Techno-economic and life cycle analyses for comparison of biorefinery scenarios for the production of succinic acid, itaconic acid and polyhydroxybutyrate (PHB) from sugarcane lignocelluloses

By

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April 2019

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ABSTRACT

The South African sugar industry is experiencing economic strain due to low international sugar prices and detrimental natural events such as droughts. Therefore, an innovative and sustainable solution is required to create new revenue streams and ensure job security. Utilization of the full potential of the lignocellulosic by-products produced, sugarcane bagasse and trash, provides such a solution. The biomass can be used in a biorefinery as feedstock for the production of valuable biofuels and bioproducts.

Succinic acid, itaconic acid and polyhydroxybutyrate (PHB) were selected as potential co-products due to their high value, wide range of applications and similar production routes. The **first objective** was to design and develop conceptual process flow sheets for the respective co-production of itaconic acid, succinic acid and/or PHB with electricity in a biorefinery, annexed to an existing sugar mill and new combined heat and power (CHP) plant. The simulations were developed from data reported in literature and simulated in Aspen Plus® v8.8.

The **second objective** was to determine which biorefinery scenarios are profitable within the South African economic conditions. The mass and energy balance results were used to develop a discounted cash flow rate of return analysis (DCFROR). A real term hurdle rate of 9.7% was used to determine the profitability of each scenario using the project internal rate of return (IRR), net present value (NPV) or minimum required selling price (MRSP). The profitable scenarios are summarized in Appendix A.

After completion of the first two objectives, **Objective 3** was included to determine whether the biorefinery profitability could be further increased by selecting an appropriate pretreatment method. Nine methods were identified and simulated. Of these, the steam explosion (STEX) with enzymatic hydrolysis pretreatment method for the co-production of succinic acid and electricity from sugarcane lignocelluloses resulted in the most profitable scenario with an IRR of 28.04%.

Although the use of a lignocellulosic feedstock can reduce our fossil resource dependency and carbon footprint, the production of these bioproducts may lead to other environmental impacts not associated with climate change or fossil resource depletion. Therefore, the **fourth objective** was to measure the environmental impacts of the respective biorefinery scenarios using a cradle-to-gate life cycle assessment (LCA), developed in SimaPro® v8.0.

In **Objective 5** the techno-economic and environmental results were used, together with the social sustainability indicator (i.e. job creation), in a multicriteria decision analysis (MCDA) tool, to determine the most sustainable solution for implementation by the South African sugar industry**.** To this end, the co-production of PHB and succinic acid with electricity in a multiproduct plant resulted in the most sustainable solution, with an IRR of 24.1% and a NPV of 447.2 million US$.

Future research could validate the multiproduct plant and succinic acid (with STEX pretreatment) co-production scenarios in a pilot scale study. In addition, possible combinations of bioproducts for multiproduct biorefineries could be investigated further to maximize economic and/or environmental sustainability. Ultimately, the implementation of a biorefinery will contribute to creating new revenue streams, ensuring job security within the sugar industry, and contribute to a developing South African green economy.

OPSOMMING

Die Suid-Afrikaanse suikerbedryf ondervind ekonomiese druk as gevolg van lae internasionale suikerpryse en natuurlike rampe soos droogtes. Daarom is 'n innoverende en volhoubare oplossing nodig om nuwe inkomstestrome te skep en werksekerheid te verseker. Die gebruik van die volle potensiaal van die lignosellulosiese neweprodukte, suikerriet pulp en afval, bied so 'n oplossing. Die biomassa kan gebruik word in 'n bioraffinadery as grondstof vir die produksie van waardevolle biobrandstowwe en bioprodukte.

Suksiensuur, itakonsuur en polihidroksiebutiraat (PHB) is gekies as potensiële medeprodukte weens hul hoë waarde, wye verskeidenheid toepassings en soortgelyke produksieroetes. Die **eerste doel** was om konseptuele prosesvloeistelle te ontwerp en te ontwikkel vir die onderskeie neweproduksie van itakonsuur, suksiensuur en/of PHB met elektrisiteit in 'n bioraffinadery wat geannekseer is aan 'n bestaande suikermeul en nuwe gekombineerde hitte- en krag (CHP) stasie. Die simulasies is ontwikkel uit data vanuit die literatuur en is in Aspen Plus® v8.8 gesimuleer.

Die **tweede doelwit** was om te bepaal watter bioraffinadery scenario's winsgewend is onder Suid-Afrikaanse ekonomiese toestande. Die massa- en energiebalans resultate is gebruik om 'n verdiskonteerde kontantvloei- en opbrengskoersanalise (DCFROR) te ontwikkel. 'n Reële termynverhogingskoers van 9.7% is gebruik om die winsgewendheid van elke scenario te bepaal deur die projek se interne opbrengskoers (IRR), netto huidige waarde (NPV) of minimum vereiste verkoopprys (MRSP) te gebruik. Die winsgewende scenario's is opgesom in Bylae A.

Na afloop van die eerste twee doelwitte is **doelstelling 3** ingesluit om vas te stel of die winsgewendheid van ‘n bioraffinadery verder verhoog kan word deur 'n gepaste voorbehandelingsmetode vir die lignosellulose te kies. Nege metodes is geïdentifiseer en gesimuleer. Hiervan het die stoomontploffing (STEX) met ensiematiese hidrolise-voorbehandelingsmetode vir die medeproduksie van suksiensuur en elektrisiteit gelei tot die winsgewendste scenario met 'n IRR van 28.04%.

Alhoewel die gebruik van 'n lignosellulosiese grondstof ons afhanklikheid van fossielhulpbronne kan verminder, kan die produksie van hierdie bioprodukte tot ander omgewingsimpakte lei wat nie verband hou met klimaatsverandering of die uitputting van fossielhulpbronne nie. Daarom was die **vierde doelwit** om die omgewingsimpakte van die

onderskeie bioraffinadery scenario’s te meet deur gebruik te maak van 'n lewensduur-siklus-assessering (LCA) wat ontwikkel is in SimaPro® v8.0.

In **doelstelling 5** is die tegno-ekonomiese en omgewingsresultate gebruik, tesame met die maatskaplike volhoubaarheidsindikator (d.w.s. werkskepping), in 'n multi-kriteria besluitanalise (MCDA) metode om die mees volhoubare oplossing vir implementering deur die Suid-Afrikaanse suikerindustrie te bepaal. Vir hierdie doelwit het die medeproduksie van PHB en suksiensuur met elektrisiteit in 'n multi-produkaanleg tot die mees volhoubare oplossing gelei, met 'n IRR van 24.1% en NPV van 447.2 miljoen US$.

Toekomstige navorsing kan die multi-produkaanleg en suksiensuur (met STEX-voorbehandeling) resultate deur behulp van ’n proefskaalstudie valideer. Daarbenewens kan moontlike kombinasies van bioprodukte vir multi-produkaanlegte verder ondersoek word om ekonomiese en/of omgewingsvolhoubaarheid te maksimaliseer. Uiteindelik kan die implementering van 'n bioraffinadery bydra tot die skep van nuwe inkomstestrome, die versekering van werksekuriteit binne die suikerbedryf, en bydra tot 'n ontwikkelende Suid-Afrikaanse groen ekonomie.

PREFACE

My perpetual curiosity about the world and all its beautifully crafted designs make me thirst to learn more. Therefore it is quite fair to say I love to learn. My first experience with science was in Grade 2, when I realised the wind was 'moving air'. Most probably I did not know about molecules then, but in my mind I must have been able to conceptualise these small bundles of 'something'. Fast forward a few years and I am in my second or third year of Chemical Engineering, sitting with Norman in his car, eating and chatting, when we noticed an elderly man scratching in the rubbish bin for something to eat.

He found an old coffee cup, emptied the last remaining sour sip into his mouth, and threw the cup to the side. Some people have questioned how ethical my next thought was, but I thought to myself: "What if he could eat the cup?" Surely if he was desperate enough to chase the last droplets of coffee-calories, he would not mind eating the cup. And there, without yet knowing it, my interest in the utilisation of lignocelluloses was born. Although the present study does not propose to solve world hunger, it does involve the breakdown of lignocelluloses into fermentable sugars for subsequent utilisation by microorganisms. Indeed, a tiny step towards eating the coffee cup.

AKNOWLEDGEMENTS

I thank my husband, Norman, from the bottom of my heart for all your love, support and dedication. You have sacrificed with me in this pursuit of knowledge. Thank you for never doubting me. Your absolute belief in me gave me the courage to believe in myself.

I acknowledge and thank Dr. Haigh and Prof. Görgens. Kate, thank you for your endless patience and commitment to read, review and comment on the work. Thank you Prof. Görgens for always asking exactly the right questions and for the opportunity to be part of a leading bioresource research group. Thank you for every grant application you reviewed and for the financial support you provided.

I also acknowledge and thank the NRF, and specifically Rozelle Petersen as designated authority, for their financial support that made it possible to have pursued my postgraduate studies.

Thank you to all my friends and family for being excited with me about sugarcane bagasse.

DEDICATION

"Your talent is God's gift to you. What you do with it is your gift back to God". I dedicate this dissertation to the One who gave me the skills, talent, support, grace and love to complete it.

