)} * (N_{W(G5)}/N_{W(G3)})$	$S_{P(X1)} = S_{P(G3)} * (N_{W(X1)}/N_{W(G3)})$
Totals	$N_{P(G3)} = X_{P(G3)} + X_{P,S,Bact(G3)} + S_{P(G3)}$	$N_{P(G5)} = X_{P(G5)} + X_{P,S,Bact(G5)} + S_{P(G5)}$	$N_{P(X1)} = P_{X1,P(X1)} + S_{P(X1)}$
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 8-12: Mass balance for Unit 3.5 Splitter: macrophyte cellulosic biomass to product stream X2 and bottoms

Carbon, Nitrogen, Phosphorus 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 Phosphorus	$N_{P(G5)}$	$N_{P(X2)} = N_{P(G5)} * r_{X2}$	$N_{P(U4)} = N_{P(G5)} * (1 - r_{X2})$
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 8-13: Mass balance for Unit 3.6 Splitter: macrophyte sediment to product stream X3 and bottoms

Carbon, Nitrogen, Phosphorus 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 Phosphorus	$N_{P(G4)}$	$N_{P(X3)} = N_{P(G4)} * r_{X3}$	$N_{P(U5)} = N_{P(G4)} * (1 - r_{X3})$
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: $(N_{C(G4)}) - (N_{C(X3)} + N_{C(U5)}) = 0$ $(N_{N(G4)}) - (N_{N(X3)} + N_{N(U5)}) = 0$ $(N_{P(G4)}) - (N_{P(X3)} + N_{P(U5)}) = 0$ $(N_{W(G4)}) - (N_{W(X3)} + N_{W(U5)}) = 0$			

8.6 Take home messages regarding the macrophyte bioreactor

The chief purpose of the macrophyte bioreactor is as polishing function. The key challenge with macrophyte bioreactors is harvesting and maintenance, and floating treatment wetlands perform best in this regard.

Considering that a factor of 192 g C/g P is needed to incorporate P into the macrophytic biomass, it becomes easy to understand why wetlands, and in extension, macrophyte bioreactors would have a high land requirement if not preceded by the bacterial and algal bioreactors. This illustrates the value and potential effectiveness of the integrated wastewater biorefinery. The mass balancing allows this to be interrogated in future scenario planning.

The carbon uptake over the year needs to be averaged to a daily value to align to the day-basis of the mass balance. Two harvests per year is assumed, and the total kg plant mass obtained annually is then divided by 365 to give the daily contribution. To incorporate a continual influx of liquid to be treated, the macrophytes need to be harvested and planted in tandem, to allow some of the growth to be in peak nutrient uptake stages at all times. This is also likely to only be feasible in warmer climates.

Seasonality and frequency of harvesting impact the material balance. Interest in wetlands - of which macrophyte bioreactors can be considered an adaptation - are currently growing, especially in the context of water sensitive urban design, but their high land requirement and maintenance needs can make them unpopular. Combining the macrophyte bioreactor with higher productive systems like the

bacterial bioreactors may reduce the land requirement for tertiary water treatment (or stormwater treatment in urban areas), and the emphasis on valuable bioproduction may change the perception of harvesting as an unpopular maintenance option. Staggering the growth and harvesting may allow for continual water treatment as well as continual harvest, this depends on the climate of the site as well. Research investigating yields of economically important, and preferably indigenous plants grown in treatment wetland environments or planted sludge drying beds may give promising results, even more so if the same plant can produce fibre, residual biomass as well as a high value product.

9 THE UNIT TRAIN OF THE SOLIDS BIOREACTOR IN THE CONTEXT OF THE WASTEWATER BIOREFINERY

A major objective for WWBR is the decoupling of solid and liquid residence times; 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 includes what is generically known as 'solid state fermentation'. Solid-state (alternatively called solid-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). It can be aerobic or anaerobic, despite the general term 'fermentation'.

In mixed solid-state fermentation, the microorganisms are varied 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. These include the static substrate bed, like the bioreactors used in the Koji process (Mitchell, et al., 2010), or the mixed bed through, for example, a rotating drum., Further, the beds may be aerated or not. SSF bioreactors are gaining interest, with novel designs becoming available, 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). SSF bioreactors show great potential for organic solid waste management (Polprasert, 2007).

Planted sludge drying beds (PDBs) can be considered as a macrophyte-assisted solids bioreactor. They are conventionally used as a dewatering strategy and not as a treatment step, consisting of shallow filters filled with sand and gravel with an under-drain at the bottom to collect leachate (Strande, et al., 2014), but could have potential in producing products from both the plants (as another macrophyte bioreactor of sorts) and using microbial biocatalysts.

Vermiculture is gaining commercial interest and represents a macro-organism-assisted solids bioreactor. Black soldier fly larvae (BSFL) is one such example where the organisms are also economically valuable (Britz, 2017). The microbial impact is still critical (Banks, 2014).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 organics in wastewater termed "non-biodegradable" can indeed be made 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 facilitate its bioconversion, both improving the characteristics of the residual solids and producing valuable products. Existing research on solid substrate fermentation on municipal sludges is scarce requiring many assumptions for the mass balance around the solids reactor. Improved research in this field is strongly recommended.

9.1 Evaluating the selection requirements

Selection of the solids bioreactor depends largely on the preferred product and biocatalyst employed. For faecal sludge treatment, this unit reactor must also affect removal of viral and bacterial pathogens, and particularly helminth, *Ascaris* and *Trichuris* eggs (Strande, et al., 2014). Research on the WWBR can contribute to novel ways of addressing the issue, through novel treatment-bioproduction steps. Table 9-1 shows that solids bioreactors are expected to generally comply well with the requirements for the WWBR.

Table 9-1: Solids bioreactor evaluation

	#	Requirement	SSF bioreactor	Planted sludge drying beds
Design Priority	1	Decouples hydraulic and solid retention times	Yes	Yes (evaporation)
	2	Continuous or semi-continuous (cannot store flows)	Possible, lower volumes of solids may make this less critical	Possible, lower volumes of solids may make this less critical
	3	Product formation in different phase	Possible	Yes
	4	Bioreactor design facilitates the recovery of the product	Possible, product-dependent	Yes
Operational Priority	5	Think big! Commodity rather than niche	Yes	Yes, sensitive to land-availability
	6	Influences microbial community, non-sterile	Yes, very well	Possible
	7	Gives advantage to product: creates ecological niche	Yes	Possible, unknown

Product formation may be in a different phase. In this model, products from the SSF bioreactor have been grouped according to their location – on the surface, in the liquor/leachate, or in the bulk of the material.

9.2 Potential products from wastewater solids

The solids bioreactor aims to generate value from the bottoms components generated in the WWBR. While not the sole biological component, solid substrate bioreactions are preferably dominated by fungi (Singhania, et al., 2009), characterised by their production of extracellular enzyme cocktails to metabolise solid organic materials as well as their invasive growth. Moisture levels that are too high lead to the unwanted dominance of bacteria.

Products from the solids bioreactor can be conceptually separated into three broad categories; crust, liquor and cake located products. The cake related products can be further split into compost and other cake related products like fungal mycelium.

9.2.1 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 (in the *Basidiomycetes* group, commonly known as mushrooms (Stamets, 1993)), black soldier fly larvae (BSFL) (Banks, 2014) or a biofilm. Products include enzymes (Stamets, 1993), surfactants (Das & Mukherjee, 2007), biopolymers (Wu, et al., 2004) and in the case of BSFL, further processing into protein.

9.2.2 Liquor related bioproduct Y2

The liquor related product stream contains products like organic acids (Pandey, et al., 2010), industrial enzymes (Viniegra-González, et al., 2003), biopesticides (for example *Bacillus thuringiensis* based biopesticides (Brar, et al., 2006) and *Trichoderma* species (Verma, et al., 2005), both of which has existing research on production from wastewater sludge). These products are most representative of the existing body of work related to SSF. Producing these products from wastewater sludge may have additional benefits, as reported in Brar et al (2006) including improved product recovery due to the sludge flocs acting as adsorbents for spores and crystal protein during centrifugation and as protectants during adverse spray drying conditions.

9.2.3 Cake-related bioproduct Y3

Cake-related product, together with compost, make up the remainder of the solids stream, The cake related product stream makes allowance for bioproducts obtained from the bulk solids that are not compost, for example brick-making or packaging material (Arifin & Yusuf, 2013; Ecovative, 2016; Corpuscoli, 2016). Small volume, high value products are also possible. Chitin and its derivative, chitosan is a promising biomaterial (Stevens & Verhe, 2004; Dhillon, et al., 2013) with utility showed in chemical conditioning in the dewatering of municipal-activated sludge (Zemmouri, et al., 2015). Extraction of chitosan from mushroom mycelia has been investigated, as fungal cell wall contains up to 50% chitin as compared to crustacean shells which contain 14–27% on dry biomass basis (Zamani et al., 2007), and production of chitosan is also possible with the co-production of a liquor-related product (citric acid), as illustrated in the Dhillon et al (2013) study. Chitin is also present in black soldier fly pupae which can feed on faecal sludge, for example (Caligiani, et al., 2018)

9.2.4 Compost bioproduct Y4

At the most basic level, the solids bioreactor contributes to stabilisation of the solid component for use as biosolids. The compost produced does not have a user-set composition but is dependent on the nutrients that remain after the entire WWBR process with an amount lost to CO_2. 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.

9.3 Solids bioreactor factors for mass balances

The model used in this thesis to explore the potential of WWBR is based on stoichiometric mass balances. It does not consider the microbial growth rates or specific product formation rates, which vary widely and would require site and situation specific analysis.

9.3.1 Solids bioreactor biomass yield and composition

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.

9.3.2 Solids bioreactor bioproduct yield and composition

Products from solid substrate bioreactors are commonly reported on a g/kg dry substrate basis. Data on production of bioproducts of high value ($1-10/kg) is limited (Susana forum on SSF (Verster, 2016) on faecal sludge in particular) despite the industry expressing the need for research in this area (Diener, et al., 2014). As with the other bioreactor units, not all products will be harvested in all cases. Decisions on which products to optimise will depend on the material processed.

Crust related bioproduct Y1

In this model BSFL was used, with an assumed elemental composition of 0.5 g C / g BSFL, 0.1 g N / g BSFL and 0.10 g P / g BSFL. Total yields per ton of waste are estimated at 25% yield on food waste and yields on faecal sludge much lower at around 12% (Dipterra, 2016; Lalander, et al., 2015). A default value of 0.12 was used.

Liquor related bioproduct Y2

The liquor related product stream contains products like organic acids. Citric acid co-produced with chitosan was reported to be in the range of 182.8 to 294 g/kg substrate (Dhillon, et al., 2013). 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 0.045 – 0.081 in different reactor configurations. A conservative yield of 0.05 g-citric acid-C/g-substrate-C was used.

Cake-related bioproduct Y3

PGA (C fraction 0.45) yield from solid substrate fermentation is in the range 36-99 mg-product/g-dry-substrate using dairy manure as substrate (Yong, et al., 2011), this translates to 0.037 – 0.1023 g-product-C/g-substrate-C. Chitosan extraction co-produced with citric acid was reported yielding 0.006 g chitosan / g fungal biomass. (Dhillon, et al., 2013). As this value is too low to play a significant role in the model, this aspect was omitted in the simulation.

Compost bioproduct Y4

Typical composition of compost nutrient values is in the range of 0.5 – 2% nitrogen, 0.3 – 1% phosphorus (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). In this model the composition is determined by the nutrients that remain,

Literature yields of potential products are summarised in Table 9-2.

Table 9-2: Literature yields of potential products from the solids bioreactor

Product	Category	Yield	**Reference**
Microbial Biomass	Biomass	28 - 52 g/kg-dry-substrate, translating to 0.019 – 0.034 g-C-biomass/g-C-substrate.	(Kalogeris, et al., 2003)
Black soldier fly larvae (BSFL)	Crust-related	0.038 - 0.22 g BSFL / g solid waste (Note: a whole product yield, not a C-based yield)	(Banks, 2014; Lalander, et al., 2015)
Citric acid	Liquor-related	0.045 – 0.081 g-product-C/g-substrate	(Prado, et al., 2005; Dhillon, et al., 2013)
Poly-glutamic acid (PGA)	Cake-related?	36-99 mg-product/g-dry-substrate, translating to 0.037 – 0.1023 g-product-C/g-substrate-C.	(Patni & Jui, 1987)
Chitosan	Cake-related	6.40% and 5.13% of dried fungal mycelium	(Dhillon, et al., 2013)
Compost after BSFL production	Compost	0.31 – 0.61 g product / g substrate (Note: a whole product yield, not a C-based yield)	(Banks, 2014)
Respiration	CO2	0.23 - 0.56 g CO2 / g substrate (Note: yield based on reduction of substrate, not a C-based yield)	(Banks, 2014)

9.3.3 Solids bioreactor respiration yield

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 biomass yield on substrate used in the model (0.028) gives a CO_2 evolution value in the range of 0.015 – 0.020 g-CO_2-C/g-substrate-C. In addition to this, the crust-related products like BSFL will also evolve CO_2 as part of their respiration. Work by Perednia et al (2017) place this value at 28%. The value used in the model is 0.30 g-CO_2-C/g-substrate-C.

9.3.4 Summary of yield factors used for Solids Bioreactor

A summary of yield values used as initial estimates is shown in Table 9-3. To convert the g BSFL-product / g substrate values to g product-C / g substrate-C, it was assumed that the carbon composition in both the products and substrate are similar, allowing the values to be used without recalculation. This simplified estimate is in keeping with early stage feasibility but need closer scrutiny especially in substrates that are highly nutrient imbalanced.

Table 9-3: Summary of Carbon-based yield values used for the Solids Bioreactor.

Conversion description	Product chosen	Symbol of factor	**Units**	**Selected factor value for start-point**
Mass of carbon reporting to microbial biomass as a fraction of that present in influent stream to reactor (U)	Microbial biomass	$Y_{C,XSolids/IN} = Y_{C,Y4/IN}$	kg biomass-C / kg influent-C	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)	Black Soldier Fly Larvae (BSFL)	$Y_{C,Y1/IN}$	kg product Y1-C /kg influent-C	0.12
Mass of carbon reporting to Product Y2 (Liquor-Related Product Stream) as a fraction of that present in influent stream to reactor (U)	Citric acid	$Y_{C,Y2/IN}$	kg product Y2-C / kg influent-C	0.05
Mass of carbon reporting to Product Y3 (Cake-Related Product Stream) as a fraction of that present in influent stream to reactor (U)	Chitosan	$Y_{C,Y3/IN}$	kg product Y3-C / kg influent-C	0
Mass of carbon lost as CO2 as a fraction of that present in influent stream to reactor (U)	-	$Y_{C,CO2,Solids/IN}$	kg CO2-C / kg influent-C	0.30
Mass of carbon remaining in final stream, product Y4, (compost) unconverted as a fraction of that present in influent stream to reactor (U)	Compost	$Y_{C,INSolids,unconverted/IN} = 1 - (Y_{C,XSolids/IN} + Y_{C,Y1/IN} + Y_{C,Y2/IN} + Y_{C,Y3/IN} + Y_{C,CO2Solids/IN})$	kg unconverted-C / kg influent-C	remainder

9.4 Solids bioreactor unit train mass balances

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 9-1, with a list of units and overall mass balance equations (

Table 9-4**Error! Reference source not found.**) and a list of stream descriptions (Table 9-5) following. The solids bioreactor yield symbols are presented in Table 9-6, with the symbols for separator and splitter factors given in Table 9-7. The detailed mass balance equations are discussed thereafter.

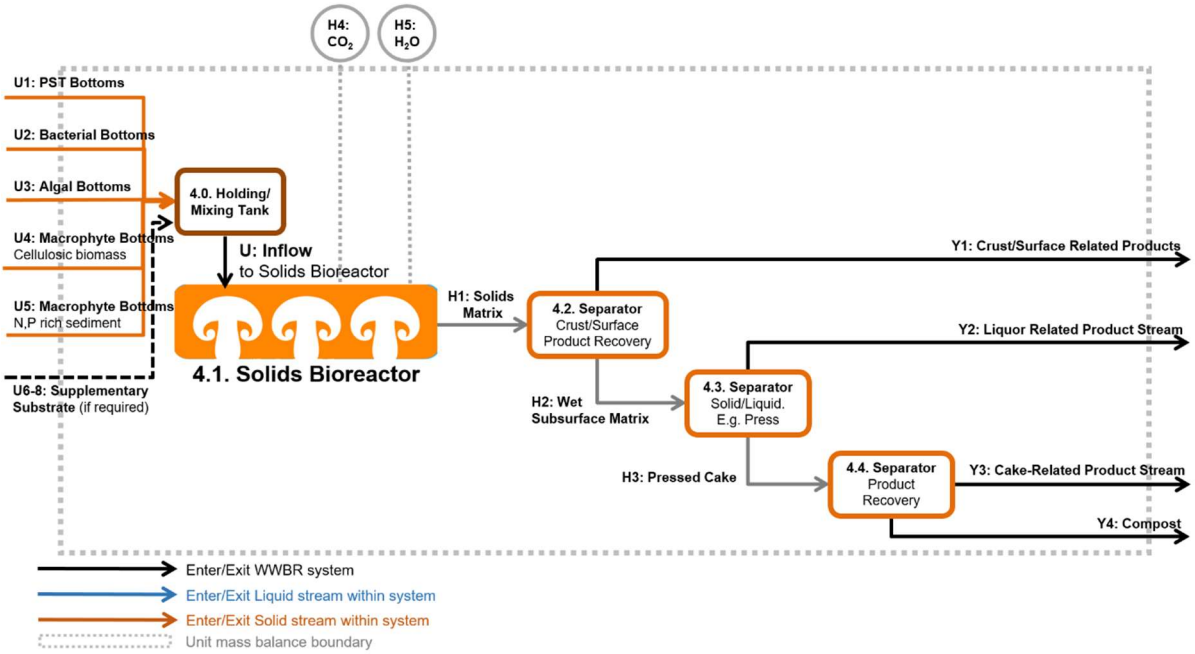

Figure 9-1: Solids bioreactor train detailed flowsheet

Table 9-4: 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, various solids streams 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, or leach and 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 9-5: 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 9-6: 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 9-7: 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}

9.4.1 Mass balances of solids bioreactor

The bottoms stream from the primary separation (0.1) of the combined influent wastewater streams (A1-4) 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, Table 9-8) in which the primary separation bottoms (U1), bacterial biomass (U2), algal biomass (U3), macrophyte biomass (U4) and macrophyte bioreactor sediment (U5) 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 (4.0, Table 9-9) 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.

Table 9-8: Mass balance for Unit 4.0 Mixing Tank: solids bioreactor inflow

Carbon, Nitrogen, Phosphorus 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 Phosphorus	$N_{P(U1)} = IN_{P(U1)liq} + IN_{P(U1)sol}$	$N_{P(U2)} = N_{P(C3)} * (1 - r_{C4})$	$N_{P(U3)} = N_{P(E4)} * (1 – r_{W3})$
Total Water	$N_{W(U1)} = N_{TOTAL(A1-4)sol} * ((1-SC_{U1})/SC_{U1})$	$N_{W(U2)} = N_{W(C3)} * (1 - r_{C4})$	$N_{W(U3)} = N_{W(E4)} * (1 – r_{W3})$
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 Phosphorus	$N_{P(U4)} = N_{P(G5)} * (1 – r_{X2})$ $N_{P(U5)} = N_{P(G4)} * (1 – r_{X3})$	$N_{P(U6-8)} = Q_{(U6)} * C_{N(U6)} + Q_{(U7)} * C_{N(U7)} + Q_{(U8)} * C_{N(U8)}$	$N_{P(U)} = N_{P(U1)} + N_{P(U2)} + N_{P(U3)} + N_{P(U4-5)} + N_{P(U6-8)}$
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 9-9: 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	$S_{C(U)} = N_{C(U)} = N_{C(U1)} + N_{C(U2)} + N_{C(U3)} + N_{C(U4)} + N_{C(U5)} + N_{C(U6-8)}$	$S_{C(H1)} = N_{C(U)} * (1- (Y_{XSolids/C} + Y_{P,Y1/C} + Y_{P,Y2/C} + Y_{P,Y3/C} + Y_{CO2Solids/C}))$		
Totals	$N_{C(U)} = S_{C(U)}$	$N_{C(H1)} = X_{C(H1)} + P_{Y1,C(H1)} + P_{Y2,C(H1)} + P_{Y3,C(H1)} + S_{C(H1)}$	$N_{C(H4)} = CO_{2Solids(H4)}$	$N_{C(H5)} = 0$
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	$S_{N(U)} = N_{N(U)} = N_{N(U1)} + N_{N(U2)} + N_{N(U3)} + N_{N(U4)} + N_{N(U5)} + N_{N(U6-8)}$	$S_{N(H1)} = S_{N(U)} - X_{N(H1)} - P_{Y1,N(H1)} - P_{Y2,N(H1)} - P_{Y3,N(H1)}$		
Totals	$N_{N(U)} = S_{N(U)}$	$N_{N(H1)} = X_{N(H1)} + P_{Y1,N(H1)} + P_{Y2,N(H1)} + P_{Y3,N(H1)} + S_{N(H1)}$		
Checks: Total stream amounts: $N_{N(U)} - N_{N(H1)} = 0$				
Phosphorus Mass Balance: Unit 4.1: Solids Bioreactor				
Phosphorus 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 Phosphorus	$S_{P(U)} = N_{P(U)} = N_{P(U1)} + N_{P(U2)} + N_{P(U3)} + N_{P(U4)} + N_{P(U5)} + N_{P(U6-8)}$	$S_{P(H1)} = S_{P(U)} - X_{P(H1)} - P_{Y1,P(H1)} - P_{Y2,P(H1)} - P_{Y3,P(H1)}$		
Totals	$N_{P(U)} = S_{P(U)}$	$N_{P(H1)} = X_{P(H1)} + P_{Y1,P(H1)} + P_{Y2,P(H1)} + P_{Y3,P(H1)} + S_{P(H1)}$		
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	$N_{W(U)} = N_{W(U1)} + N_{W(U2)} + N_{W(U3)} + N_{W(U4)} + N_{W(U5)} + N_{W(U6-8)}$	$N_{W(H1)} = N_{W(U)} + N_{W(H5)}$		$N_{W(H5)} = N_{W(U)} * (F_{precip} - F_{evap})$
$(N_{W(U)} + N_{W(H5)}) - (N_{W(H1)}) = 0$				

9.4.2 Mass balances for separations of solids bioreactor

The solids bioreactor (4.1, Table 9-9) produces a solids matrix (H1) which is most likely harvested periodically. This matrix goes to the first separator (4.2, Table 9-10,) in the solids train which recovers the crust/surface related product (Y1) and sends the subsurface matrix (H2) to the second separator (4.3, Table 9-11). Here a liquor related product stream (Y2) is retrieved, with the pressed cake (H3) going to the final separator (4.4, Table 9-12) yielding cake related product (Y3) and compost (Y4). All these product streams exit the WWBR.

Table 9-10: 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	$S_{C(H1)} = N_{C(U)} * (1- (Y_{XSolids/C} + Y_{P,Y1/C} + Y_{P,Y2/C} + Y_{P,Y3/C} + Y_{CO2Solids/C}))$	$S_{C(H2)} = S_{C(H1)} * (N_{W(H2)}/N_{W(H1)})$	$S_{C(Y1)} = S_{C(H1)} * (N_{W(Y1)}/N_{W(H1)})$
Totals	$N_{C(H1)} = X_{C(H1)} + P_{Y1,C(H1)} + P_{Y2,C(H1)} + P_{Y3,C(H1)} + S_{C(H1)}$	$N_{C(H2)} = X_{C(H2)} + P_{Y1,C(H2)} + P_{Y2,C(H2)} + P_{Y3,C(H2)} + S_{C(H2)}$	$N_{C(Y1)} = X_{C(Y1)} + P_{Y1,C(Y1)} + P_{Y2,C(Y1)} + P_{Y3,C(Y1)} + S_{C(Y1)}$
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	$S_{N(H1)} = S_{N(U)} - X_{N(H1)} – P_{Y1,N(H1)} – P_{Y2,N(H1)} – P_{Y3,N(H1)}$	$S_{N(H2)} = S_{N(H1)} * (N_{W(H2)}/N_{W(H1)})$	$S_{C(Y1)} = S_{C(H1)} * (N_{W(Y1)}/N_{W(H1)})$
Totals	$N_{N(H1)} = X_{N(H1)} + P_{Y1,N(H1)} + P_{Y2,N(H1)} + P_{Y3,N(H1)} + S_{N(H1)}$	$N_{N(H2)} = X_{N(H2)} + P_{Y1,N(H2)} + P_{Y2,N(H2)} + P_{Y3,N(H2)} + S_{N(H2)}$	$N_{N(Y1)} = X_{N(Y1)} + P_{Y1,N(Y1)} + P_{Y2,N(Y1)} + P_{Y3,N(Y1)} + S_{N(Y1)}$
Checks: Total stream amounts: $(N_{N(H1)}) – (N_{N(H2)} + N_{N(Y1)}) = 0$			

Phosphorus Mass Balance: Unit 4.2: Separator			
Phosphorus 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 Phosphorus	$S_{P(H1)} = S_{P(U)} - X_{P(H1)} – P_{Y1,P(H1)} – P_{Y2,P(H1)} – P_{Y3,P(H1)}$	$S_{P(H2)} = S_{P(H1)} * (N_{W(H2)}/N_{W(H1)})$	$S_{P(Y1)} = S_{P(H1)} * (N_{W(Y1)}/N_{W(H1)})$
Totals	$N_{P(H1)} = X_{P(H1)} + P_{Y1,P(H1)} + P_{Y2,P(H1)} + P_{Y3,P(H1)} + S_{P(H1)}$	$N_{P(H2)} = X_{P(H2)} + P_{Y1,P(H2)} + P_{Y2,P(H2)} + P_{Y3,P(H2)} + S_{P(H2)}$	$N_{P(Y1)} = X_{P(Y1)} + P_{Y1,P(Y1)} + P_{Y2,P(Y1)} + P_{Y3,P(Y1)} + S_{P(Y1)}$
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	$N_{W(H1)} = N_{W(U)} + N_{W(H5)}$	$N_{W(H2)} = N_{W(H1)} - N_{W(Y1)}$	$N_{W(Y1)} = (N_{C(Y1)}/C_{comp,solids}) * ((1\text{-}SC_{Y1})/SC_{Y1})$
Checks: Total stream amounts: $(N_{W(H1)}) – (N_{W(H2)} + N_{W(Y1)}) = 0$			