"Far better it is to dare mighty things, to win glorious triumphs, even though checked by failure, than to take rank with those poor spirits who neither enjoy much nor suffer much, because they live in the grey twilight that knows neither victory nor defeat" - Theodore Roosevelt

NOMENCLATURE

Table i: List of Symbols

SYMBLOLS	DESCRIPTION
˚C	Degrees centigrade
atm	Pressure measured in atmosphere
CO_2	Carbon dioxide
CO_2e	Carbon dioxide equivalent
CH_4	Methane
$FPU.g^{-1}$	Filter paper units per gram cellulignin
$g.g^{-1}$	Grams per gram
$g_{SA}.g_{GLU}^{-1}$	Grams succinic acid produced per gram glucose
$g.L^{-1}$	Grams per litre
$g.L^{-1}.hr^{-1}$	Grams per litre per hour
kW	kilowatt
kWh	Kilowatt-hour
$L.min^{-1}$	Litres per minute
m^3	Cubic meters
$m^3.hr^{-1}$	Cubic meters per hour
mg	milligram
MW	megawatt
MWh	Megawatt-hour
N_2O	Nitrous oxide
rpm	Rotations per minute
$R.kg^{-1}$	Rand per kilogram
$R.t^{-1}$	Rand per tonne
t	tonnes
$t\ CO_2e.hr^{-1}$	Tonnes carbon dioxide equivalent per hour
$t.hr^{-1}$	Tonnes per hour
wt%	Percentage by weight

Table ii: List of abbreviations and acronyms

ACRONYMS	DESCRIPTION
1G; 2G; 3G	First generation; second generation; third generation
ACC	Annual capital charge
AD	Anaerobic digestion
ADM	Archer Daniels Midland
AFEX™	Ammonia Fibre Expansion
BDO	1,4 - butanediol

BFD	Block flow diagram
BPST	Back pressure steam turbines
CAPEX	Capital expenditure
CCOP	Cash cost of production
CDW	Cell dry weight
CEPCI	Chemical Engineering Plant Cost Index
CEST	Condensing Extraction Steam Turbine
CHP	Combined heat and power plant
COM	Cost Of Manufacturing
DAT	Dilute acid treatment
DCFROR	Discounted cash flow rate of return
DM	Dry Mass
DPBP	Discounted payback period
DSP	Downstream Process
ESKOM	Electricity Commission of South Africa
EU	Endotoxin level
FCI	Fixed capital investment cost
FCOP	Fixed cost of production
FPU	Filter paper units
GAC	Granular Activated Carbon
GBL	γ-butyrolactone
GHG	Greenhouse gas
GWI	Global warming impact
HHx	3-hydroxyhexanoate
HMF	5-hydroxymethylfurfural
HP	High pressure
HPT	High Pressure Turbine
HPU	High Pressure Utility steam
IA	Itaconic Acid
IA-e	Itaconic acid with enzymatic hydrolysis scenario
IA-ee	Itaconic acid with a high initial glucose concentration (180 $g.L^{-1}$) and enzymatic hydrolysis scenario
IA-w	Itaconic acid without enzymatic hydrolysis scenario
ICI	Imperial chemical industries
IRR	Internal rate of return
ISBL	Inside battery limits
ISPR	In-situ process recovery
LCA	Life cycle analysis
LHW	Liquid hot water
LPT	Low Pressure Turbine

LPU	Low Pressure Utility steam
MCL	Medium Chain Length
MMA	Methacrylate monomer
MP	Multiproduct plant scenario
MPT	Medium Pressure Turbine
MRSP	Minimum required selling price
NPCM	Non-PHA Cell Mass
NPP	New Product Plant
NPV	Net present value
OPEX	Operational expenditure
PA-xxx	Plant area
PFD	Process flow diagram
PHA	Polyhydroxyalkanoates
PHB	Poly-3-hydroxybutyrate, PHB scenario
PLA	Polylactic acid
PSD	Particle size distribution
RK EOS	Redlich-Kwong equation of state
RO	Reverse osmosis
ROI	Return on investment
RW	Representative weighting
SA	Succinic acid
SA-e	Succinic acid with enzymatic hydrolysis scenario
SA-w	Succinic acid without enzymatic hydrolysis scenario
SB	Sugarcane bagasse (stems)
SCL	Short Chain Length
SG	Standard gravity
SHF	Separate hydrolysis and fermentation
STEX	Steam Explosion
SmF	Submerged fermentation
SMRI	Sugar Milling Research Institute
SsF	Solids-state fermentation
SSCF	Simultaneous saccharification and co-fermentation
sp.	Species
ssp.	Subspecies
ST	Sugarcane tops (leaves)
TCA	Tricarboxylic acid cycle
TCI	Total capital investment
TCOP	Total cost of production
TDC	Total direct costs

THF	Tetrahydrofuran
US$	United States of America dollar
VCOP	Variable Cost of Production
v/v	Volume per volume
WO	Wet oxidation
WWT	Waste water treatment
w/v	Weight per volume

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Chapter 1

1. Introduction

1.1 Background

The South African sugar industry makes a significant contribution to the national economy. It produces an average of 2.3 million tons of sugar per annum and directly employs approximately 430 000 people, resulting in an estimated 1 million people who depend on the industry for their livelihoods, such as farmers, farm workers, factory workers and their families. However, the industry is faced with challenges such as low international sugar prices, increasing production costs and droughts (Gorgens *et al*., 2016). To overcome these challenges for sustainable future operations, innovative solutions are required. One solution is the valorisation of sugar mill by-products for diversified and additional income streams in a biorefinery.

This approach means that changes will need to be made to current harvesting and mill practises to liberate sugarcane by-products which can be valorised. Typically, sugarcane is harvested by burning the dry leaves and tops, after which the cane is sent to the sugar mill. The sugar juice is extracted from the cane and the milled and crushed fibre residue (i.e. bagasse) is treated as a waste or by-product and burnt in low-efficiency boilers to generate steam and energy for the sugar mill (Mbohwa, 2013). However, by introducing green harvesting practises and replacing the existing boiler with a high pressure and efficient unit, excess bagasse can be made available (Ali Mandegari *et al*., 2017; Venkatesh and Roy, 2011). This bagasse, together with the leaves and tops, can be used as a lignocellulosic biomass feedstock for the production of biofuels, biochemicals and bioproducts, as illustrated in Figure 1-1 (Carvalheiro *et al*., 2008; Werpy and Petersen, 2004).

Biomaterials and -chemicals can be produced in a new production facility annexed to an existing sugar mill and new co-generation plant, as shown in Figure 1-2. Sugarcane is fed to the sugar mill, from which sugar, molasses and bagasse (a by-product) are produced. The sugarcane bagasse and trash feedstock is split between the biorefinery (new products plant, NPP) and the combined heat and power (CHP) plant. The energy requirements of the NPP and existing sugar mill need to be met by the CHP plant for an energy self-sufficient biorefinery and sugar mill. Consequently, the feedstock split or bypass ratio from the NPP to the CHP plant

will be determined by the NPP energy requirement. Ultimately a low bypass ratio is desired for the maximum NPP capacity and bioproduct production, with associated economies-of-scale benefits.

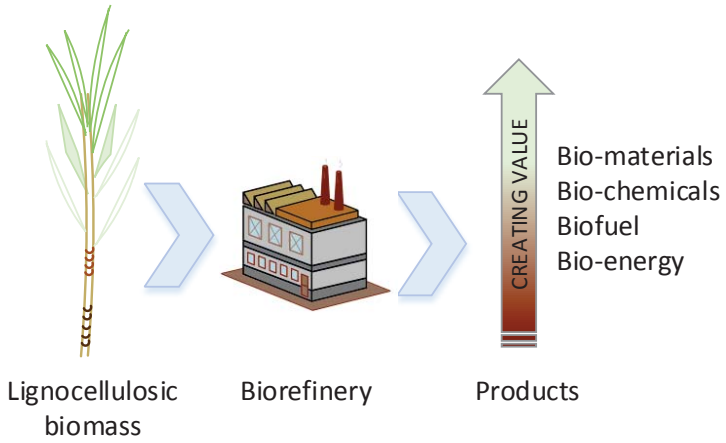

Figure 1-1: Biorefinery options (drawn from web diagram Werpy and Petersen, 2004)

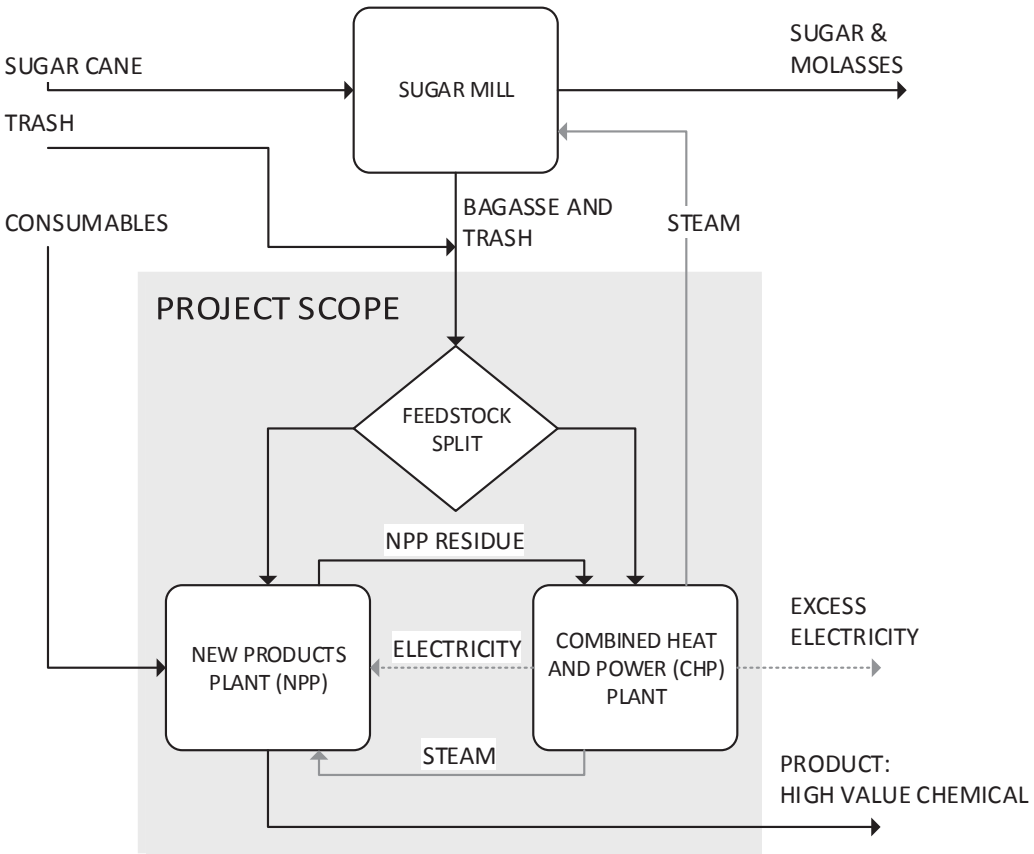

Figure 1-2: Bypass rate concept of feedstock from the NPP biorefinery to the CHP

The range of biochemicals and -materials that can be produced from lignocelluloses have been identified (Kapanji 2016) and subsequent screening shortlisted three products: itaconic acid,

succinic acid and the polyhydroxyalkanoate, polyhydroxybutyrate (PHB). These products were selected due to their wide range of applications and similar production route, i.e. the bioconversion of fermentable sugars. Fermentable sugars are produced from lignocelluloses by pretreating the lignocellulosic biomass followed by detoxification and enzymatic hydrolysis.