Table 9-11: 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	$S_{C(H2)} = S_{C(H1)} * (N_{W(H2)}/N_{W(H1)})$	$S_{C(H3)} = S_{C(H2)} * (N_{W(H3)}/N_{W(H2)})$	$S_{C(Y2)} = S_{C(H2)} * (N_{W(Y2)}/N_{W(H2)})$
Totals	$N_{C(H2)} = X_{C(H2)} + P_{Y1,C(H2)} + P_{Y2,C(H2)} + P_{Y3,C(H2)} + S_{C(H2)}$	$N_{C(H3)} = X_{C(H3)} + P_{Y1,C(H3)} + P_{Y2,C(H3)} + P_{Y3,C(H3)} + S_{C(H3)}$	$N_{C(Y2)} = X_{C(Y2)} + P_{Y1,C(Y2)} + P_{Y2,C(Y2)} + P_{Y3,C(Y2)} + S_{C(Y2)}$
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	$S_{N(H2)} = S_{N(H1)} * (N_{W(H2)}/N_{W(H1)})$	$S_{N(H3)} = S_{N(H2)} * (N_{W(H3)}/N_{W(H2)})$	$S_{N(Y2)} = S_{N(H2)} * (N_{W(Y2)}/N_{W(H2)})$
Totals	$N_{N(H2)} = X_{N(H2)} + P_{Y1,N(H2)} + P_{Y2,N(H2)} + P_{Y3,N(H2)} + S_{N(H2)}$	$N_{N(H3)} = X_{N(H3)} + P_{Y1,N(H3)} + P_{Y2,N(H3)} + P_{Y3,N(H3)} + S_{N(H3)}$	$N_{N(Y2)} = X_{N(Y2)} + P_{Y1,N(Y2)} + P_{Y2,N(Y2)} + P_{Y3,N(Y2)} + S_{N(Y2)}$
Checks: Total stream amounts: $(N_{N(H2)}) – (N_{N(H3)} + N_{N(Y2)}) = 0$			
Phosphorus Mass Balance: Unit 4.3: Separator			
Phosphorus 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 Phosphorus	$S_{P(H2)} = S_{P(H1)} * (N_{W(H2)}/N_{W(H1)})$	$S_{P(H3)} = S_{P(H2)} * (N_{W(H3)}/N_{W(H2)})$	$S_{P(Y2)} = S_{P(H2)} * (N_{W(Y2)}/N_{W(H2)})$
Totals	$N_{P(H2)} = X_{P(H2)} + P_{Y1,P(H2)} + P_{Y2,P(H2)} + P_{Y3,P(H2)} + S_{P(H2)}$	$N_{P(H3)} = X_{P(H3)} + P_{Y1,P(H3)} + P_{Y2,P(H3)} + P_{Y3,P(H3)} + S_{P(H3)}$	$N_{PY2)} = X_{P(Y2)} + P_{Y1,P(Y2)} + P_{Y2,P(Y2)} + P_{Y3,P(Y2)} + S_{P(Y2)}$
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 9-12: 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	$S_{C(H3)} = S_{C(H2)} * (N_{W(H3)}/N_{W(H2)})$	$S_{C(Y3)} = S_{C(H3)} * (N_{W(Y3)}/N_{W(H3)})$	$S_{C(Y4)} = S_{C(H3)} * (N_{W(Y4)}/N_{W(H3)})$
Product P_{Y4}			$P_{Y4,C(Y4)} = X_{C(Y4)} + P_{Y1,C(Y4)} + P_{Y2,C(Y4)} + P_{Y3,C(Y4)} + S_{C(Y4)}$
Totals	$N_{C(H3)} = X_{C(H3)} + P_{Y1,C(H3)} + P_{Y2,C(H3)} + P_{Y3,C(H3)} + S_{C(H3)}$	$N_{C(Y3)} = X_{C(Y3)} + P_{Y1,C(Y3)} + P_{Y2,C(Y3)} + P_{Y3,C(Y3)} + S_{C(Y3)}$	$N_{C(Y4)} = P_{Y4,C(Y4)}$
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	$S_{N(H3)} = S_{N(H2)} * (N_{W(H3)}/N_{W(H2)})$	$S_{N(Y3)} = S_{N(H3)} * (N_{W(Y3)}/N_{W(H3)})$	$S_{N(Y4)} = S_{N(H3)} * (N_{W(Y4)}/N_{W(H3)})$
Product P_{Y4}			$P_{Y4,N(Y4)} = X_{N(Y4)} + P_{Y1,N(Y4)} + P_{Y2,N(Y4)} + P_{Y3,N(Y4)} + S_{N(Y4)}$
Totals	$N_{N(H3)} = X_{N(H3)} + P_{Y1,N(H3)} + P_{Y2,N(H3)} + P_{Y3,N(H3)} + S_{N(H3)}$	$N_{N(Y3)} = X_{N(Y3)} + P_{Y1,N(Y3)} + P_{Y2,N(Y3)} + P_{Y3,N(Y3)} + S_{N(Y3)}$	$N_{C(Y4)} = P_{Y4,N(Y4)}$
Checks: Total stream amounts: $(N_{N(H3)}) - (N_{N(Y3)} + N_{N(Y4)}) = 0$			
Phosphorus Mass Balance: Unit 4.4: Separator			
Phosphorus 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 Phosphorus	$S_{P(H3)} = S_{P(H2)} * (N_{W(H3)}/N_{W(H2)})$	$S_{P(Y3)} = S_{P(H3)} * (N_{W(Y3)}/N_{W(H3)})$	$S_{P(Y4)} = S_{P(H3)} * (N_{W(Y4)}/N_{W(H3)})$
Product P_{Y4}			$P_{Y4,P(Y4)} = X_{P(Y4)} + P_{Y1,P(Y4)} + P_{Y2,P(Y4)} + P_{Y3,P(Y4)} + S_{P(Y4)}$
Totals	$N_{P(H3)} = X_{P(H3)} + P_{Y1,P(H3)} + P_{Y2,P(H3)} + P_{Y3,P(H3)} + S_{P(H3)}$	$N_{P(Y3)} = X_{P(Y3)} + P_{Y1,P(Y3)} + P_{Y2,P(Y3)} + P_{Y3,P(Y3)} + S_{P(Y3)}$	$N_{P(Y4)} = P_{Y4,P(Y4)}$
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: $(N_{W(H3)}) – (N_{W(Y3)} + N_{W(Y4)}) = 0$			

9.5 Closing remarks on the solids bioreactor

This Chapter contributes a more in-depth understanding of solids bioprocessing through literature overview, contextualised through mass balancing. More research is needed on bioproduct productivities from solid raw materials, current values cover a large range without a good understanding of what influences productivity. The solid materials need improved characterisation. Like the preceding reactor trains, not all products will be harvested in all cases i.e. decisions on which products to maximise will depend on the material processed.

Solid substrate bioreactors need significantly more research and can benefit from the work done for biopesticide production (Brar, et al., 2006). The current interest in black soldier fly production from organic wastes are also contributing greatly to this area. Solids bioprocesses have challenges in scale-up, but they are also poorly researched in terms of adequate process control and monitoring (Chen, 2013). More applied research is required to understand and employ the potential of low water activity biological environments.

10 COMBINING THE REACTOR TRAINS TO FORM A WASTEWATER BIOREFINERY

The reactor units were discussed in the preceding chapters. This chapter discusses how they relate to each other, how supplementary raw material may be introduced, and how the linking units – separators and splitters - integrate into the model. The chapter concludes with considerations relating to the water mass balance

10.1 Early Stage Considerations for Integration into the Wastewater Biorefinery

In conceptualising and developing the WWBR, both understanding of individual unit operations and awareness of the interrelationship between unit operations is needed. The principles of industrial ecology dictate that the components of this 'ecosystem' are optimised to function as an integrated system both spatially and from a process engineering perspective, rather than maximised with respect to individual unit productivities (Graedel & Allenby, 2010). These principles are followed with, and within, the WWBR. 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 facilitate the unit producing the (main) commercially relevant product, as well as producing final compliant water as a product of worth.

10.1.1 Supplementary raw materials

Supplementary raw materials are important from two perspectives: availability of supply and optimisation of productivity. The successful integration of processes into a WWBR is largely dependent on the availability of the appropriate amounts of biomass feedstock as well as their nutrient composition. Special attention needs to be given to potential seasonality of wastes such as agricultural and food processing by-products and how they interface with continually produced waste streams. Feedstocks may need to be stored and managed to ensure efficient use of the equipment and production of 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 a well-established practice to add reagents to obtain better treatment performance, which can be re-interpreted to facilitate increased productivity in the WWBR. As example, 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). Methanol contaminants may typically include methoxide and high pH which can limit 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 carbon source and electron donor, the wastewater biorefinery may need more sophisticated additives (Ferry & Giljova,

2015; Olguín, 2012). One example is nutrient streams to enable a more appropriate C:N:P ratio, as would be required for intensive bioproduct formation in bacterial reactors or algal production. A second is 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 these should be considered to enhance productivity. In particular, the sourcing of additional complex waste streams rich in these supplements may be appropriate, in keeping with the industrial ecosystem approach. At the same time, while it is tempting to design an eco-industrial park to tailor the effective use of waste streams, designing co-placement of industries to provide complementary waste streams (greenfield development) have proven to be less successful to date, than shaping processes (and products) in response to the existing streams and potential synergies (brownfield development) (Desrochers & Sautet, 2008).

10.1.2 Optimising for the main economic units

Overall process optimisation is a key factor with focus on both the economic product and the water product, as the WWBR has the dual objective of water treatment and bioproduction. The range of unit operations, type of mixed culture (aerobic or anaerobic, for example) catalysts, conversion efficiency, yield and productivity, amongst others, significantly affect the overall sustainability and economic aspects of a WWBR. 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 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 2.2.3. (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 (Ferry & Giljova, 2015) combine waste streams (most frequently municipal solid waste and sewage) in order to optimise the feedstock and C:N:P ratio 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. Energy produced (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.

Figure 10-1 is a very early stage attempt at facilitating the decision making 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 to optimise exiting water quality, only a few units are likely to be optimised for bioproduct productivity, depending on the composition of the wastewater processed. This heuristic process is intended to be a guideline only, to be further developed as more information becomes available, as well as 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.

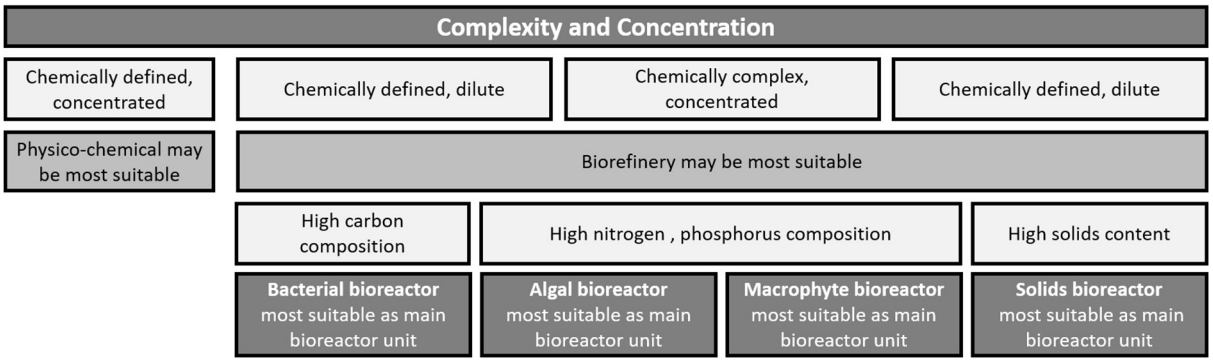

Figure 10-1: Decision making matrix to guide selection of priority bioreactor

10.2 Downstream processing in the wastewater biorefinery

Separation and purification processes, grouped as downstream processing (DSP) 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). DSP and fractional separations are well developed for the biotechnology and chemical engineering industries. Conventionally reactor design is focused on maximising productivity, and seldom integrated with reduction in downstream processing costs. In wastewater treatment DSP is also well developed but focused on constituent removal from the water product. For the WWBR, these processes need to be further adapted along with integration of the appropriate reactor designs as outlined in the preceding chapters. The end goal is to focus on product recovery, bearing in mind the large, dilute system.

The quality of DSP governs the marketability of a product by affecting potency and aiding in further processing during formulation development. Formulation is a crucial link between production and application and dictates economy, longer shelf life, ease of application and enhanced field efficacy (Brar et al 2006). Approaches used in wastewater treatment as well as in mining of specifically low-grade ores give some indication of the requirements for the product recovery aspect of downstream processing for dilute streams. The first consideration is to increase the product concentration and reduce the total volume by orders of magnitude i.e. to recover the product while, at the same time, ensuring limited losses or further contamination of the water stream to be further processed. The majority of primary industrial wastewater-treatment solids-separation process units operate with clarifiers and flotation devices (Theobald, 2015). With the progress in filtration (specifically reverse osmosis) and possibly adsorption technologies, these are also expected to become more suitable product concentration processes. Once the product is recovered in a more concentrated form, product purification can utilise existing DSP options, depending on the specific product and stream contaminants.

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

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.

10.3 Separator efficiency factors for the WWBR bioreactor unit trains

Typical generic separation factors are listed next. Specific considerations and factors relevant to the different reactor unit trains are discussed in the following subsections.

For clarification of the water component, 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 is used in the model.

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 10-1.A more comprehensive list of solids concentrations relevant to wastewater treatment can be found in Tchobanoglous, et al. (2003).

Table 10-1: 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

Separators and downstream processing units are generally well developed and well understood. Obtaining 100% separation between e.g. solids and liquids is theoretically 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 10-2 (Harding, 2009). Where no specific information was available, a product recovery efficiency fraction value of 0.9 was used in the model (Chapter 11).

Table 10-2: 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

10.3.1 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 10-3.

Table 10-3: 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

10.3.2 Algal bioreactor train separator efficiencies

The model does not specify specific downstream processing options, but this section does suggest likely recovery methods, in keeping with the design for downstream processing approach, discussed in Chapter 5. 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).

The 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, like storage lipids, or antioxidant production, 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 10-4.

Table 10-4: 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 content in 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

10.3.3 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. These values are summarised in Table 10-5.

Table 10-5: 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

10.3.4 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 is 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. These values are summarised in Table 10-6.

Table 10-6: 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	eff_{H2}	0.8 - 1.0	0.8
	Crust to Top	eff_{Y1}	0.8 - 1.0	0.9
	Slurry solids contents	SC_{H2}	design specific	0.5
4.3	Solids to Bottoms H3	eff_{H3}	0.8 - 1.0	0.9
	Pressed cake solids contents	SC_{H3}	0.12 – 0.32	0.3
4.4	Product Y3 to product stream	eff_{Y3}	unknown	0.6
	Product Y4 to product stream	eff_{Y4}	unknown	0.9
	Solids contents: Product Y4	SC_{Y4}	0.4 – 0.6	0.5

10.4 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 11.3.

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 10-7.

Table 10-7: Splitter ratios for 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

10.5 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 (Muller, 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 a closed tunnel to aid in increasing temperature in composting, but likely will be open for at least some of the time (or total area) to remove excess moisture. It needs to be deep enough to generate enough heat, but at greater depths mass transfer becomes challenging. A default value of 1 m depth 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.

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 South Africa 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, as illustrated in Table 10-8 and Table 10-9. Note that the evaporation and precipitation data need to be converted to a daily value, to fit with the basis of the model. The values are only applied to the reactor units, and not to other process units, which represents an underestimation.

Table 10-8: 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 10-9: 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

10.6 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 (Chapter 5.3), 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 (Chapter 6.4, 7.4, 8.4 and 9.4), 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 presented in Chapter 11. 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.

11 SIMULATION FOR PRELIMINARY EXPLORATION OF POTENTIAL WASTEWATER BIOREFINERY DESIGN

The simulation presented in this chapter 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 for resource recovery using the limited information available at the early stage.

The flowsheets and mass balances for this approach are presented in Chapter 5 and Appendix B, listing the required factors. In Chapters 6 to 9, a range of default values is determined from literature for each factor. These values are informed estimates and can be changed by the user depending on the scenario being investigated and its best representation.

In Section 11.1 the model is demonstrated across a single bioreactor producing PHA from confectionary wastewater taken with values from an experimental study reported (Tamis, et al., 2014). This is to determine how well the model replicates existing experimental findings. Section 11.2 reports a hypothetical integrated WWBR mass balance using municipal wastewater as feedstock.

The utility of the model lies in its linking of four different biocatalyst groups – the heterotrophic microbial, the photo-mixotrophic. the macrophytic and the solids bioreactor, while allowing consideration of different scenarios to explore the consequences of changing the various factors and configurations used. The model is then applied to poultry abattoir wastewater and papermill wastewater in Section 11.3. This establishes the value of this tool as an initial consideration of application of the WWBR concept to any local setting.

11.1 Demonstration of Simulation for a Simple Bioreactor Train: PHA production from confectionary wastewater

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 (Tamis, et al., 2014) and paper industry effluents (Bengtsson, et al., 2008) may be considered suitable substrates for waste-based PHA production.

The model for the heterotrophic bioreactor was developed in Chapter 6 and its applicability is demonstrated here for the PHA case, before embedding it in the wastewater biorefinery case study. The confectionary wastewater is a literature example to test if the model can be applied to other scenarios, and to demonstrate the model reaching similar outcomes than the literature example.

11.1.1 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. The PHA was poly-β-hydroxybutyrate (PHB) and was produced in an aerobic conversion using a mixed microbial enrichment culture in three sequential fermentation steps. The wastewater from the confectionary factory was pre-treated in a flotation-based fat separation unit before entering the influent tank of the pilot installation. No primary settling of solids was employed.

The concentration of the ammonium ion 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 the ammonium ion 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, including feed conversion to VFAs, enrichment for PHA producers and accumulation of PHA under limitation (refer to Appendix Section B.2). To adapt this to the model used

here, two further assumptions are made. Firstly the model considers the influent stream as already converted to VFA. Secondly, the reported experiment was run as a fed-batch system. To use the model an assumption of continuous operation is needed, with a reference value of 1 000 m^3/day incoming substrate. PHA is made under limiting conditions when cell growth is constrained, but a continuous reactor system needs a positive cell growth rate to prevent washout. This is often achieved using fed-batch. To adapt to a continuous system a two-stage continuous culture is employed. The cells formed in the first reactor enter the second reactor at the rate at which they leave. The second reactor is responsible for PHA production. These two steps are included in a 'black box' where only the overall yield is used.

The average soluble COD (sCOD) of the wastewater varied strongly over time (intrinsic to factory operation, e.g. semi-periodic cleaning of equipment) with an average soluble COD concentration of 7.8 ± 4.1 g-COD/ℓ (average ± standard deviation over the dataset) and a particulate COD concentration of 0.8 ± 0.5 g-COD/ℓ present as solids not passing a 0.45 µm pore size filter. Full conversion of the fermentable COD to VFA were achieved (Tamis, et al., 2014). For this model this assumes both the soluble and particulate COD was converted completely to give a total of 8.6 g COD/ℓ.

For the stoichiometric model used in this project, the carbon (equivalent to TOC) yield values of the PHB and biomass are required, thus these values need to be converted. While the greatest fraction of the VFA consisted of acetic acid (32%), propionic acid was more representative of the weight distribution of the VFAs and was used to represent VFAs in the elemental mass balance with a calculated ratio of 0.486 g C / g propionic acid. Converting the COD to g propionic acid using the theoretical oxygen demand of 1.51 g COD / g propionic acid gives an influent substrate carbon concentration of 2.77 g C(propionic acid) /ℓ.

The soluble nitrogen concentration in the wastewater was negligible (<1 mg/ℓ) and the nutrients were supplemented with a urea and phosphate stream. These values are summarised in Table 11-1, and incorporated into the model along with the separation and splitter values, as summarised in Table 11-3 and visualised in the flow sheet in Figure 11-1.

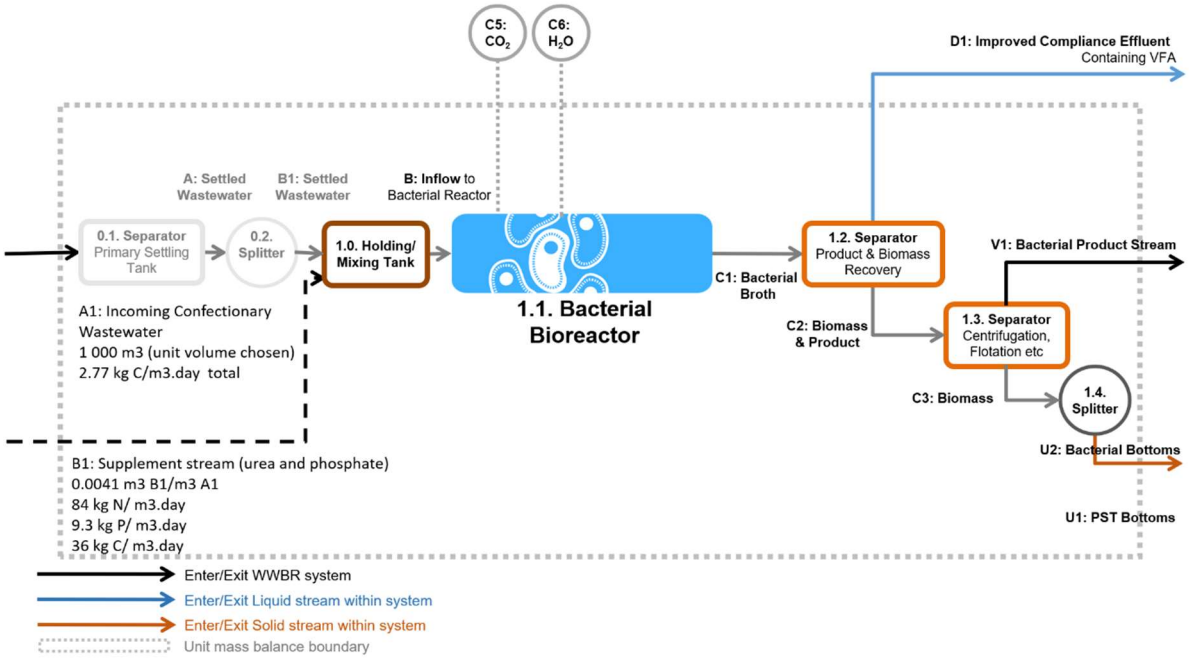

Figure 11-1: Flow diagram for PHA production from confectionary wastewater case study

Table 11-1: Values for incoming streams in PHB production (adapted from Fernández-Dacosta et al. (2015) and Tamis et al. (2014))

Stream	Value	Comments
A1: Mars candy bar factory	1 000 m^3 (unit volume chosen) 2.77 kg-C/m^3.day total	7.8 ± 4.1 g-COD/ℓ soluble + 0.8 ± 0.5 g-COD/ℓ solids = 8.6 g-COD/ℓ Assume all COD is converted to propionic acid, 1.51 g COD/ propionic acid
B1: Supplement nutrient stream (Urea + PO_4)	0.0041 m^3/m^3 B1 84 kg-N/ m^3.day 9.3 kg-P/ m^3.day 36 kg-C/ m^3.day	See Appendix Section B.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_2 MoO_4) was provided to the bioreactor.

The PHB yield was reported as a biomass-accumulation of 0.76 g PHB / g biomass (as VSS) (Table 11-2). It is assumed that the biomass values reported in Table 11-2 **Error! Reference source not found.** is non-PHA-biomass.

Table 11-2 Parameters reported in (Tamis, et al., 2014). Calculations were performed using assumptions 1.7 gCOD / g PHA, 1.4 g COD/gX

Parameter	Value	Unit
$Y_{PHA/S}$	0.37	g PHA/ g substrate COD
$Y_{X/PHA}$	0.49	g biomass/ g PHA COD
$Y_{X/COD0}$	0.28	g biomass / g COD
Final PHA content	0.76	g PHA / g VSS

Back-calculating the yields to incorporate the COD values of PHA and biomass gives 0.62 g PHA-COD / g substrate COD and 0.38 g biomass-COD / g substrate COD. This means all substrate was converted into either PHA or biomass (0.62 + 0.38 = 1), and as the bioprocess was anaerobic no C is lost to CO_2. PHA for the purposes of this model is assumed to be composed of poly-hydroxybutyrate (PHB) only.

Table 11-3: Factors for units in PHA production to use in the model (adapted from Fernández-Dacosta et al. (2015) and Tamis et al. (2014))

Process Unit	Conversion	Units	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	$Y_{C,XBact/IN} = 0.38$	g biomass-COD/g substrate-COD	**Error! Reference source not found.**
1.1. Bacterial reactor: Product V1: PHA	$Y_{C,V1/IN} = 0.62$	g-PHA-COD / g-substrate COD	**Error! Reference source not found.**
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	$Y_{C,CO2Bact/IN} = 0$	g CO2-C / g substrate C	anaerobic bioprocess
1.1. Bacterial reactor: Unconverted			Remainder
1.2. Separator	$eff_{C2} = 0.99$ $SC_{C2} = 0.06$		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.
(Overall PHA recovery)	0.735		Fraction of PHA in stream I / PHA in stream C1, bacterial broth. Figure 11-1

11.1.2 The output values of model demonstration run

Table 11-4 shows the results obtained using the Tamis et al (2014) yield values in the stoichiometric WWBR model. Urea contains 0.2 g-C / g urea which contributed a small amount of carbon to the feed supplement stream B2.

Table 11-4: Inventory of carbon, nitrogen, phosphorus and water for bacterial bioreactor train using 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	2 770	0	0	997 230
Urea supplement stream B2	3M Urea, 0.3M PO_4	147	344.40	38	3 570
Precipitation/ Evaporation		0	0	0	8149
Incoming (total)		**2917**	**344**	**38**	**1 008 949**
CO_2 (out)		0	0	0	0
Bacterial product V1 stream (not 100% pure)	PHA (impure, after recovery – without biomass)	1811	27	3.1	12 148
D1: Improved compliance effluent		29	92	8.5	973 361
U2: Bacterial bottoms	Cell debris after PHA recovery and a small amount of PHA from product recovery losses	1077	225	26.5	23 439

Item	Stream Description	C kg/day	N kg/day	P kg/day	W kg/day
Total outgoing		2917	344	38	1008949
Difference	*(should be 0)*	0	0	0	0
Difference (%)		0	0	0	0

Table 11-5: Percentage distribution of carbon, nitrogen, phosphorus and water for bacterial bioreactor train using confectionary factory wastewater

Item		% C of total	% N of total	% P of total	% Water of total
Raw, unsettled wastewater A1 to mixing tank	Mars confectionary factory wastewater	95.0	0	0	98.85
Urea supplement stream B2	3M Urea, 0.3M PO4	5.04	100	100	0.35
Incoming (total)		100	100	100	100
CO_2 (total)		0.00	0.00	0.00	0.00
Precipitation/ Evaporation		0.00	0.00	0.00	0.81
Bacterial product V1	PHA	62.08	7.85	8.21	1.20
Improved compliance effluent D1		0.99	26.74	22.37	96.47
Bacterial bottoms U2		36.92	65.41	69.45	2.32
Difference	*(should be 0)*	0.00	0.00	-0.03	0.00

11.1.3 Concluding remarks on simulating a single unit system

Section 11.1 successfully demonstrates the use of the model for resource recovery for a single-unit system. The use of elemental compositions is motivated on their direct usefulness in the mass balance; reporting in COD (typical in wastewater studies) requires an additional assumption about the organic nature of the substrate. This becomes more apparent when the algal, macrophyte and solids bioreactors are included, for which literature data in terms of COD is often not given. The model can be expanded to allow for an electron balance in the future.

The model is a theoretical, stoichiometric elemental mass balance that is fully specified. The differences are included to indicate if errors were propagated leading to the mass balance not closing. Further, the yields (for example $Y_{C,V1/IN}$ = 0.62g-PHA-C / g-substrate C) and the final percentage allocation of the elements reporting to the streams (for example 62.08% C reporting to PHA) do not correlate completely because the model incorporates imperfect separations and hence losses and impurities. The evaporation and precipitation assumptions (Section 10.5) are too general to be useful but serve as a reminder to keep the influence of the climate in mind.

From the results of the simulation, it can be seen that the majority of the C from the C-rich wastewater is captured into the bacterial product PHA – 63%. The remaining C will need to be removed in a secondary step e.g. bioenergy through AD or mixotrophic algal processes. The N (and P) are not removed substantially in the bacterial reactor producing an organic polymer. A secondary step is required as acknowledged in the integrated flowsheet if the incoming stream is high in N and P, but if low then this may not be required. The water quality is significantly improved, reducing the load on further treatment or potentially fit for purpose water.

This section considered a single unit, the Bacterial Bioreactor, using the studies by Fernández-Dacosta et al. (2015) and Tamis et al (2014) which were not designed for a multi-unit system. Such single unit bioproduction processes from wastewater limits the resilience of the reactors, and the reactor system in its current configuration cannot absorb shock loads of high nutrient containing waters. It may be a

suitable system for a highly-defined, intensively managed waste stream like confectionary, or more widely a food industry's wastewater, but less suitable for a complex wastewater. In Section 11.2, the model is demonstrated for an integrated WWBR and some examples of more complex wastewaters are evaluated in Section 11.3.

11.2 Demonstration of Simulation for an Integrated System

The single unit simulated in Section 11.1 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 polymer, is now the chosen bacterial product.

This simulation considers the most conservative approach to the WWBR, to acknowledge the use of conventional systems to be a first stepping stone in changing paradigms:

1. No supplementary streams were used, although the model makes provision for this in future.
2. A conservative approach to bioproduct yields were used, to take into account the unoptimized nature of initial WWBR pilots.
3. No major redesign of hypothetical plant layouts were assumed.

11.2.1 Municipal wastewater as feedstock for integrated WWBR simulation

A hypothetical municipal wastewater stream was used, drawing from the design ranges used in Henze et al (2008) and Tchobanoglous et al. (2003), with data relating to the sludge adapted from Strande et al. (2015). These values are listed in Table 11-6, and the values for two treatment works in Cape Town, South Africa are included for comparison. Municipal wastewater as biorefinery feedstock is reviewed in Section 3.3. For the purposes of comparison with the wastewaters introduced in Section 11.3, the incoming flow was standardised to a basis of 1 000 m^3 per day (1 Mℓ/day) and in Section **Error! Reference source not found.** also to 1 000 kg C per day. Owing to the use of a stoichiometric model, the outputs can readily be scaled to the incoming flowrate of interest by introducing a new basis. For the demonstration of the integrated unit process, no supplementary streams were added.

Table 11-6: Summary of incoming domestic municipal wastewater values used to demonstrate an integrated multi-unit process, with two WWTPs in greater Cape Town for comparison

Incoming (Stream A1)	**Total flow** (m^3/day)	**C as COD** (kg/m^3)	**N** (kg/m^3)	**P** (kg/m^3)
Liquid component	1 000	0.750 Athlone: 0.880 Mitchells Plain: 1.465	0.050 0.056 0.092	0.008 0.009 0.019
	Solids (kg/m^3)	**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

11.2.2 Values of factors for units in the integrated WWBR used in simulation

The overview flowsheet of the integrated WWBR is given in Figure 5-3 in Chapter 5.3. Detailed flowsheets for the component process operations are given in Chapters 6 to 9 as

Figure 6-1, Figure 7-1, Figure 8-1 and Figure 9-1**Error! Reference source not found.**. A summary of the factors used in this simulation of the integrated WWBR is listed in the following Tables:

4. Table 11-7: the biomass composition and product compositions,
5. Table 11-8: the yield factors and
6. Table 11-9: the separator efficiencies.