The production of these bioproducts were simulated in Aspen Plus® whereafter the mass and energy balances were used to determine both the economic outcome and environmental impact of the respective biorefinery scenarios. For the production of each bioproduct the various process flow sheet configuration options and process technologies were considered and evaluated. These various options were presented as different biorefinery scenarios. More information on these scenarios are provided in the thesis outline in section 1.3. In the end these biorefinery scenarios will be added to the range of developed scenarios, which include an ethanol plant, ethanol with lactic acid, ethanol with furfural, methanol, Fisher-Tropsch products, butanol (Gorgens *et al*., 2016), citric acid, glutamic acid, and xylitol (Ozudogru, 2018) simulations to compare and identify the most viable biorefinery scenario solution for the sugar industry.

1.2 Project Aim and Objectives

The overall project aim was to investigate whether a lignocellulose biorefinery is a sustainable and viable investment option to revive the sugar industry and associated farming communities. The primary objectives are provided below, with the aim and novel contribution outlined for each chapter in section 1.3.

1.2.1 Objective 1: Design and develop conceptual biorefinery process designs for the production of biobased chemicals

Since no techno-economic studies have been reported for the co-production with electricity of succinic acid, itaconic acid or PHB from sugarcane lignocellulosic bagasse and trash, a suitable process flow sheet for the production of each bioproduct was designed from literature and simulated in Aspen Plus®. Such scenarios also considered integration of such biorefinery scenarios into an existing sugar mill, in particular through the sharing of steam and electricity supply. A base case for the co-generation of electricity only was also included. This objective was addressed in Chapter 3 for itaconic acid and in Chapter 4 for succinic acid, PHB, and electricity-only production.

1.2.2 Objective 2: Determine which biorefineries are profitable in accordance with South African economic conditions

Economic indicators, such as the net present value (NPV), internal rate of return (IRR) and minimum product selling price (MPSP), were used to assess the investigated scenarios and determine whether a biorefinery is a viable investment opportunity for the sugar industry. This objective was addressed in Chapter 3 for itaconic acid and Chapter 4 for succinic acid and PHB production.

1.2.3 Objective 3: Determine which pretreatment method will maximise the valorisation of sugarcane bagasse and trash lignocelluloses, for one of the preferred biorefinery scenarios

It is well-known that the pretreatment step contributes significantly to the capital costs of lignocellulose bioprocessing facilities, and that the profitability is sensitive to the bioproduct yield on fermentable sugars. Therefore this objective was included after the fact to determine whether the biorefinery profitability could be further improved by selecting an appropriate pretreatment method. The available pretreatment technologies for sugarcane bagasse and/or trash were screened and nine methods were identified, simulated and compared for the co-production of one preferred bioproduct. This was based on the completed deliverables of Objectives 1 and 2, in a sugarcane bagasse and trash biorefinery annexed to an existing sugar mill. This objective was addressed in Chapter 5.

1.2.4 Objective 4: Determine the environmental impact of succinic acid, itaconic acid and PHB production from sugarcane lignocelluloses.

Even though the feedstock is renewable, the process may have a detrimental impact on the environment. Therefore the environmental impact of each bioproduct was determined using a life cycle assessment, simulated in SimaPro®. The environmental impact of the selected biorefinery scenarios were compared to one another, as well as to equivalent products, such as polylactic acid (PLA) for PHB and maleic anhydride for succinic acid. This objective was addressed in Chapter 6 for the selected biorefinery scenarios.

1.2.5 Objective 5: Determine which biorefinery is the most sustainable solution for implementation by the South African sugar industry.

A scenario can be profitable, but not necessarily sustainable. A green engineering solution requires a sustainable design, i.e. where the economic, environmental and social design factors are taken into consideration. Therefore a multi-criteria decision analysis was used to compare the selected scenarios and identify the most sustainable solution for implementation by the South African sugar industry. This objective was addressed in Chapter 6.

1.3 Thesis outline and original contribution summary

After the introductory chapter and literature review, Chapters 2 – 6 are presented as individual studies, prepared as articles for publication. The major conclusions and recommendations are summarised in Chapter 7. In Figure 1-3 the connection between the objectives and the respective chapters is shown together with the summarised novel contribution of each chapter.

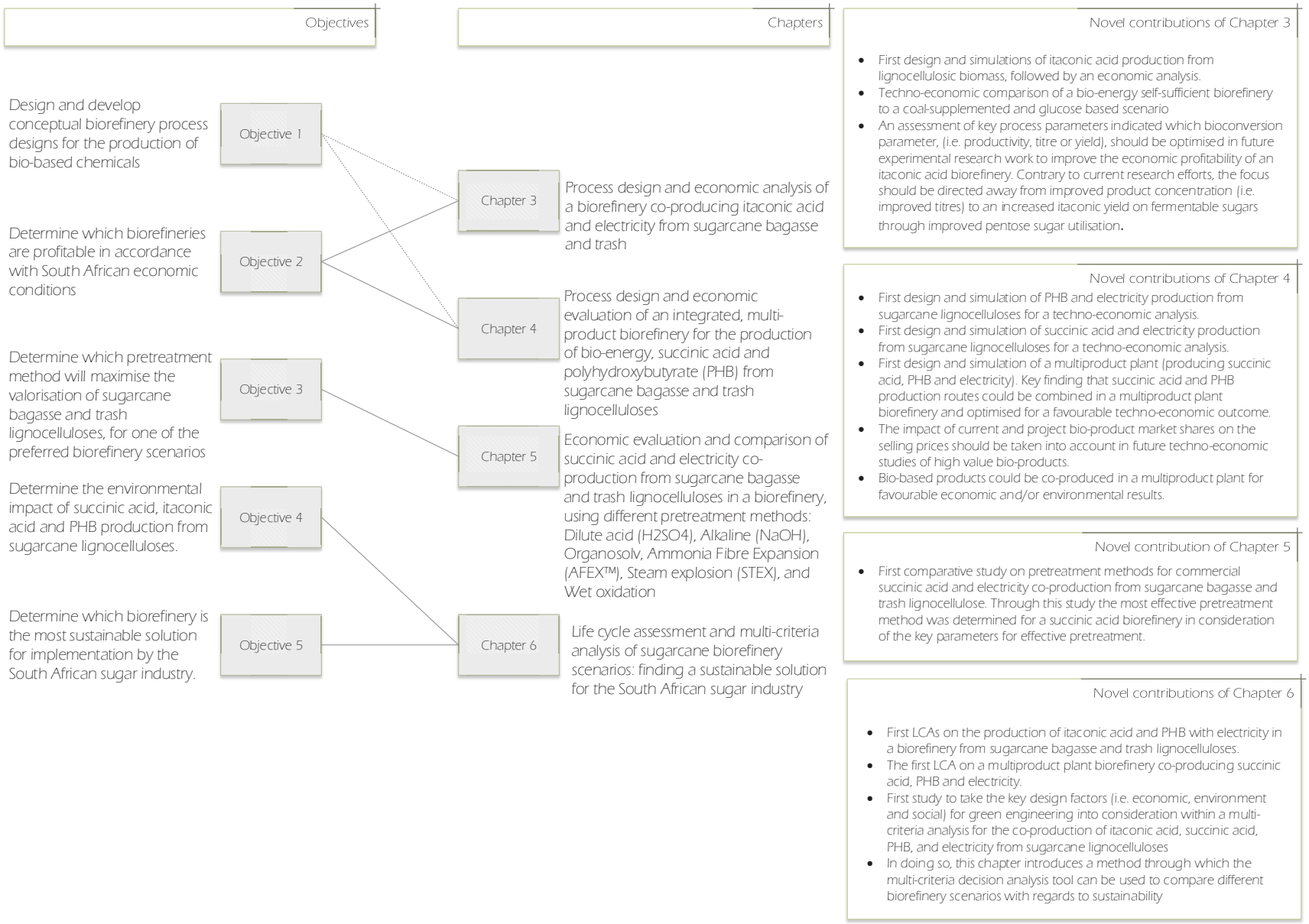

Figure 1-3: Thesis outline and novel contribution of work by chapter

Chapter 2

2. Literature review

The bioproduct selection, i.e. succinic acid, itaconic and PHB, together with the respective process steps required for the manufacturing of these bioproducts, are expanded on in this chapter. The production challenges and techno-economic evaluation of the biorefinery scenarios are also reviewed, together with the environmental considerations and benefits of the biorefinery concept.

2.1 Overview of the product selection

Succinic acid, itaconic acid and PHA's are three of twelve shortlisted potential building blocks and chemical products for sugarcane biorefineries (Kapanji 2016). The shortlisted products are shown in Figure 2-1. Building block products are used as co-monomers for the manufacturing of other valuable products for a wide range of applications. Chemical products are used industrially without further processing. Glutamic acid, itaconic acid, glucaric acid, polyhydroxyalkanoates (PHAs'), succinic acid, levulinic acid, sorbitol and xylitol are all building block products that can be produced from sugarcane lignocelluloses.