Table 11-7: Summary of biomass and product composition values used to demonstrate an integrated multi-unit process

Biomass Composition (g / g total dry biomass)	**Description**	**C**	**N**	**P**	**Section Reference**
Bacteria	$C_{100}H_{180}O_{50}N_{20}P$	0.48	0.11	0.013	Section 6.3.1
Algal	$C_{106}H_{181}O_{46}N_{16}P$	0.52	0.092	0.013	Section 7.3.1
Macrophyte	$C_{494}H_{824}O_{412}N_7P$	0.44	0.00735	0.0023	Section 8.4.1
Solids	$C_{100}H_{180}O_{50}N_{20}P$	0.48	0.11	0.013	Section 9.3.4
Product Composition (g / g total dry product)		**C**	**N**	**P**	
Bacterial Bioproduct V1	Polyglutamic acid (PGA) $(C_5H_7O_3N)_n$	0.465	0.109	0	Section 6.3.2
Algal Bioproduct W1	Phycocyanin $C_{165}H_{185}O_{30}N_{20}$	0.68	0.096	0	Section 7.3.2
Algal Bioproduct W2	Algal lipids $C_{16}H_{32}O$	0.75	0	0	Section 7.3.2
Algal Bioproduct W3	Algal biomass $C_{106}H_{181}O_{46}N_{16}P$	0.52	0.092	0.013	Section 7.3.1
Macrophyte Bioproduct X1	Long fibre biomass $C_{494}H_{824}O_{412}N_7P$	0.44	0.00735	0.0023	Section 8.4.1
Macrophyte Bioproduct X2	Short fibre biomass $C_{494}H_{824}O_{412}N_7P$	0.44	0.00735	0.0023	Section 8.4.1
Macrophyte Bioproduct X3	Sediment	determined by process	determined by process	determined by process	Section 8.4.3
Solids Bioproduct Y1	Black Soldier Fly Larvae (BSFL)	0.5	0.1	0.01	Section 9.3.4
Solids Bioproduct Y2	Citric acid $C_6H_8O_7$	0.375	0	0	Section 9.3.4
Solids Bioproduct Y3	n/a	0	0	0	Section 9.3.4
Solids Bioproduct Y4	Compost	determined by process	determined by process	determined by process	Section 9.3.4
Compliant Effluent Z	Water	determined by process	determined by process	determined by process	Section 8.5

Table 11-8: Summary of outgoing yield values used to demonstrate an integrated multi-unit process

Conversion value (Y)		**Units**
1.1.Bacterial bioreactor		Section 6.3.4
$Y_{C,XBact/IN}$	0.164	g-biomass-C/g-substrate-C
$Y_{C,V1/IN}$	0.123	g-productV1-C/g-substrate-C
$Y_{C,VFA/IN}$	0.7- $Y_{V1/IN}$ - $Y_{C,XBact/IN}$ - $Y_{C,CO2Bact/IN}$	g-product VFA-C/g-substrate-C
$Y_{C,CO2Bact/IN}$	0.33	g-CO_2-C/g-substrate-C
$Y_{C,INBact,unconverted/IN}$ = 1 – ($Y_{C,XBact/IN}$ + $Y_{C,V1/IN}$ + $Y_{C,CO2Bact/IN}$)	remainder	g-unconverted-C/g-substrate-C
2.1. Algal bioreactor		Section 7.3.3
$Y_{N,XAlgal/IN}$	0.80	g N algal biomass / g N influent stream
$Y_{W1/,XAlgal}$	0.0034	g product W1 / g algal biomass
$f_{C,W1/C,XAlgal}$	1.3	g C (product W1) / g C (algal biomass)
$Y_{W2/,XAlgal}$	0.23	g product W2 / g algal biomass
$f_{C,W2/C,XAlgal}$	1.4	g C (product W2) / g C (algal biomass)
$f_{C,XAlgal/N,XAlgal}$	5.7	g C algal biomass / g N algal biomass
$X_{C,CO2Algal/IN}$	$f_{C,XAlgal/N,XAlgal}$ * $X_{N,IN}$ – $N_{C(D)}$	gC
3.1.Macrophyte Bioreactor		Section 8.4.4
$Y_{P,XMacrophyte/IN}$	0.70	g P macrophyte biomass / g P influent stream
$Y_{X1/,XMacrophyte}$	0.25	g product X1 / g macrophyte biomass
$f_{C,X1/C,XMacrophyte}$	1	g C (product X1) / g C (macrophyte biomass)
$Y_{X2/,XMacrophyte}$	0.75	g product X2 / g macrophyte biomass
$f_{C,X2/C,XMacrophyte}$	1	g C (product X2) / g C (macrophyte biomass)
$Y_{C,CO2Macrophyte/IN}$	192	g C (CO_2) / g P (macrophyte biomass)
4.1 Solids bioreactor		Section 9.3.4
$Y_{C,XSolids/IN}$ = $Y_{C,Y4/IN}$	0.028	kg biomass-C / kg influent-C
$Y_{C,Y1/IN}$	0.12	kg product Y1-C /kg influent-C
$Y_{C,Y2/IN}$	0.05	kg product Y2-C / kg influent-C
$Y_{C,Y3/IN}$	0	kg product Y3-C / kg influent-C
$Y_{C,CO2,Solids/IN}$	0.30	kg CO2-C / kg influent-C
$Y_{C,INSolids,unconverted/IN}$ = 1 – ($Y_{C,XSolids/IN}$ + $Y_{C,Y1/IN}$ + $Y_{C,Y2/IN}$ + $Y_{C,Y3/IN}$ + $Y_{C,CO2Solids/IN}$)	remainder	kg unconverted-C / kg influent-C

Table 11-9: Summary of separator and splitter values used to demonstrate an integrated multi-unit process

Process Unit	Conversion value		Comments
0.1. Separator	SC_{U1} =	0.06	Table 10-3
	eff_{U1} =	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} =	0.008	Table 10-3
	eff_{C2} =	0.5	
1.3 Separator: Centrifugation	SC_{C3} =	0.08	Table 10-3
	eff_{C3} =	0.9	
	eff_{V1} =	0.5	
1.4. Splitter	r_{C4} =	0.1	Table 10-7, Assumption: 10% of biomass is recycled.
2.2. Separator	SC_{E2} =	0.02	Table 10-4
	eff_{E2} =	0.5	
2.3. Separator: Centrifugation	SC_{E4} =	0.08	Table 10-4
	eff_{E3} =	0.5	
	eff_{E4} =	0.9	
2.4. Separator	eff_{W1} =	0.9	Table 10-4
	eff_{W2} =	1	
	SC_{W2}	0.05	
2.5. Splitter	r_{W3} =	0.9	Table 10-7, Assumption: 10% of biomass is directed to solids bioreactor for nutrient supplementation
3.2. Separator	SC_{G2} =	0.6	Table 10-5
	eff_{G2} =	0.99	
3.3. Separator: Centrifugation	SC_{G3} =	0.6	Table 10-5
	eff_{G3} =	0.9	
	eff_{G4} =	0.9	
3.4. Separator	SC_{X1} =	0.8	Table 10-5
	eff_{X1} =	0.8	
3.5. Splitter	r_{X2} =	0.9	Table 10-7, Assumption: 10% of cellulosic biomass directed to solids bioreactor to supplement structural matrix
3.6. Splitter	r_{X3} =	0.9	Table 10-7, Assumption: 10% of biomass is directed to solids bioreactor for nutrient supplementation
4.2. Separator	SC_{Y1} =	0.8	Table 10-6
	eff_{Y1} =	0.9	
	eff_{H2} =	0.5	
4.3. Separator: Centrifugation	SC_{H3} =	0.3	Table 10-6
	eff_{H3} =	0.9	
4.4. Separator	SC_{Y4} =	0.5	Table 10-6
	eff_{Y3} =	0.6	
	eff_{Y4} =	0.9	

11.2.3 Results for the simulation of an integrated WWBR on domestic wastewater

The model output for an integrated flowsheet using four reactor unit trains is summarised in Table 11-10 and visualised in Figure 11-2, noting that N has been scaled by a factor of 5, P by a factor of 100 and water by a factor of 1 000 to allow visualisation on the same axis.

Table 11-10: 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	Water kg/day
Raw, unsettled wastewater A1 to mixing tank	domestic wastewater	824	163.0	8.29	998658
CO_2 uptake		1663	0.0	0.00	64363
Total incoming		2487	163.0	8.29	998659
CO_2 out		311	0.0	0.00	-1362
Lost to DSP (incl evaporation)		33	5.7	0.00	102846
Bacterial product stream V1		25	6.0	0.15	12154
Algal oil W1		297	0.0	0.00	0
Algal bioproduct stream W2		143	0.0	0.11	0
Algal digestible waste W3		1420	27.6	8.06	24735
Cellulosic fibre X1		0	0.4	0.00	0
Cellulosic biomass X2		0	0.1	0.00	0
N,P rich sediment X3		0	3.9	0.00	73765
Crust/surface related product stream Y1		86	24.0	0.66	5373
Liquor related product stream Y2		20	0.9	0.02	19341
Cake-related product stream Y3		0	0.0	0.00	215
Compost Y4		152	50.6	1.64	1934
Compliant effluent Z		0	43.8	0.00	829856
Concentration of effluent Z, mg/L		0.000	0.053	0.000	n/a
Authorisation standard, mg/L		30	2	1	n/a
Total outgoing		2487	163.0	10.63	1004495
Difference (should be 0)		0	0.0	-2.34	-5836
Difference (%)		0.00	0.00	-28.27	-0.58
Item	Stream Description	% C of total	% N of total	% P of total	% Water of total
Raw, unsettled wastewater A1 to mixing tank	domestic wastewater	33.12	100.00	100.00	100.00
CO_2 uptake		66.88			
Incoming (total)		100.00	100.00	100.00	100.00
CO_2 (total)		12.50	0.00	0.00	-0.14
Lost to DSP (incl evaporation)		1.33	3.52	0.00	10.30
Bacterial product V1		1.01	3.67	1.82	1.22
Algal bioproduct W1		11.94	0.00	0.00	0.00
Algal oil W2		5.73	0.00	1.37	0.00
Algal digestible waste W3		57.07	16.91	97.21	2.48
Cellulosic fibre X1		0.00	0.27	0.00	0.00
Cellulosic biomass X2		0.00	0.06	0.00	0.00
N, P rich sediment X3		0.00	2.42	0.00	7.39
Crust/surface related product stream Y1		3.47	14.73	7.91	0.54
Liquor related product stream Y2		0.82	0.55	0.23	1.94
Cake-related product stream Y3		0.00	0.00	0.00	0.02
Compost Y4		6.11	31.03	19.73	0.19
Compliant effluent Z		0.00	26.85	0.00	83.10
Total % (Should be 100)		100.00	100.00	128.27	107.03
Difference (should be 0)		0.00	0.00	-28.274	-7.029

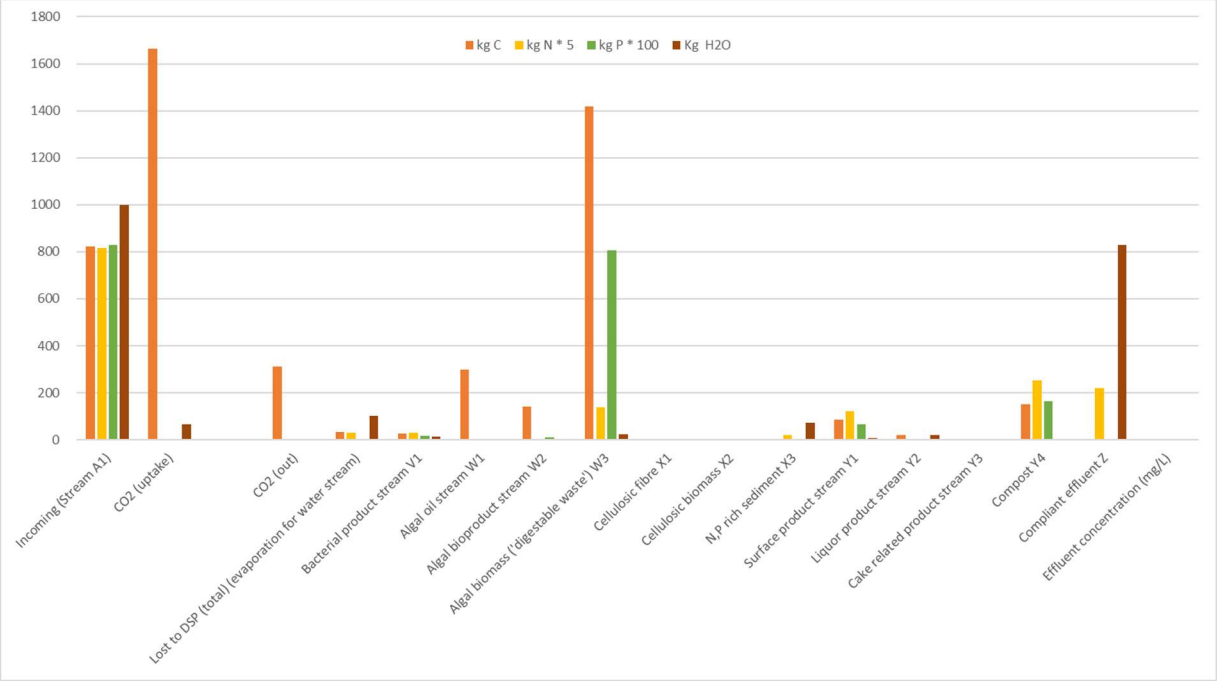

Figure 11-2 : Visualisation of Inventory of Carbon, Nitrogen, Phosphorus and Water for generic WWBR using municipal wastewater as listed in Table 11-10.

Even in this conservative scenario, the potential for the wastewater biorefinery is significant. The value of the bacterial product is low because the biomass has already been removed and directed to the solids bioreactor, where it eventually exists as compost. The bacterial reactor could do much better and is a reflection of the low conversion values of the experimental stage of PGA production from Madonsela (2013) and may be expected with early-stage application of the biorefinery concept from more traditional bioprocessing. Emerging work, however, hints at higher values possible (van der Hoek, et al., 2016).

The algal reactor becomes phosphorus limited, but the model has a shortcoming in that this cannot currently be accounted for, leading to the error in the overall phosphorus balance. The large amount of algal biomass represents the choice to keep algal biomass for a digestible waste rather than diverting it to compost. The products are not pure, the values reported needs to be distinguished from the potential yield of pure product. For an early stage feasibility analysis, the value can be used as a first estimate.

In this simulation it is apparent that the water is already phosphorus deficient by the time it reaches the macrophyte bioreactor, showing that the preceding bioreactors are sufficient for effluent removal. In reality it is likely that some residual nutrients may remain due to the Ks values becoming limiting (it is harder and harder to remove more dilute nutrients). For this reason, a small polishing pond should still be included, but it is clear this is unlikely to contribute economically.

Even though the effluent is compliant, almost a quarter of the nitrogen passes through the system unutilised. This indicates the need for a better understanding of the nutrient balance, as in this case it may be the phosphorus limiting the uptake. Optimising for nitrogen containing polymers like PGA (bacteria) or cyanophycin (algae) may be an option, but overall preventing the nutrients from entering the system through example urine diversion remains the best approach.

11.3 Contextualisation of an Integrated WWBR for Possible Scenarios

11.3.1 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 11.2). Two further examples of wastewaters have been selected, using data from Chapter 3 and are compared in terms of bioproduction potential per 1000 m^3/day (1 ML/day). Poultry abattoir waste (Section 3.3.3) is used as the first example representative of a complex, more concentrated and highly variable wastewater with a high nitrogen content. The pulp and paper wastewater is used as an example of a more chemically defined and less variable process stream, high in carbon and low in N and P. 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 the pulp and paper industry is covered by four major producers in South Africa (Section 3.3.2). The wastewater compositions used are listed in Table 11-11, with the range of reported values indicated in brackets.

The yield, composition and efficiency values used in the demonstration of the model for municipal wastewater (Section 11.2) were used in this section, except where noted.

Table 11-11: Summary of incoming wastewater characterisation used to compare an integrated multi-unit process using different wastewaters

Incoming (Stream A1)	**Domestic municipal**	**Poultry abattoir**	**Pulp and paper**
Soluble component, total flow 1 000 m^3/day			
C (kg/m^3) **as COD**	0.750	(range 0.5 – 6.0) 1.85 (Kiepper: 13.2)	(range 0.7 – 1.2) 0.95
N (kg/m^3)	0.050	(0.026 – 0.050) 0.038 (Kiepper: 0.175)	(0.0087 (ammonia) + 0.00152 (nitrate)) 0.00904
P (kg/m^3)	0.008	(0.005 – 0.010) 0.0075 (Kiepper: 0.057)	0.004
Solids component			
Solids (kg/m^3)	0.72	(0.051 – 1.500) 0.86	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 11.2	(Pocock, 2017)	(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)

11.3.2 Poultry abattoir wastewater as feedstock for integrated WWBR simulation

The poultry sector has seen a threefold increase in output since 1989. The average specific volume index (SVI) of water use per bird has decreased from an average of 17 L/bird to 12.8 L/bird with about 85% discharged as wastewater, and together with investments in water management, the industry has illustrated its commitment to sustainable production (Pocock, 2017).

Poultry abattoir wastewater contributes a small amount of wastewater in the national context but is highly polluting. The data reported in Table 11-11 are in the same order of magnitude as municipal wastewater but may be pre-treated already or reported to be in line with municipal By-Laws for discharge into sewers, because equivalent data from the US is an order of magnitude higher (Kiepper, 2003). 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 waste-water streams. This wastewater normally passes through screens which remove the larger solids either for treatment or final disposal. 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. 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. The Molapo study (2009) reports that 42% of abattoirs interviewed discharge into a wetland or dam to be used for irrigation, indicating that there may be particular interest to include a macrophyte bioreactor in a poultry abattoir based WWBR application, while solids bioreactors may be suitable for the manure (Chen, et al., 2005).

Table 11-12 shows the result of the simulation and visualised in Figure 11-3, noting that N has been scaled by a factor of 40, P by a factor of 200 and water by a factor of 500 to allow visualisation on the same axis.

Table 11-12: 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	Water kg/day
Raw, unsettled wastewater A1 to mixing tank	poultry abattoir wastewater	1672	38.4	8.02	997579
CO_2 uptake		0	0.0	0.00	64174
Total incoming		1672	38.4	8.02	997579
CO_2 out		535	0.0	0.00	-1362
Lost to DSP (incl evaporation)		3	0.4	0.00	8783
Bacterial product stream V1		51	11.8	0.16	13035
Algal oil W1		25	0.0	0.00	0
Algal bioproduct stream W2		12	0.0	0.01	0
Algal digestible waste W3		120	2.3	0.85	2064
Cellulosic fibre X1		458	7.6	2.41	0
Cellulosic biomass X2		103	1.7	0.54	0
N,P rich sediment X3		52	0.2	0.39	83081
Crust/surface related product stream Y1		74	11.7	0.54	5522
Liquor related product stream Y2		18	0.8	0.02	19880
Cake-related product stream Y3		0	0.0	0.00	221
Compost Y4		131	7.5	1.31	1988
Compliant effluent Z		85	-5.5	1.78	934661
Concentration of effluent Z, mg/L		0.091	-0.006	0.002	n/a
Authorisation standard, mg/L		30	2	1	n/a
Total outgoing		1668	38.3	8.02	1003698
Difference (should be 0)		5	0.0	-0.00	-6119
Difference (%)		0.27	0.01	-0.00	-0.61
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
CO_2 uptake		0.00	0.00	0.00	6.43
Incoming (total)		100.00	100.00	100.00	100.00
CO_2 (total)		32.01	0.00	0.00	-0.14
Lost to DSP (incl evaporation)		0.16	0.91	0.00	0.88
Bacterial product V1		3.07	30.76	2.01	1.31
Algal bioproduct W1		1.48	0.00	0.00	0.00
Algal oil W2		0.71	0.00	0.12	0.00
Algal digestible waste W3		7.15	5.92	10.66	0.21
Cellulosic fibre X1		27.41	19.78	30.06	0.00
Cellulosic biomass X2		6.17	4.45	6.76	0.00
N, P rich sediment X3		3.12	0.64	4.90	8.33
Crust/surface related product stream Y1		4.45	30.40	6.74	0.55
Liquor related product stream Y2		1.05	2.00	0.21	1.99
Cake-related product stream Y3		0.00	0.00	0.00	0.02
Compost Y4		7.82	19.52	16.36	0.20
Compliant effluent Z		5.11	-14.40	22.18	93.69
Total % (Should be 100)		99.73	99.99	100.00	107.05
Difference (should be 0)		0.27	0.01	-0.001	-7.046

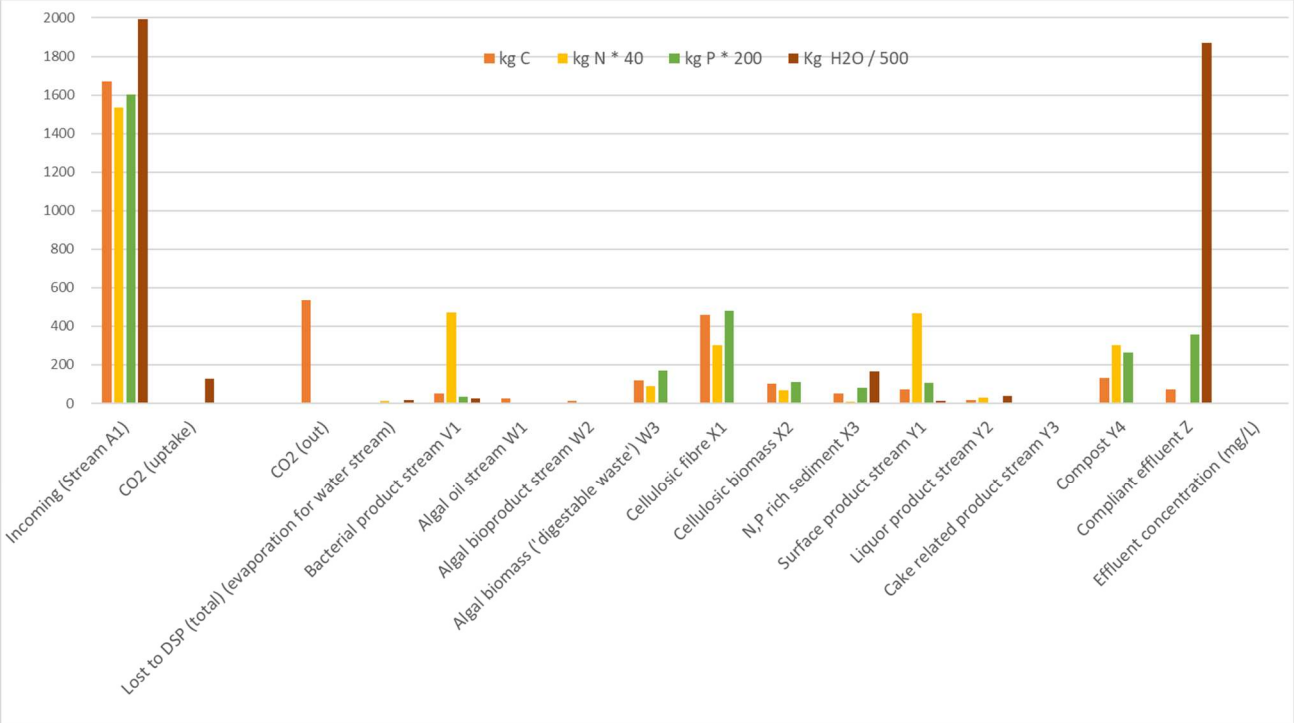

Figure 11-3 Visualisation of Inventory of Carbon, Nitrogen, Phosphorus and Water for generic WWBR using abattoir wastewater as listed in Table 11-12.

Using both the Pocock and Kiepper data, the poultry wastewater data shows nitrogen limitation, likely due to most of the nutrients being captured in solid wastes, indicating that a single unit system like an anaerobic digester which requires higher C:N ratios, followed by irrigation and/or macrophyte bioreactor is the most suitable WWBR configuration, with cellulosic biomass absorbing a third of the C. Table 11-12 shows the simulation using the Pocock (2017) values, with $Y_{C,XBact/IN}$ halved to 0.08 g biomass-C/ g-substrate-C to allow the model to complete. In the current approach of end-of-pipe treatment, the small productivity of the bacterial unit will not be feasible in terms of effort or money. Treatments should also consider pathogens reduction.

11.3.3 Paper wastewater as feedstock for integrated WWBR simulation

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, sizing 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 withhold 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 tons 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.

A quarter of the incoming carbon remains in the compliant 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 11-4 shows the result of the simulation and visualised in Figure 11-4, noting that N has been scaled by a factor of 10, P by a factor of 200 and water by a factor of 500 to allow visualisation on the same axis.

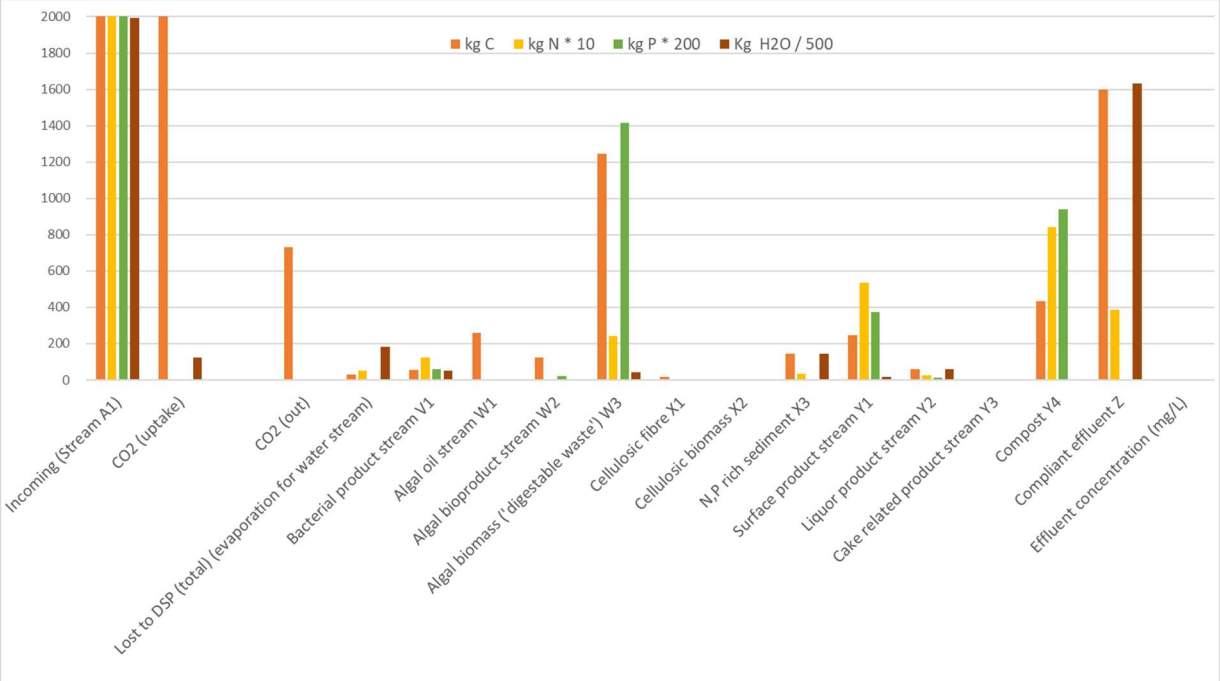

Figure 11-4 Visualisation of Inventory of Carbon, Nitrogen, Phosphorus and Water for generic WWBR using papermill wastewater as listed in Table 11-13.