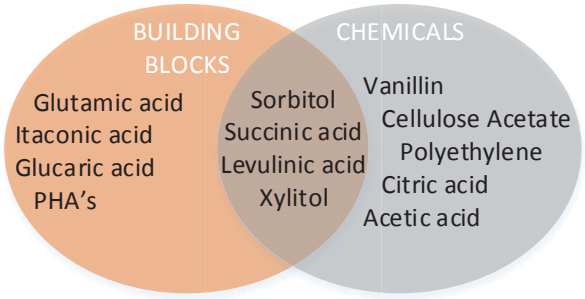

Figure 2-1: Shortlisted products for potential biorefinery scenarios

Succinic acid, levulinic acid, sorbitol and xylitol can be used as building block chemicals. Cellulose acetate, vanillin, acetic acid, citric acid and polyethylene are high value chemicals (Kapanji 2016).

The products are produced by either chemical or biological processing routes (Table 2-1). The chemical route includes the use of other chemicals, catalysts and thermochemical processes to convert the substrates into products. The biological route utilises bioconversion (fermentation) as the key process step to convert the biomass-derived sugar substrates into the desired product.

This study will focus on the use of biological processing routes, since a common route will allow a better basis for comparison within the project.

Itaconic and succinic acid are indicated as the building blocks with the widest range of applications. Itaconic acid can be produced with a chemical route from citric acid through pyrolytic distillation, but the low cost difference between citric acid and itaconic acid makes the chemical route of itaconic acid production unviable (Klement and Büchs, 2013; Mondala, 2015). Even the biological route must be cost competitive to its chemical, fossil-based equivalents before it will be utilised commercially for all its applications.

Table 2-1: Product selection for techno-economic analysis (Werpy *et al.* 2004, Chandel *et al.* 2012, de Jong *et al.* 2012)

	INDUSTRY RELATED PRODUCTS															PROCESS		
BUILDING BLOCKS	Food	Pharmaceutical	Polymers	Polyesters	Absorbent	Herbicide	Resin	Cosmetic	Detergent builders	Corrosion ingibuitors	Cements	Solvents	Oil additives	Paints	Rubbers	APPLICATIONS	Fermentation	Chemcial	SELECTED
SORBITOL	✓		✓	✓			✓									4		x	
LEVULINIC ACID		✓	✓	✓		✓	✓					✓				6		x	
XYLITOL	✓	✓						✓								3	x	x	
SUCCINIC ACID	✓	✓	✓	✓		✓			✓	✓						7	x		YES
ITACONIC ACID			✓	✓	✓		✓		✓		✓		✓	✓	✓	9	x	x	YES
GLUTAMIC ACID	✓	✓	✓													3	x	x	
GLUCARIC ACID			✓						✓	✓						3		x	
PHA		✓	✓			✓								✓		4	x		YES

Although levulinic acid can be used in six different industries and sorbitol in four, both are produced via a chemical route. Since succinic acid and itaconic acid are produced through a biochemical route, the next suitable candidate produced through fermentation is polyhydroxyalkanoate (PHA). PHA's can be used in four different industries and is used in the polymer industry and for biomedical applications.

Succinic acid, itaconic acid and PHA's are building block products that have been identified as having potential as part of the green economy (Kapanji 2016). The green economy is a system that promotes social, environmental and economic balance for a sustainable future. A short overview will be given of each product on their characteristics, applications, market relevance and commercial production.

2.1.1 Succinic Acid

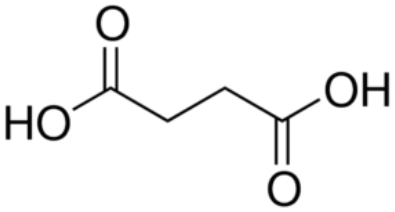

Figure 2-2: Succinic Acid structure

Succinic acid is a C_4 dicarboxylic acid ($C_4H_6O_4$), shown in Figure 2-2. It is a white, water-soluble crystalline solid, also known as butanedioic acid or amber acid, from which it was first derived (Jiang *et al.*, 2013). It has a molecular weight of 118.09 $g.mol^{-1}$, decomposes at 188˚C and has a density (SG) of 1.572 at 25˚C. It has been listed in the United States Department of Energy's list of the top 12 value-added chemicals that can be derived from biomass in 2004 and was reselected for the 2010 revised list (Werpy and Petersen, 2004; Luo, van der Voet and Huppes, 2010; J. Cheng *et al.*, 2012; Salvachúa *et al.*, 2016).

Succinic acid has the potential to replace industrially relevant chemicals such as benzene-derived chemicals, maleic anhydride and tetrahydrofuran (Pandey *et al.*, 2015). Succinic acid is also a building block for 1,4-butanediol (BDO), γ-butyrolactone (GBL) and polybutylene succinates (Okino *et al.*, 2008; Lin *et al.,* 2012; Cheng *et al.*, 2012; Orjuela *et al.*, 2013).
Succinic acid has several existing industries or markets. In the first market, it is used as solvent, detergent extender, foaming agent, surfactant and additive. In the second market, it is used as ion chelator to prevent pitting and corrosion in the plating and metals industry (Akhtar, Idris and Abd. Aziz, 2014; Pandey *et al.,* 2015). The third market is the food industry where it is used as flavouring additive and agent, pH regulator, antimicrobial agent and acidulate (Akhtar, Idris and Abd. Aziz, 2014; Pandey *et al.,* 2015). The fourth defined market is the pharmaceutical industry (Akhtar, Idris and Abd. Aziz, 2014). It is also widely recognised in the polymer and agricultural industry (Lin *et al.,* 2012).

Polybutylene succinate (PBS) can be used to manufacture biodegradable plastic, further increasing the interest in succinic acid bioproduction (van Heeden and Nicol, 2013a). Companies such as BASF-Purac, Bioamber, Reverdia, Mitshubishi-PPT and Myriant technologies are actively developing and implementing industrial and pilot scale plants (Van Heerden and Nicol, 2013).

Bio-amber has launched their plant for the production of 30 000 tonnes of succinic acid per year at the end of 2015. Bio-amber is the commercial leader for biobased succinic acid production and will consequently increase the awareness for the utilisation of this product. The study done by Tan *et al.* (2014) also states that the reduced cost and environmental benefit from succinic acid will change its status from a speciality to a commodity building block chemical. Table 2-2 shows the companies that produce succinic acid (adjusted from Tan *et al.,* (2014)).

Table 2-2: Commercial production of biobased succinic acid (adjusted from Tan *et al.,* 2014)

COMPANY	CAPACITY (tonnes per year)	LOCATION	OPERATIONAL DATE
GENERAL			
BASF-Purac JV	25 000	Barcelona, Spain	2013
Reverdia	10 000	Cassena, Italy	2012
Research Institute of Innovative Technology	50 000	-	2014 (Akhtar, Idris and Aziz 2014)
BIO-AMBER GROUP			
ARD	2 000	Pomace, France	2010
Mitsui & Co	30 000	Sarnia, Canada	2015
Bio-amber	70 000	North America	2018
MYRIANT GROUP			
Myriant	13 600	Louisiana, USA	2010
Uhde	500	Germany	2012
TOTAL	**201 100**	-	Date of study

From Table 2-2 it is concluded that the industrial presence of succinic acid is good, with a selling price range of 1 145 – 4995 US$.t^{-1} (Luo, van der Voet and Huppes, 2010; Vlysidis *et al.*, 2011).

2.1.2 Itaconic Acid

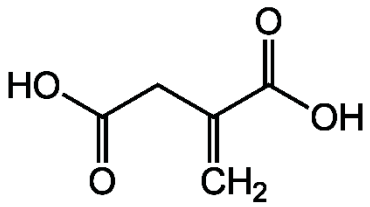

Figure 2-3: Itaconic acid structure

Itaconic acid is an unsaturated C_5 dicarboxylic acid ($C_5H_6O_4$) shown in Figure 2-3 with a white crystalline appearance (Klement and Büchs, 2013; Paranthaman, Kumaravel and Singaravadivel, 2014). Itaconic acid has a molecular weight of 130 g. mol^{-1}, it decomposes at 162-164˚C and has a density (specific gravity, SG) of 1.632. It was first discovered by Baup in

1836 as a product of pyrolytic distillation of citric acid (Okabe *et al.*, 2009; Weastra, 2011; Klement and Büchs, 2013). The biological production route was first reported by Kinoshita in 1931 using the fungal strain *Aspergillus itaconicus.* The cost difference between the chemical and biological production route of itaconic acid production makes the biological route the favoured option (Willke and Vorlop, 2001; Kuenz *et al.*, 2012). Since the 1960's, when the industrial production of itaconic acid started, the preferred fungal strain was *Aspergillus terreus* (Klement and Büchs, 2013; Steiger *et al.*, 2013; Huang *et al.*, 2014; Mondala, 2015). Itaconic acid can also be produced via genetically modified potatoes and switch grass, but little investigation has gone into this route due to numerous separation and purification challenges (Klement and Büchs, 2013).

Itaconic acid, together with succinic acid, were selected for the 2004 US DOE's list of the top 12 value-added chemicals that can be derived from biomass, but have since been removed from the subsequent list (Klement and Büchs, 2013). Itaconic acid is primarily used as a co-monomer for the production of styrene-butadiene rubber and acrylate latexes in the coating and paper industry. It is also used for the production of polymers, lubricant, surface active agents, paints, dye intermediates, resins, pesticides, synthetic rubbers, acrylic plastics, synthetic latex, detergent builders and chemical fibres (Okabe *et al.*, 2009; Weastra, 2011; Hu, 2012; Mondala, 2015).