Table 11-13: 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	Water kg/day
Raw, unsettled wastewater A1 to mixing tank	paper wastewater	2144	224.4	10.74	996720
CO_2 uptake		2805	0.0	0.00	62480
Total incoming		4949	224.4	10.74	996720
CO_2 out		731	0.0	0.00	-1362
Lost to DSP (incl evaporation)		29	4.9	0.00	90447
Bacterial product stream V1		54	12.5	0.30	24562
Algal oil W1		261	0.0	0.00	0
Algal bioproduct stream W2		125	0.0	0.10	0
Algal digestible waste W3		1247	24.2	7.08	21720
Cellulosic fibre X1		16	0.4	0.00	0
Cellulosic biomass X2		4	0.1	0.00	0
N,P rich sediment X3		144	3.5	0.00	72573
Crust/surface related product stream Y1		247	53.5	1.88	8137
Liquor related product stream Y2		58	2.5	0.06	29295
Cake-related product stream Y3		0	0.0	0.00	325
Compost Y4		434	84.2	4.70	2929
Compliant effluent Z		1600	38.6	0.00	816447
Concentration of effluent Z, mg/L		1.960	0.047	0.000	n/a
Authorisation standard, mg/L		30	2	1	n/a
Total outgoing		4949	224.3	14.12	1002594
Difference (should be 0)		0	0.0	-3.38	-5874
Difference (%)		0.00	0.00	-31.44	-0.59
Item	**Stream Description**	**% C of total**	**% N of total**	**% P of total**	**% Water of total**
Raw, unsettled wastewater A1 to mixing tank	paper wastewater	43.33	100.00	100.00	100.00
CO_2 uptake		56.67	0.00	0.00	6.27
Incoming (total)		100.00	100.00	100.00	100.00
CO_2 (total)		14.77	0.00	0.00	-0.14
Lost to DSP (incl evaporation)		0.59	2.18	0.00	9.07
Bacterial product V1		1.09	5.55	2.76	2.46
Algal bioproduct W1		5.27	0.00	0.00	0.00
Algal oil W2		2.53	0.00	0.93	0.00
Algal digestible waste W3		25.19	10.77	65.97	2.18
Cellulosic fibre X1		0.32	0.17	0.00	0.00
Cellulosic biomass X2		0.07	0.04	0.00	0.00
N, P rich sediment X3		2.91	1.55	0.00	7.28
Crust/surface related product stream Y1		4.98	23.85	17.50	0.82
Liquor related product stream Y2		1.18	1.13	0.51	2.94
Cake-related product stream Y3		0.00	0.00	0.00	0.03
Compost Y4		8.76	37.55	43.77	0.29
Compliant effluent Z		32.33	17.20	0.00	81.91
Total % (Should be 100)		100.00	100.00	131.44	106.86
Difference (should be 0)		0.00	0.00	-31.443	-6.858

The results show that this stream is phosphorus limited. While the phosphorus in the dissolved component is adequate, the solids fraction is highly nutrient deficient. This supports the current preference for anaerobic digestion for biogas production, but it is still possible to produce higher value products to improve the overall economics of the system.

11.3.4 Evaluation of using different wastewaters in integrated WWBR scenarios

The products produced by the three wastewaters investigated are summarised using a basis of 1 000m^3 in Table 11-14 and visually compared in a bar graph in Figure 11-5. In addition, the values have been normalised to a basis of 1 000 kg-C/day incoming, as summarised in Table 11-15 and Figure 11-6. 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 total product mass values were determined by dividing the total C of the product by the C fraction. The sediment product X3 and compost product Y4 does not have a fixed composition and were estimated by adding the C,N,P and water amounts, as the composition of these are dependent on the process.

Table 11-14: Comparison of total amount of each product produced by three wastewater streams investigated, per 1 000m^3 incoming wastewater. All units are kg/day except where indicated otherwise.

kg/day	Domestic municipal wastewater	Poultry wastewater	Paper mill wastewater
Bacterial product V1	54	110	116
Algal bioproduct W1	437	37	384
Algal oil W2	191	16	167
Algal digestible waste W3	2731	231	2398
Cellulosic fibre X1	0	1041	36
Cellulosic biomass X2	0	234	9
N,P rich sediment X3 *	4	53	148
Crust/surface related product stream Y1	172	148	494
Liquor related product stream Y2	53	48	155
Compost Y4 *	204	140	523
Compliant effluent Z C (mg/L)	0.000	0.091	1.96
Compliant effluent Z N (mg/L)	0.053	0.000	0.047
Compliant effluent Z P (mg/L)	0.000	0.002	0.000

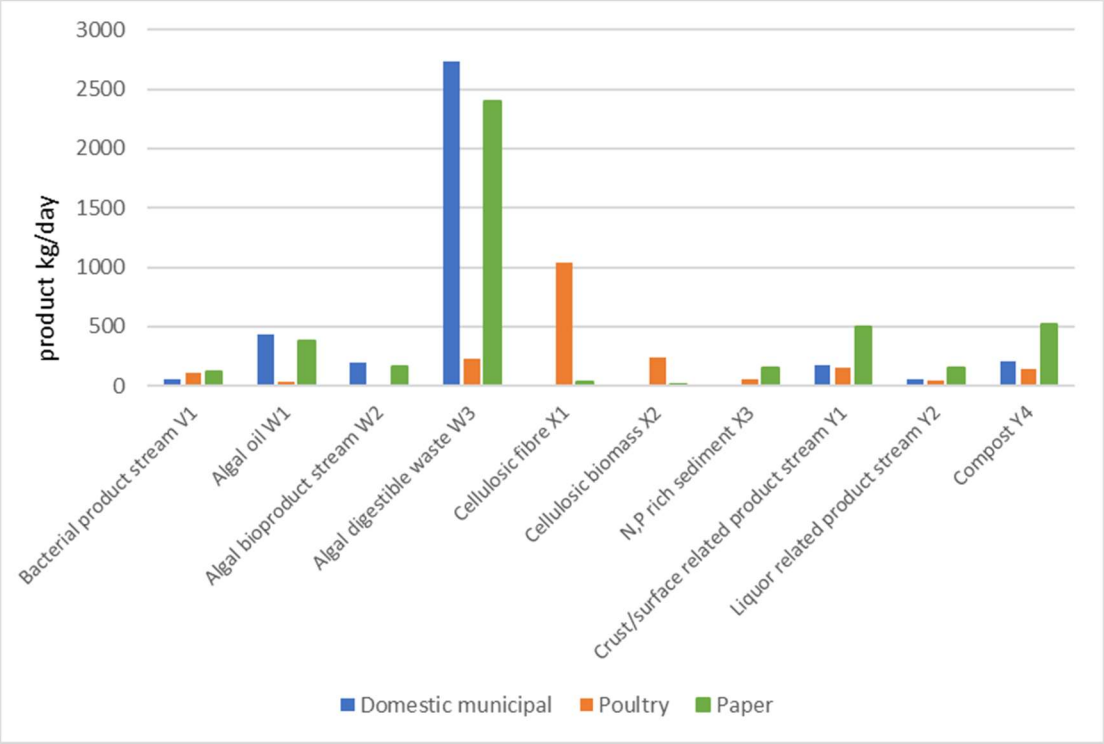

Figure 11-5: Bar graph comparing total amounts of products produced (kg/day) by each wastewater stream investigated, per 1000m^3/day incoming wastewater

Table 11-15: 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	65	66	54
Algal bioproduct W1	530	22	179
Algal oil W2	231	10	78
Algal digestible waste W3	3314	138	1119
Cellulosic fibre X1	0	623	17
Cellulosic biomass X2	0	140	4
N,P rich sediment X3 *	5	31	69
Crust/surface related product stream Y1	209	89	230
Liquor related product stream Y2	65	29	72
Compost Y4 *	248	84	244

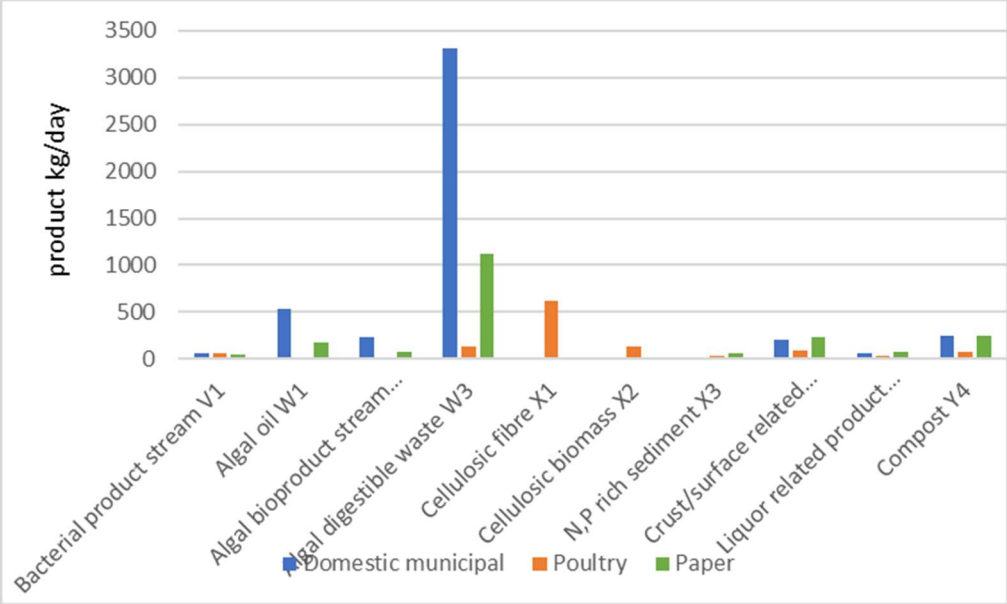

Figure 11-6: Bar graph comparing total amounts of products produced by each wastewater stream investigated, per 1000 kg-C/day incoming substrate

Supplementation of nutrients to achieve optimal C:N:P rations need to be considered in each case, necessitating industrial symbioses in order to improve the economic and environmental sustainability of this approach. The wastewaters considered here do not have optimal nutrient balancing, which impacts the selection of main bacterial bioreactor. Without supplementation, there seems little point in including the first bacterial reactor in the WWBR. To include it, more disruptive thinking and optimisation is needed.. A clear consequence of using primary settling and directing waste biomass to the solids bioreactor is that a large portion of nutrients and potential products is associated with the solids bioreactor and ultimately the Compost product Y4. As mentioned in Chapter 10 this can do with more research into biotransformation-based beneficiation. From a cleaner production perspective this promotes greater focus on being more water efficient up-stream and aiming to separate streams at source, to enable more appropriate and directed biotransformation of each stream.

11.4 Closing remarks on the wastewater biorefinery simulation model

To pursue the potential for WWBRs in the South African industrial and municipal wastewater context (and other national or regional contexts), a mass balancing tool centred on a generic WWBR flowsheet has been developed to allow a first order evaluation of specific opportunities. It is intended to serve both for early stage feasibility assessment and as a communication and facilitation tool between potential industry partners. It is aimed at stimulating 'future-thinking' and assessing potential benefit and is not intended as a (proprietary) modelling tool.

Building on the material balance tool set up in this thesis 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 focussed 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.

Future work need to include the use of sensitivity analyses, which will allow a more thorough unpacking where innovation and optimisation must be done. Sensitivity analyses on sources and amounts of supplementary substrates can facilitate industrial partnerships for specific case studies. Overall working within the current end-of-pipe paradigm is not sufficient to truly mobilise the value contained in wastewaters. There is a clear need to carefully design complex waste biorefinery systems to shift from an environmental protection to bioprocess engineering mindset.

12 CONCLUSIONS AND RECOMMENDATIONS

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. The WWBR differs from the biomass biorefinery as the latter typically still produces a wastewater stream for adequate bioremediation. The WWBR addresses the wastewater treatment to 'fit for purpose' water while recovering value from the stream. This thesis was about how to use conventional systems to be a stepping stone in changing paradigms. This hypothesis still works if the water is cleaned only to regulatory standards, but as Chapter 11 shows, that still means a lot of nutrients are lost. The concept is that there is more 'carrot' in terms of producing value to incentivise removing nutrients, than merely the 'stick' of regulation. The thesis contributes to the current body of knowledge in the following ways:

1. Introduction of the concept of the wastewater biorefinery (WWBR)
2. Provision of a potential preliminary guide for classification of wastewaters for use in the WWBR
3. Development of criteria for reactor evaluation for use in the WWBR
4. Development of an integrated model to interrogate bioproduction from wastewater and determine product yields associated with wastewater treatment
5. Creation of new knowledge through the interpretation of the model on different wastewater systems.

Chapter 1 and 2 highlight the strongly emerging themes 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. This has traditionally been focussed more on producing energy or energy carriers, but this thesis argues that the opportunity is greater, with potential to deliver both value-add products and energy products, and has more potential to galvanise investment in the sector than a mere request for compliance. Through characterisation of a range of wastewaters in South Africa in Harrison, et al. (2017), 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.

12.1 Overall conclusions

This thesis shows that inclusion of more units in an integrated process allows optimisation of more than one product while allowing for resilience of the overall system in removing nutrients and producing a compliant water stream as an additional, but essential, product. The novelty of this thesis is its integration of the units, by recognising the value that each unit adds to the overall process. This thesis integrates biology to underpin the concept of the ecological niche in the engineering context and provides a simple way to contribute understanding of why the different units are needed, applicable to a variety of wastewaters. It emphasises the need to create ecological niches which are more appropriate for bioproduction using large volumes of fluids than aiming to sterilise the bulk fluid and control the species producing the chosen product. This thesis argues that reactor design which facilitates downstream processing of the high value bioproducts is critical to improve overall productivity and is the largest difference between the WWBR and conventional wastewater treatment. Implementing ecological niches and reactor design for product recovery in this context promotes the beneficiation of wastes and contributes to the industrial ecology context. This provides incentive to improve the operation of wastewater treatment plants generally. The overall effect of considering wastewater as a resource is a reduction in the cost of disposal of the wastewater treatment products like the sludges, and a move towards resource efficiency in which value derived from the resource is maximised while environmental burden created in minimised.

12.2 A preliminary guide for classification of wastewaters for use in the WWBR

Wastewaters need to be classified and better understood in order to establish what valorisation is most suitable, and to understand what pretreatment may be required to improve the consistency, and suitability in general for use as a raw material. Wastewaters are receptacles, meaning that their composition is, for the most part, unpredictable and uncontrollable. From an overview of wastewaters in South Africa, a tremendous challenge is the availability of data and the format in which the available data is reported. The classification rests on three axes, that of volume, composition, and complexity. The volume needs to be known for both the national scale to inform investment incentive, and site specific to inform process design. While the smaller municipalities and smaller industries may be less attractive to investment, government needs to incentivise these smaller stakeholders to explore the WWBR, as this may contribute to attracting skills to smaller metros and contribute to capacity building in industry. More information about the composition, the type and concentration of the components (nutrients, and contaminations) in the stream is needed. Wastewater streams need better characterisation which includes reporting on the concentrations of all major nutrients e.g. C, N, P as well as the potential complicating inhibitors or compounds. Complexity includes the number of constituents in the stream, their concentration as well as how these change over time. To work towards the classification of wastewaters, a common reporting framework, and greatly improved disclosure of waste streams are sorely needed, with cognisance of the dual approach i.e. both water treatment and product creation.

12.3 Criteria for reactor evaluation for use in the WWBR

Several factors inform product selection and affect the bioreactor design. 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. This requires bioreactors to function in continuous or semi-continuous mode. 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 reactor design for application in the WWBR, addressing the provision of a niche environment for desired biocatalysts to avoid sterilisation is required. Further, reactor 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 reactor evaluation criteria developed in Chapter 5 and used to interrogate all four of the bioreactor types used in the WWBR was helpful to articulate the strengths and weaknesses of the bioreactors. 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. 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.

12.4 Development of an integrated model to interrogate bioproduction from wastewater

The development of the wastewater biorefinery concept 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. To assist in communicating this integration, a generic flowsheet was developed, showing a product spectrum which 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 MS Excel-based model has been established using this generic flowsheet It has been populated with typical performance data for these biological systems. The provision of this typical performance data provided the framework for early design stage material inventories to be developed for early design stage decision making without the collection of tailored performance data. The generic flowsheet model forms the key tool for the exploring of WWBR scenarios to investigate the potential of this approach.