The industrial production of itaconic acid was initiated by Pfizer Co. in 1955 using submerged fermentation (Okabe *et al.*, 2009). Since then numerous attempts have been made to optimise the production and reduce the economic cost. However, in 2011 it was reported that the industrial relevance of itaconic acid was low (Weastra, 2011). The annual production is estimated at 41 400 tonnes per annum, which is half (51.8%) of the global production capacity (Weastra, 2011). Most of the current production has moved from the USA, Japan and France to China, due to the lower manufacturing costs (Klement and Büchs, 2013). However, in 2005 China's production capacity led to overproduction of itaconic acid, causing a further decline in market price (Okabe *et al.*, 2009; Weastra, 2011).

As seen from Table 2-3, the largest producers are located in China, with only Zheijiang Guoguang Biochemistry reported to produce at nameplate capacity. There are 11 other companies that have been in production since the time range 1995 – 1999, mostly in China with two in the USA and one each in France and Japan (Okabe *et al.*, 2009).

Table 2-3: Commercial production of itaconic acid (adjusted from Weastra, 2011)

COMPANY	CAPACITY (tonnes per year)	PRODUCTION (tonnes per year)	LOCATION
Zhejiang Guoguang Biochemistry	10 000	10 000	China, since 1995
Qingdao Kehai Biochemistry	20 000	15 000	China, since 2000
Jinan Huaming Biochemistry	15 000	1 000	China
Alpha Chemika	8 000	Contract production	India
Others	27 000	7 400	-
TOTAL	**80 000**	**41 400**	

Although itaconic acid can be used in various markets, it is currently seen as a niche building block chemical with a limited current market, due to its high cost relative to fossil based equivalents (petro-chemicals) (Weastra, 2011). However, the production demand of itaconic acid is expected to grow once the price reduces and the potential market and range of applications increase. Another drive for the production of itaconic acid is the drive towards more sustainable product use by the customer, and the need for environmental conservation (Okabe *et al.*, 2009).

If itaconic acid succeeds in replacing its fossil-based equivalent and causes an expansion in the range of applications, the market will grow a 100-fold. It is estimated that such a future potential market will be 31% for detergent builders, 15% for unsaturated polyester resins, 26% for super absorbent polymers and 27% for thermoplastics. These forecasts are shown in Figure 2-4. One example for the thermoplastic market potential is the replacement of methyl methacrylate ($C_5H_8O_2$), which is used for the production of resins, polymers and plastics (Weastra, 2011).

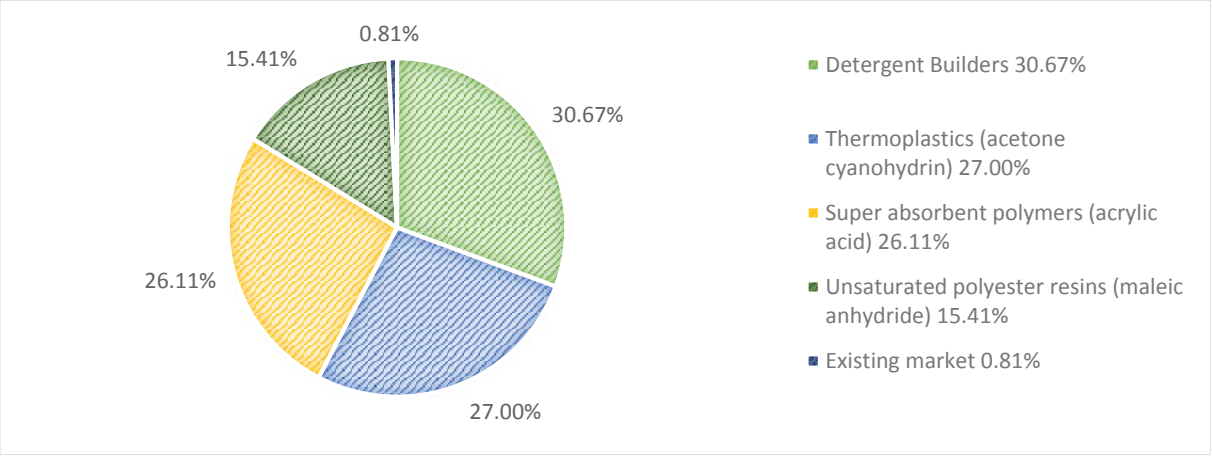

Figure 2-4: Itaconic acid potential future market (redrawn from Weastra, 2011)

Novel applications for itaconic acid in polymers, pharmaceuticals and agriculture are currently being investigated by Lucite International, DSM and Itaconix with the aim of expanding and developing the end use applications for itaconic acid.

No indication could be found of itaconic acid being produced in South Africa, and therefore it is assumed that itaconic acid have not been considered locally for use within the different potential markets, and that the petro-chemical equivalents are currently used instead. Thus, there is potential to replace itaconic acid's chemical equivalents in South Africa. The company *ISEGEN* produces maleic anhydride ($C_4H_2O_3$) and *Atlantic Trading Enterprise Pty Ltd* produces methyl methacrylate monomer (MMA), which are both fossil-based equivalents for itaconic acid. This indicates that there will be a market demand for itaconic acid once it becomes economically viable, and is accepted by the industry as a green bio-based chemical, suitable to replace the fossil-based chemical equivalents. The current price for itaconic acid varies between 1800 and 2000 US$.t^{-1} (Mondala, 2015; Weastra, 2011).

2.1.3 Polyhydroxyalkanoates

Polyhydroxyalkanoates (PHA's) are produced by bacterial fermentation. PHA's were first discovered in 1926 and are not excreted, but accumulated intracellularly in the microorganism's cytoplasm as energy and carbon reserve, and can reach up to 80-90 % of the cell dry weight (CDW) (Sudesh, Abe and Doi, 2000; Suriyamongkol *et al.*, 2007; Verlinden *et al.*, 2007; Tripathi *et al.*, 2012; Ramos *et al.*, 2015).

A large number of microorganisms (approximately 150) can be utilised for PHA production. Verlinden *et al.* (2007) tabulated more than 20 strains for which investigations have been reported. These strains can be classified into two groups based on their PHA production characteristics. The microorganisms in the first group produce PHA when they experience oxygen or nutrient stress such as nitrogen, phosphate, magnesium or oxygen limitations in the presence of an excess carbon substrate (Sudesh, Abe and Doi, 2000; Reddy *et al.*, 2003; Silva *et al.*, 2004; Suriyamongkol *et al.*, 2007; Verlinden *et al.*, 2007; Tripathi *et al.*, 2012). The second group of microorganisms can produce PHA during the growth and stationary phase. PHA can also be synthesized at low yields <10% (w/w) in transgenic plants and crops (Verlinden *et al.*, 2007).

Polyhydroxyalkanoates (PHA's) are water insoluble, crystalline, optically active, isotactic, non-toxic piezoelectric polyesters of various hydroxyalkanoates (Reddy *et al.*, 2003). PHA's

are thermoplastics and are regarded as the bio-based equivalent of polypropylene (Lopes *et al.*, 2014). It is also very similar to polyethylene (Verlinden *et al.*, 2007). Although it can replace fossil-based plastics, industrial application has been limited due to the high production cost of PHA's (Khanna and Strivastava, 2004). However, with the increased consumer awareness for bio-based products utilisation, PHA's has potential for industrial application (Verlinden *et al.,* 2007).

PHA structures are uncontrollable, due to various metabolic activities, which results in an inconsistent and extensive range of properties (Reddy *et al.*, 2003; Pandey *et al.*, 2015). This is remedied through metabolic engineering approaches, synthetic biology procedures or chemical grafting (Sudesh, Abe and Doi, 2000). The general chemical structure of PHA's are shown in Figure 2-5. The pendant [R] group can vary from methyl (C_1) to tridecyl (C_{13}) and is dependent on the specific substrate (Verlinden *et al.*, 2007).

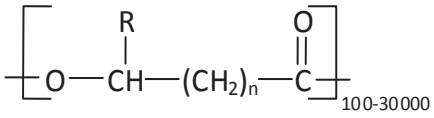

Figure 2-5: The general structure of polyhydroxyalkanoates (adapted from Reddy *et al.,* 2003)

The plastic properties, such as elasticity and crystallinity, depend on the chain lengths and specific type of PHA (Reddy *et al.*, 2003). PHA's can be classified according to monomer- and microstructures. According to monomer structures, PHAs are classified via chain length. Short side chain lengths consist of monomers of 3-5 (C_3-C_5) carbon chain lengths and medium side chain lengths consist of monomers 6-14 (C_6-C_{14}) chain lengths (Khanna and Strivastava, 2004; Pandey *et al.,* 2015). Short chain length (SCL-PHA) polymers are stiff, brittle and more crystalline, and include PHA's such as poly-lactide, PHB (poly-3-hydroxybutyrate) and PHV (poly-3-hydroxyvalerate). Middle side length (MCL-PHA) polymers are semi-crystalline thermoplastic elastomers, which can be used as biodegradable rubbers when cross-linked. According to the microstructures, PHA's can be classified as graft polymers, copolymers, block copolymers or homo-polymers, and possess over 150 monomer variations (Lopes *et al.*, 2014; Wang, Yin and Chen, 2014).

The differences in monomer structures and microstructures mean there are endless possibilities for manipulating PHA structures and properties (Pandey *et al.,* 2015). However, to date only a PHA copolymer of 3-hydroxybutyrate and 3-hydroxyhexanoate (P3HB & HHx) has been successfully produced in sufficient quantities for application research. P3HB, also written as

PHB, is the first discovered, best described and the most widely produced PHA (Kapritchkoff *et al.*, 2006; Suriyamongkol *et al.*, 2007; Khanna and Srivastava, 2004; Pandey *et al.,* 2015). PHA's are used for various applications: biodegradable plastics, paints, printing, packaging, commodity plastics (e.g. razors, diapers, and cosmetic containers), biofuels, biomedical equipment and implants. The chiral intermediates of PHA's are also used as carriers in medical or fine chemical applications such as pesticides (Reddy *et al.*, 2003; Chen, 2009; Pandey *et al.,* 2015). In the biomedical field, PHA's are used as surgical sutures (i.e. structures that hold body tissue together), blood vessel replacements and bone growth stimulation, due to its piezoelectric properties.

PHA production started in 1970 (Verlinden *et al.*, 2007; Pandey *et al.,* 2015), when Imperial Chemical Industries (ICI) developed Biopol PHA, and was the first company that commercially produced PHBV using *Ralstonia eutropha* (Pascault *et al.,* 2012, Lopes *et al.,* 2014). Since then, companies such as Monsanto, Metabolix and Archer Daniels Midland (ADM) manufactured PHA's. From 1995, Chinese companies started to exploit the overcapacity of the bioconversion (fermentation) market. Pandey *et al.* (2015) lists 24 companies worldwide that are involved in PHA research and are producing various types of PHA's. The production scales vary from pilot scale, to 100 tonnes per annum and up to a maximum of 50 000 tonnes per annum by ADM in the USA. The two most recent companies to produce PHA's are Qingdae V Land and Shandong Baisheng, both located in China and operating on a pilot scale, from 2012 to the present time.

PHA's have a good industrial presence, but commercial application is challenged by high production costs. The cost of the final product is influenced by several factors: the operational costs of the feedstocks (raw materials), chemicals and energy required, as well as the capital expenditure (related to equipment size) and the technological robustness (how new a piece of technology or equipment is). These factors are grouped into the feedstock (substrate) and biorefinery process design, of which the substrate cost influences PHA cost the most (Lopes *et al.,* 2014, Silva *et al.,* 2014). The price of the technology and operation thereof must either be decreased, or a high value PHA should be selected for a high-end market, such as high purity PHB for biomedical applications.

For these products to be considered as part of the green economy, they should be environmentally friendly and sustainable. This is required for both the manufacturing process and the feedstock selected.

2.2 Feedstock considerations

Bagasse is produced as a waste or by-product of sugar cane processing (Mashoko, Mbohwa and Thomas, 2013). For every 100 tonnes of sugarcane harvested, 28-30 tonnes of bagasse is produced, which is then used to produce steam and electricity for the sugar mill (Mbohwa, 2013). However, it is possible to increase the sugar mill energy efficiency (Mbohwa, 2013; Ali Mandegari, Farzad and Görgens, 2017), so that excess bagasse is available to use in a biorefinery. This is done by increasing the sugar mill process efficiency, and/or operating the boiler at a higher pressure (Mashoko, Mbohwa and Thomas, 2013). Therefore, a typical South African sugar mill that is fed at 300 $t.hr^{-1}$ sugarcane for 8 to 9 months of the year, and is equipped with a high pressure boiler (63 atm), can have 45 $t.hr^{-1}$ dry matter (DM) excess bagasse (Ali Mandegari, Farzad and Görgens, 2017).

Furthermore, the sugarcane green harvesting method, where the leaves and tops are left in the field rather than burned, allows for an additional 45 $t.hr^{-1}$ DM trash, of which 25 $t.hr^{-1}$ DM trash (green tops) must remain in the field, and 20 $t.hr^{-1}$ DM trash (brown leaves only) is available for use (Ali Mandegari, Farzad and Görgens, 2017). To this end, 65 $t.hr^{-1}$ DM bagasse and trash is available for use as a lignocellulosic feedstock for the production of bio-based chemicals (Tan *et al.*, 2014; Ali Mandegari, Farzad and Görgens, 2017).

Lignocellulose is composed of three major fractions: cellulose, hemicellulose and lignin (Gao *et al.*, 2013; Benjamin, 2014). Cellulose and hemicellulose are polysaccharides that can be hydrolysed to hexose and pentose mono-sugars, such as D-glucose, xylose, arabinose, mannose and galactose (Wyman, 2013). Lignin is a non-carbohydrate fraction of the lignocellulose. Although these fractions vary between the different species of sugarcane, with 66.6 – 77.6 %wt DM for carbohydrates and 14.4 – 23.1 %wt DM for lignin (Benjamin, 2014), the composition for the 45 $t.hr^{-1}$ bagasse and 20 $t.hr^{-1}$ DM trash is reported as 40.7% cellulose, 27.1% hemicellulose (67.8% carbohydrates), 21.9% lignin, 6.7% extractives and 3.5% ash (Ali Mandegari, Farzad and Görgens, 2017). The lignocellulosic bagasse and trash can be processed via pretreatment and hydrolysis, to convert the cellulose into hexose sugars (glucose and cellobiose), and the hemicellulose into pentose sugars (xylose, mannose, arabinose). These can then be used to produce bio-based products in the new products plant (NPP).

Pretreatment modifies the crystalline cellulose structure, and its close association with hemicellulose and lignin in the plant cell wall, to such an extent that the polymer chains of

cellulose and hemicellulose become accessible to enzymes in the subsequent hydrolysis step. This increased enzymatic digestibility results in improved efficiency for a higher sugar yield (Chandel *et al.*, 2012; Neto *et al.*, 2013; Akhtar, Idris and Abd. Aziz, 2014; Benjamin, 2014; Nanda *et al.*, 2014), as shown in Figure 2-6.

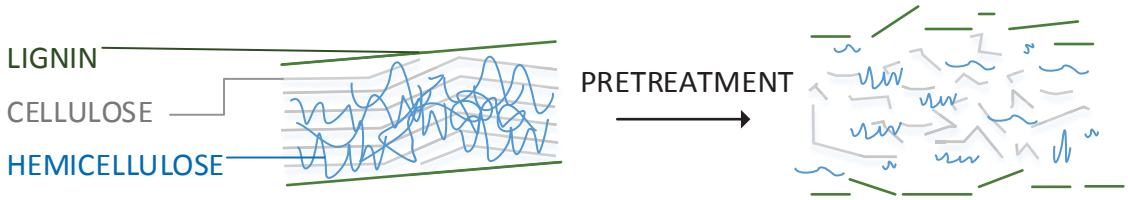

Figure 2-6: Pretreatment of lignocellulose (redrawn from Benjamin, 2014)

Figure 2-6 shows how the crystalline structure is disturbed by dilute acid pretreatment, where the hemicellulose fraction is solubilised during pretreatment, while the cellulose and lignin remain in the solid phase. The solid phase is referred to as cellulignin (Benjamin, 2014).

2.3 New products plant design considerations

The NPP has four (4) major process steps: pretreatment, hydrolysis, fermentation and downstream process recovery. Each of these process steps are discussed below.

2.3.1 Pretreatment and Enzymatic Hydrolysis overview

The lignocellulose pretreatment and enzymatic hydrolysis are considered together, since the pretreatment method impacts the enzymatic digestibility, and thus the sugar yields and hydrolysis efficiency.

Although acid hydrolysis can replace both process steps (i.e. pretreatment and enzymatic hydrolysis), the disadvantages of this method include a high amount of fermentation inhibitors produced and corrosion of equipment (Benjamin, 2014). Fermentation inhibitors lead to sub-optimal fermentation performance and thus process economics, and require additional process equipment units to remove them from the fermentation feed stream, further increasing process equipment costs.

To avoid these disadvantages, a combined pretreatment and enzymatic hydrolysis step is preferred. The enzymatic hydrolysis process is selective, produces no inhibitors, and results in higher glucose yields (Benjamin, 2014). Furthermore, it is also possible to combine the

enzymatic hydrolysis and fermentation process steps, known as simultaneous saccharification and co-fermentation (SSCF), shown in Figure 2-7 below.

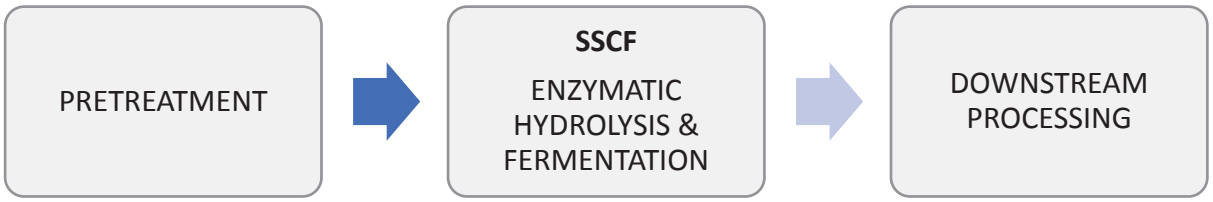

Figure 2-7: New products plant process diagram (SSCF scheme)

However, the type of microorganism will determine if this scheme is possible or not, due to potentially different process conditions required for enzymatic hydrolysis and by-product fermentation.

2.3.1.1 Enzymatic hydrolysis

The type of enzyme used during enzymatic hydrolysis of cellulose, is called cellulase, which releases glucose by cleaving the β-1-4-glucosydic bonds (Benjamin, 2014). There are three different categories of cellulases: endoglucanase, exoglucanase and β-glucosidase. These enzymes all target different regions of the cellulose. Endoglucanase reduces the degree of polymerisation, exoglucanase enables the release of cellobiose and β-glucosidase enables the release of glucose from cellulose (Benjamin, 2014). Enzymatic hydrolysis can be optimised by using a blend of enzymes (Akhtar, Idris and Abd. Aziz, 2014), which include cellulases, hemicellulases and oxidative enzymes (Benjamin, 2014), to hydrolyse the cellulose and hemicellulose polysaccharides in the lignocelluloses. Enzymatic hydrolysis efficiency depends on various factors, such as the type of polysaccharide, solids loading, enzyme dosage, inhibitors present, temperature, residence time, and pH (Humbird, 2011; Akhtar, Idris and Abd. Aziz, 2014).

For SHF the enzymatic hydrolysis temperatures and residence times vary from 48 – 50 ˚C and 72 - 84 hours, respectively (Humbird, 2011; Diedericks, Van Rensburg and Görgens, 2012; Harrison *et al.*, 2013; Benjamin, 2014). The enzyme dosage is measured in filter paper units (FPU) per gram of cellulose treated. Experimental dosages vary from 2.5 to 60 FPU/g, but a low dosage in the range of 10 – 20 $FPU.g^{-1}$ is preferred from an economic viewpoint (Benjamin, 2014). For an economic analysis, the cost of enzymes are expressed per mg enzyme protein added. The FPU relates to the amount of enzyme protein, depending on the assay procedure followed to determine the enzyme activity.