12.5 New knowledge through the interpretation of the model on different wastewater systems.

The generic flowsheet and material balance model assembled in this study provides 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, its usefulness and potential for refinement has been demonstrated. The use of elemental compositions of the wastewater in terms of C, N and P is used 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 and macrophyte products. Further potential exists for refinement of effluent compliance, with future work in 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. Most of the bioproducts are directed to the solids streams, and all suffer from nutrient limitation, showing the need for supplementary substrates through, for example, industrial partnerships. 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 balancing tool.

WWBRs incorporate multiple unit operations to ensure removal of all nutrients and the combined optimisation of multiple products, including platform chemical, 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 PHA and PGA used as a flocculant, for metal removal and for water retention, have been highlighted as products of interest.

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. Thus the importance and potential of wastewater biorefineries is highlighted as a way to close the nutrient and energy cycles in the urban metabolism, to maximise resource productivity and to address water scarcity in nations such as South Africa.

12.6 Recommendations

The potential of wastewater biorefineries in general, and specifically in South Africa, is clearly demonstrated through this study. This is seen through the provision of a substantial feedstock with potential for bioconversion, their 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, both in broad ecological terms and in detailed analytical studies. Specific recommendations include:

The first requirement for reactor design is to decouple the hydraulic and solid retention times. A logical extension of this is to reduce the amount of liquid entering the stream. Therefore the wastewater biorefinery approach is complementary to other water reducing and recycling, and cleaner production approaches. For domestic municipal wastewater, the largest contributor to wastewater in South Africa, this may mean considering urine diversion, dry sanitation and new approaches to greywater management.

Current regulated parameters still allow too much nutrients and other compounds to remain in the discharged streams. Albeit very low, the concentration of nutrients in the effluent still adds up to significant total amounts of nutrients, which most receiving water bodies can simply not absorb any more, contributing to anoxic or eutrophic conditions. In the industrial context separating waste streams within the plant are better suited for WWBRs, allowing the streams to be blended to meet nutrient specs and avoiding cross-contamination of process specific inhibitors.

Targeted research on the relevant product spectrum obtainable from, in particular, the macrophyte bioreactor systems needs to be conducted, with a specific focus on indigenous species and consortia. Limited research has been conducted on these unit operations, specifically on their operation and harvesting, sensitive to the context of a functioning bioreactor. Research on hemp, as a source of fibre and by-products, and its legalisation should be investigated.

Nutrient and sediment removal with floating wetlands needs to be investigated in the context of bioproduction (macrophyte bioreactor), as the floating treatment wetlands show particular promise in the WWBR context.

Targeted research on the relevant product spectrum obtainable from solid substrate fermentation on wastewater sludges need to be conducted. There is limited understanding of the biological behaviour within these systems, which include nutrient removal and pathogen behaviour. Harvesting and downstream processing needs to be improved.

Bioreactor design studies in the context of the WWBR should be done for all the bioreactor units: the bacterial bioreactors, algal bioreactors, macrophyte bioreactors, sludge digesters and solid substrate fermentation bioreactors for sludge utilisation. This includes individual bioreactor design and optimisation to function in an integrated unit, as well as considerations towards product recovery and downstream processing.

Downstream processing needs to be investigated in the context of the WWBR. Technologies like reverse osmosis and new bioreactor designs like the AGS need to be investigated and adapted to promote product recovery.

Conventional process monitoring, analysis and control for wastewater treatment may be inadequate for bioproduction from wastewater, while process analysis for conventional bioprocessing (especially as used in the pharmaceutical sector) may be economically unfeasible. Therefore the need exists to develop appropriate process control, analysis, monitoring and/or automation in the context of the WWBR. This will also add value to better reporting on wastewater classification.

The model could be expanded to include an energy balance which could use COD directly. Further detail through iron, sulphur and possible other balances may be useful for specialist applications.

12.7 Post note

This thesis was developed in conjunction with the Water Research Commission (WRC) project “Introducing the wastewater biorefinery concept: A scoping study of polyglutamic acid production from a Bacillus-rich mixed culture using municipal waste water” (Verster, et al., 2014) and Water Research Commission (WRC) K5/2380 project titled “Towards Wastewater Biorefineries: integrated bioreactor

and process design for combined water treatment and resource productivity" (Harrison, et al., 2017). While the project focused on a global and national review on research on wastewater biorefineries and wastewater as a resource, this thesis explores in greater depth the requirements of each of the reactor units and their integration.

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A ANALYSIS OF SOUTH AFRICAN WASTEWATER STREAMS FOR BIOREFINERY FEEDSTOCK

There were several issues encountered in compiling data which would explicate the current status of South African wastewaters. These are explained in Chapter 3. 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. 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.

A.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 (mass flows) of C, N and P (Sections 3.2 and 0. However this is seldom how these are reported and the desired form was calculated from reported forms as follows.

A.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: A-1.

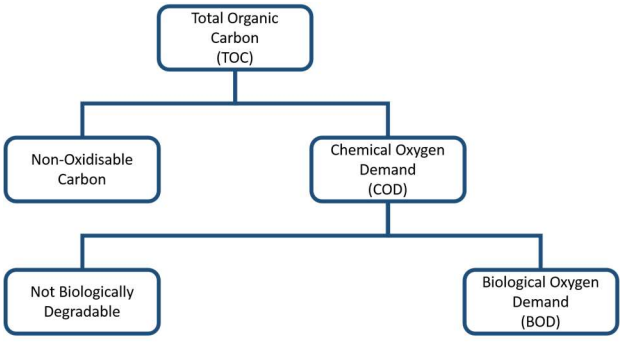

Figure: A-1: Relationship between measures of carbon concentration in organic wastewaters (adapted from Davies, 2005)

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, and not 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
$CH_3COOH + 2\ O_2 => 2CO_2 + 2H_2O$
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
$CH_4 + 2O_2 => CO_2 + 2H_2O$
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
$CHOOH + ½\ O_2 => CO_2 + H_2O$
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 formic acid is equivalent to 1/0.6667 x 20 mg/ℓ C = 30 mg/ℓ C
This value forms a minimum for this ratio.

Table: A-1: Some theoretical ratios of COD to TOC

				ratio calculation	COD/C
Acetate:	$CH_3COOH + 2\ O_2$	$=> 2CO_2 + 2H_2O$	1 mole acetate is 64 gCOD	64/(2*12)	2.667
Propionate:	$CH_3(CH_2)COOH + 3½\ O_2$	$=> 3CO_2 + 3H_2O$	1 mole propionate is 112 gCOD	112/(3*12)	3.111
Butyrate:	$CH_3(CH_2)_2COOH + 5\ O_2$	$=> 4CO_2 + 4H_2O$	1 mole butyrate is 160 gCOD	160/(4*12)	3.333
Valerate:	$CH_3(CH_2)_3COOH + 6½\ O_2$	$=> 5CO_2 + 5H_2O$	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: A-2.

Table: A-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: A-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.

A.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

A.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

A.2 General Data for Industrial Wastewaters

A.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: A-3. This table should be read together with the table in Section **Error! Reference source not found.**.

Table: A-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)

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
						(i) (Brito, et al., 2007)
TKN Total Kjeldahl Nitrogen TN Total nitrogen TP Total phosphorus TSS Total supended solids nl not listed						

A.2.2 Additional general data for industrial wastewaters

Data compiled from Cloete et al. (2010) is shown in Table: A-4 and from Burton et al. (2009) in Table A-5.

Table: A-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: A-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

A.3 Municipal wastewater (Section 0)

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: A-6. The composition of typical raw municipal wastewater with the normal contribution of industrial wastewaters is given ((Henze, et al., 2008)) in Table: A-7. Local data was also obtained from the City of Cape Town for two specific wastewater treatment works, Athlone and Mitchell's Plain WWTWs in Cape Town, and given in Table: A-8.

Table: A-6: 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: A-7: 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: A-8: Athlone and Mitchell's Plain municipal WWTW composition data

	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)		

A.4 Data for Specific Industrial Wastewaters (Section 0)

The source data used to calculate the values presented in Section **Error! Reference source not found.** are tabulated here.

A.4.1 Pulp and Paper industry (Section 3.3.2)

Table: A-9: 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	–	–
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	–	–

Mill	Wastewater (ML)	Est wastewater (ML)	COD (mg/L)	pH	Temperature (°C)
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					

A.4.2 Poultry abattoirs industry (Section 3.3.3)

From the Molapo (2009) study, the composition of poultry abattoir wastewater is given in Table: A-10, The slaughtering capacity of these plants is given in Table: A-11. The estimated wastewater generated from the number of plants with their C, N and P content has been calculated and is summarized in Table: A-12.

Table: A-10: Poultry abattoir wastewater content concentration 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
FOG (fat, oil & grease) (mg/L)	100 – 1 000

Table: A-11: 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: A-12: 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				

Note: The suspected incorrect values – repeated in the N, P and Fats columns also occurs like this in the Molapo report.

B SUPPLEMENTARY DATA FOR SELECTION OF MASS BALANCE FACTORS

B.1 Supporting data for Section 8.1 Unit Mass Balances

B.1.1 Bacterial Bioreactor Factors for Mass Balances

These factors are all enumerated in Chapter 6.3 as a full example of the requirements.

B.1.2 Algal Bioreactor Factors for Mass Balances

Table: B-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	224	glucose	60.42	89.60	0.674
109	182	acetic acid	56.68	72.80	0.779
166	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	130	glucose	60.94	52.00	1.172
22	22	glucose	11.49	8.80	1.306
51	n	glucose	26.62		not used
48	n	glucose	24.96		not used
84	n	glucose	43.68		not used
52	n	glucose	26.94		not used
42	n	carob	21.84		not used
48	n	glucose	24.96		not used
40	n	ethanol	20.54		not used
Average					
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 Chapter 7.3

Table: B-2: Conversion of composition to mass percent for algal biomass

Element	**Composition: Normalised to P** (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	0.0127 = g P / g algal biomass
H	181	1	181	0.074
O	46	16	736	0.300
Total			**2 444**	**1.000**

Table: B-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**
Freshwater, N starvation	42 - 60
Freshwater, N deficient	43
Freshwater, nutrient sufficient	21 - 38
Heterotrophic culture	20 - 50
Marine, N starvation	41- 73
Marine, nutrient sufficiency	29 - 67

B.1.3 Macrophyte Bioreactor Factors for Mass Balances

The tabulated factors for the macrophyte bioreactor mass balances follow. These tables match those in Section 8.4.1 and Section 8.4.2 refers.

Table: B-4: Cellulose carbon content

	C	**H**	**O**	**Total**	**Fraction C** (g-C/g-c**ellulose**)
molecular mass element	12	1	16	-	
cellulose mol formula	6	10	5	$(C_6H_{10}O_5)_n$	
cellulose mass fraction	72	10	80	162	0.444

Table: B-5: Macrophyte (flax) carbon content

0.00735	fraction N average flax
0.00023	fraction P grass
assume remainder cellulose	
0.99242	cellulose
0.444	C fraction in cellulose
0.441	fraction C of total

Table: B-6: Macrophyte (flax) plant biomass (Dodkins & Mendzil, 2014b)

shoots	**roots**	**total**	**g/m²**

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
		2.0000	harvests per year
		0.31	kg per m^3 influent

Table: B-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
2.0000	harvests per year
0.306	kg total plant mass per m^3 influent, per year
0.81	C composition of macrophyte
0.2480	kg C per m^3 influent, per year
365	days per year (averaged growth)
0.00068	kg C uptake per m^3 influent, per day

B.1.4 Solids Bioreactor Factors for Mass Balances

The tabulated factors for the solids bioreactor mass balances follow. These tables match those in Section **Error! Reference source not found.**.

Table: B-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: B-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

B.2 Supplementary information for Chapter 11.1

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

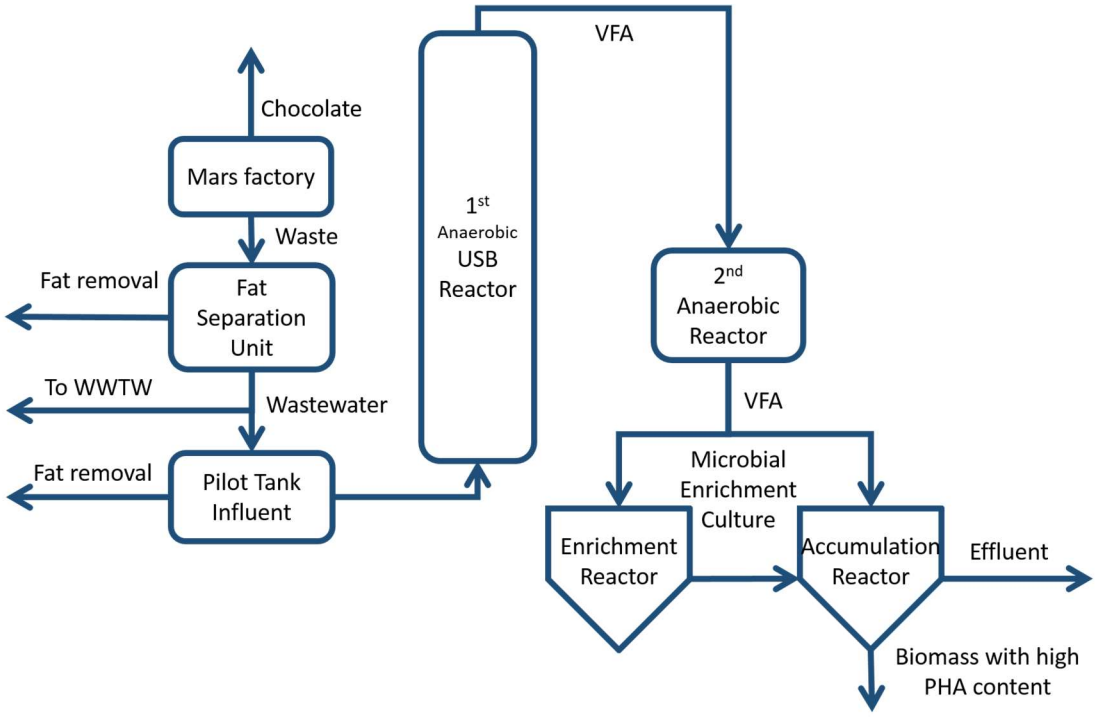

Figure: B-1: Three-step process to produce PHA from Mars factory wastewater (Tamis, et al., 2014)

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: B-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)		
0.00312	g/L P delivered with N		