The enzyme blend of cellulase, β-glucosidase and xylanase, together with the enzyme dosage, should be optimised for the substrate and pretreatment method used (Novozymes, 2010). A high enzyme dosage results in a reduced residence time, which mean smaller process equipment is required. Therefore, a high enzyme dosage will have an advantageous impact on the capital cost (Novozymes AS, 2010), but the increased cost of a high enzyme dosage will have a detrimental impact on the operational cost (Novozymes AS, 2010). Benjamin (2014) used 32.31 mg enzyme protein per gram cellulignin, but the dosage recommended by Novozymes for their cellulosic ethanol enzyme kit, ranges from 5 mg to 25 mg enzyme protein per g cellulignin (Novozymes AS, 2010). The enzyme dosage used in the 2011 NREL report is 20 mg protein per g DM (Humbird *et al.,* 2011). If the enzyme dosage is reduced, the same results for enzymatic hydrolysis can be obtained, but it will require a longer hydrolysis residence time (Novozymes AS, 2010).

The solids loading has an impact on the conversion efficiency and capital cost required. The conversion efficiency is favoured by a low solids loading (i.e. 5 %wt). However, a low solids loading causes a diluted product stream, which requires larger process equipment (e.g. heat exchangers and hydrolysis reactors), and substantial downstream costs for concentration of sugars before bioconversion. This will increase operational and capital costs. Alternatively, a higher solids loading (i.e. 20 %wt) will favour the economic impact, and the process design should be such that the conversion efficiency is not affected by mass and heat transfer effects due to stirring limitations and product inhibition (high glucose concentrations) (Du *et al.*, 2014).

A potential pretreatment technology is the use of a twin screw reactor. Duque *et al.* (2014) reported combined alkali and enzymatic extrusion to pretreat barley straw. Subsequent enzymatic hydrolysis could then be carried out at a high solids loading of 30% (w/v) producing 32 g glucose (96 g/L) and 18 g xylose (52 g/L) per 100 g extruded material, resulting in 50 g combined sugar yield per 100 g DM. This is comparable to AFEX™ and enzymatic hydrolysis (SHF) pretreatment of sugarcane lignocelluloses with 53.7 g combined sugars per 100 g DM. The extruded material has been subject to physical disruption due to the shearing forces as well as mixing mechanism of the enzymes with the biomass during the extrusion and prior to subsequent incubation for enzymatic hydrolysis (Daque *et al.*, 2014).

Cellulase, including endoglucanases, exo-glucanases and β-glucosidase, can be produced on an industrial scale by using fungi such as *Trichoderma reesei* (Humbird *et al.,* 2011). The

National Renewable Energy Laboratory (NREL) reports are widely used and referred to for techno-economic studies, due to the extent of research and industry involvement on the studies. The 1999 NREL report includes an on-site enzyme production plant in the model discussed, which used a fraction of the hydrolysate stream as feedstock (Humbird *et al.*, 2011). The 2002 design report did not include an on-site production plant, but rather included a 'purchased-enzyme model' where the enzyme cost was calculated as a fixed production cost per unit of ethanol produced (Humbird *et al.*, 2011).

However, for the 2011 design report, an on-site enzyme plant is included to increase the transparency of determining the cost of enzymes for a large scale biorefinery. Since the cellulase titre is increased when glucose is used as feedstock, the capital and utility costs are lower, even though the feedstock cost is higher (Humbird *et al.*, 2011). The flow diagram of the enzyme production plant is shown in Figure 2-8. Ten %(wt) of the feed stream is sent to the seed train for *T. reesei* growth, and the rest is sent to the fermentation tanks for cellulase production.

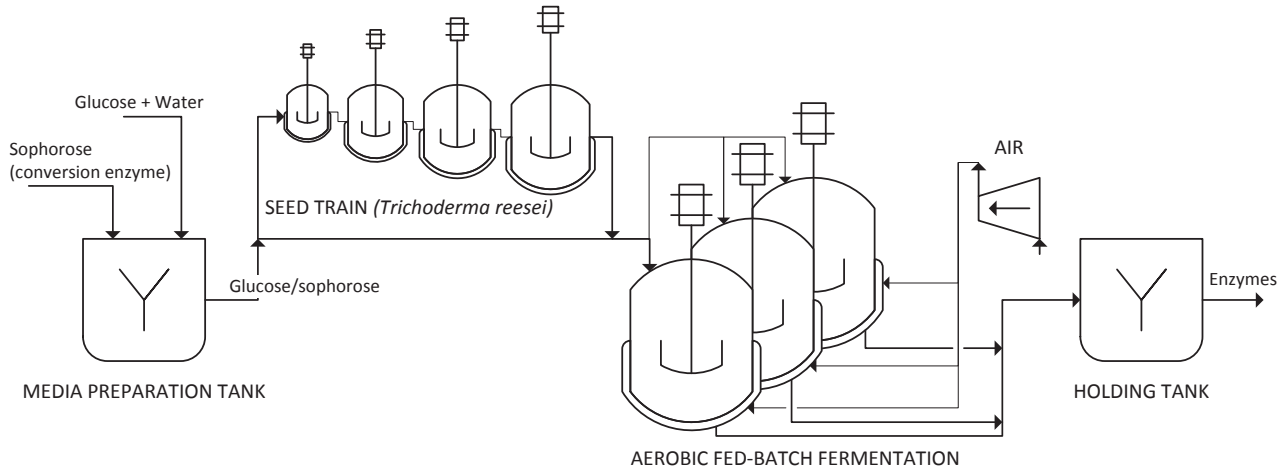

Figure 2-8: Flow diagram of the enzyme production plant (redrawn from Humbird *et al.* 2011)

2.3.1.2 Pretreatment methods available

Different pretreatment methods are available for sugarcane bagasse and trash, and include chemical-, physicochemical-, hydrothermal- or a combination of these methods (Carvalheiro, Duarte and Gírio, 2008). Physical pretreatment methods (thermal treatment, radiation and size reduction) and biological pretreatment methods (white rot fungi) are excluded from the table, since they cannot compete with the process efficiency, time efficiency, selectivity and cost of chemical pretreatment methods (Wyman, 2013). The chemical pretreatment methods, i.e.

chemical, physicochemical and hydrothermal pretreatment steps, are summarised in Table 2-4.

Table 2-4: Pretreatment methods (Nanda *et al.*, 2014, Tan *et al.*, 2014, Su *et al.*, 2015)

PRETREATMENT STEPS	METHODS FOR SUGARCANE LIGNOCELLULOSE
Chemical	Alkaline hydrolysis (NaOH, Na_2SO_3, NH_4OH and H_2O_2) Acidic hydrolysis (HCl, H_2SO_4, H_3PO_3, HCl & HNO_3) Solvent based (Ionic liquid and Organosolv)
Physicochemical	Ammonia fibre expansion (AFEX™) Ozonolysis Steam explosion
Hydrothermal	Carbon dioxide explosion (Supercritical CO_2) Liquid hot water (LHW) Wet oxidation (WO)

The pretreatment method will determine how the lignocellulose is disrupted and the type and quantity of by-products produced, such as weak acids, furan derivatives and phenolics (Carvalheiro, Duarte and Gírio, 2008; Silva *et al.*, 2014). The desired end-product of the lignocellulose will determine which fraction the pretreatment should target, since a trade-off usually exists between cellulose, hemicellulose or lignin recovery and degradation.

Only some of the available pretreatment methods can be applied on an industrial scale due to environmental and economic considerations (Akhtar, Idris and Abd. Aziz, 2014). The advantages and disadvantages for the major pretreatment methods reported for sugarcane bagasse are compared in Table 2-5 for an optimised combined sugar yield. Each advantage is indicated by a green check mark and awarded 1 point. Each disadvantage is indicated by a red cross and awarded -1 points. A mild influence receives a 0. All the considerations receive equal weighting. For example, if the temperature is below 200 °C it receives a 0 (mild influence), and above 200 °C receives an -1 (disadvantageous influence).

From the grading system, it can be seen that alkaline and acid hydrolysis methods (chemical pretreatment methods) have the highest score of three. Acid and alkaline pretreatment methods are followed by the Ammonia Fibre Expansion (AFEX™), Ionic liquid solvent pretreatment and Steam explosion hydrothermal pretreatment methods at a score of two. The solvent based pretreatment, Organosolv, is a good pretreatment method to choose when lignin recovery is important (e.g. to sell lignin as a product or use it as an intermediate feedstock to produce a high-value lignin-based product), although it receives a low score. The pretreatment method with the lowest score is supercritical carbon dioxide (CO_2), which has not been widely

investigated thus far and therefore little information is available on the sugar yield, degradation and enzymatic digestibility on sugarcane bagasse (Nanda *et al.*, 2014).

Table 2-5: Advantages and disadvantages of different pretreatment methods (Wyman, 2013)

STEP 2 PRETREATMENTS Advantage Disadvantage Mild influence	High sugar yield	Little/ no inhibitor production	High hemicellulose recovery/yields	Cost effective	Low temperature	Low pressure	High enzymatic digestibility	Low residence time (<30min)	Literature specific for sugarcane	Low sugar yield	Produces inhibitors	Low hemicellulose recovery e.g.	Expensive	High temperature (>200?)	High pressure	High enzymatic hydrolysis load	Neutralisation/detoxification required	Wyman 2013
PHYSIOCOCHEMICAL																		
AFEX	✓	✓					✓	✓	✓			!	✗	!	✗	✗		**2**
CHEMICAL																		
Alkaline Hydrolysis	✓	✓			✓	✓	✓		✓	!		✗	✗	!			✗	**3**
Acid Hydrolysis	✓		✓	✓		✓	✓		✓		✗		✗	!	!		✗	**3**
Solvent: Organosolv					!	✓	✓		✓		✗	✗	✗	!	!		✗	**-1**
Solvent: Ionic Liquid*	✓	✓					✓	✓	✓		✗		✗	!			✗	**2**
HYDROTHERMAL																		
Steam explosion	✓	✓		✓			✓	✓	✓		✗	✗		✗	✗			**2**
Supercritical CO_2		✓											✗	✗	✗			**-2**

*does not produce inhibitors, but the solvent itself is toxic for DSP

In mild alkaline pretreatment, the bonds between lignin and hemicellulose are cleaved, the cellulose swells, and the hemicellulose is solubilised. High severity alkaline treatment will also solubilise lignin. By removing some or all of the hemicellulose and lignin, the process exposes the cellulose, which makes the biomass more accessible for enzymatic hydrolysis (Wyman, 2013). Compared to acid pretreatment, alkaline pretreatment is a less aggressive process: the capital costs are lower (no corrosion resistance required) and the control of the operating conditions is less critical (Wyman, 2013). However, the major disadvantages of alkaline and AFEX™ pretreatment methods are the high capital costs, and the fact that additional downstream conditioning is required due to solubilised lignin and hemicellulose before it can be used in the boiler in the CHP plant.

Acid pretreatment solubilises the hemicellulose fraction into a liquid hydrolysate fraction, which exposes the cellulose fraction. The cellulose and lignin (cellulignin) fraction remains in the solid fraction (Benjamin, 2014). The cellulignin's crystalline structure is weak and porous, which causes increased cellulose surface area, and allows access to the cellulose for enzymatic

hydrolysis (Benjamin, 2014). The preferred pretreatment method used for sugarcane bagasse, is steam explosion (STEX) and dilute acid treatment (DAT) (Mesa *et al.*, 2010; Wyman, 2013). In Humbird *et al.* (2011), these two pretreatment methods are combined in a dilute acid steam explosion pretreatment, since they are chemically similar.

2.3.2 Dilute acid treatment

Dilute acid treatment (DAT) is widely researched and has potential for commercial application (Benjamin, 2014). The pretreatment conditions can be varied to increase the enzymatic digestibility of the pretreated lignocellulose. The process conditions required for high enzymatic digestibility are more severe and cause more biomass degradation. The severity factor (R˚) can be calculated from the temperature, residence time and pH used (Benjamin, 2014), as shown in Equation E2-1.

$$\log(R^0) = \log\left[t.exp\left(\frac{T-T_b}{14.75}\right)\right] - pH \qquad \text{[E2-1 Wyman 2013]}$$

Where (T) is the treatment temperature, (T_b) is the reference temperature of 100˚C, (t) is the residence time and (pH) is the pH of the final mixture, which is a function of the acid concentration used. Table 2-6 shows reported DAT pretreatment conditions and severity (acid concentration, ratio of solid biomass to dilute acid solution, residence time and temperature) for sugarcane bagasse pretreatment.

Table 2-6: Severity Factor reported for decreasing acid strength (%) at different solid to liquid ratios (w/v)

ACID	RATIO	TIME & TEMPERATURE	SEVERITY FACTOR log(R˚)	REFERENCES
1% H_2SO_4	1:2 w/v[a]	40 min, 121 ˚C	1.5	Borges and Pereira, 2011
2% H_2SO_4	1:5 w/v	150 min, 121 ˚C	2.4	Liu *et al.,* 2013b
2% H_2SO_4	1:5 w/v	150 min, 125 ˚C	2.5	Xi *et al.,* 2013
2% H_2SO_4	1:10 w/v	150 min, 121 ˚C	2.4	Liang *et al.,* 2013
0.5% H_2SO_4	1:2 w/w	15 min, 165 ˚C	2.1	Koekemoer, 2018
0.75% H_2SO_4	1:10 w/v[b]	120 min, 115 ˚C	1.7	Yu and Stahl, 2008
0.65% H_2SO_4	1:20 w/v[c]	10 min, 180 ˚C	2.5	Benjamin, 2014

[a] solid: liquid ratio; [b] 1g per 10mL; [c] 1.5g in 30mL = 1g per 20mL

The higher the severity factor, the higher the enzymatic digestibility of the cellulose, and the higher the degree of biomass degradation. However, the severity factor does not take the solids loading into account. This is evident from the severity factor of 2.4 for both Liang *et al.* (2013) and Liu *et al.* (2013), for the same residence time, temperature and pH, but different solid loadings (10% and 20%, respectively). The impact of a higher solids loading and scale-up on the process performance (i.e. yield) should be measured and could be mitigated with dedicated

reactor design. It should be designed to prevent mass transfer limitations between the acid catalyst and the lignocellulosic biomass.

Xi *et al.* (2013) and Benjamin (2014) both have a severity factor of 2.5, and should provide good enzymatic digestibility. Koekemoer (2017), Yu and Stahl (2008) and Borges and Pereira (2011) have severity factors of 2.1, 1.7 and 1.5 respectively, and will result in lower enzymatic digestibility. This trend is evident in the glucose yield from enzymatic hydrolysis, of 0.66 and 0.76 g glucose per g cellulose, for Koekemoer (2017) and Benjamin (2014) respectively.
More severe DAT's also result in higher hemicellulose degradation into by-products, which are inhibitory to the fermentation process (Benjamin, 2014). High temperatures will cause the rate of reaction of xylose into degradation products (k_2) to exceed the rate of reaction of xylan into xylose (k_1).

$$\boldsymbol{Xylan_s \rightarrow (k_1) \rightarrow Xylose_{aq} \rightarrow (k_2) \rightarrow Degradation\ products}$$

[E 2-1 Benjamin 2014]

$$Cellulose \rightarrow (k_1) \rightarrow Glucose \rightarrow (k_2) \rightarrow Decomposition\ products\ (HMF)$$

Note: High temperatures: (k_2) >> (k_1) [E2-2 (Cardona, Quintero and Paz, 2010)]

Lignin degrades into phenolics, cellulose into HMF, xylan (hemicellulose) into oligosaccharides and furan derivatives and these degradation products are all considered by-products. They can be categorised as (Benjamin, 2014):

i. Aliphatic acids (levulinic acid, formic acid, acetic acid)
ii. Phenolic compounds
iii. Furan derivatives (furfural and 5-hydroxymethylfurfural (HMF))

For a DAT with a severity factor of 2.4%, 6.4% acetic acid, 1.3% furfural and less than 1% HMF are produced (Liang *et al.*, 2013; Liu *et al.*, 2013). The amount of levulinic acid, formic acid and phenolic compounds are negligible (Carvalheiro, Duarte and Gírio, 2008; Liang *et al.*, 2013; Liu *et al.*, 2013). If these by-products are detrimental to the fermentation step, an additional detoxification step is required to remove the inhibitors.

Alternatively, the microorganisms can be genetically engineered to be resistant against inhibitors, in which case no detoxification may be required, resulting in lower capital and/or operational costs.

2.3.3 Detoxification

By-products, such as acetic acid, furfural and HMF, can be removed through the process of detoxification.

Detoxification steps include

i. Alkaline detoxification (over-liming with $Ca(OH)_2$),
ii. Electrodialysis,
iii. Evaporation
iv. Ion-exchange resin,
v. Adsorption (activated carbon) and
vi. Enzymatic detoxification (Hodge *et al.*, 2009; Cardona, Quintero and Paz, 2010).

For hydrolysate (pentose rich stream after pretreatment) detoxification, ion-exchange is used with over-liming to remove acetic acid, furans (45.8 %) and phenolics (35.9 %) (Wooley *et al.*, 1999; Cardona, Quintero and Paz, 2010). However, in over-liming, the reagent cannot be reused, which results in sugar losses of up to 13% and does not significantly alter the acetic acid concentration (Cardona, Quintero and Paz, 2010; Humbird, 2011). Electrodialysis together with evaporation removes acetic acid and furfural with low sugar losses (Cardona, Quintero and Paz, 2010).

Electrodialysis also allows the reuse of the dilute acid stream, which will decrease operational costs, but has a high initial capital cost (Cardona, Quintero and Paz, 2010). Ion-exchange resins can remove 63.4%, 85.2% and 75.8%, respectively, of all furans, acetic acid and phenolics present in the sugarcane bagasse hydrolysate (Cardona, Quintero and Paz, 2010). Adsorption using activated carbon also targets these inhibitors and removes more HMF and phenolics (57%) than over-liming (Hodge *et al.*, 2009; Cardona, Quintero and Paz, 2010).

The amount of granular activated carbon (GAC) used is 2% (w/v) at 30 - 50˚C for 120 min (Liu *et al.*, 2013; Xi, Dai *et al.*, 2013) and for PHA detoxification the AC weight to volume ratio of 20% w/v at 30 ˚C for 180 min is recorded (Silva *et al.*, 2004). Enzymatic detoxification utilises enzymes such as laccase to remove phenolic compounds (77.5%), but does not remove furans or acetic acid (Cardona, Quintero and Paz, 2010).

2.3.4 Bioconversion (fermentation), Separation and Purification of product

The three major parameters that determine whether the production of a bioproduct is economically viable are all related to the fermentation process step (Tan *et al.*, 2014). However,

these are also highly dependent on the properties of the sugar stream generated from the pretreatment (and hydrolysis) sections.

The primary process performance characteristics are (Tan *et al.*, 2014):

i. product yield on sugar ($g.g^{-1}$),
ii. final product concentration (titre, $g.L^{-1}$) and the
iii. volumetric productivity ($g.L^{-1}.hr^{-1}$).

The *yield* relates to the cost of the feedstock and the *concentration* and *productivity* relate to the operating cost, fixed capital cost and total investment (Cheng *et al.*, 2012; Akhtar, Idris and Abd. Aziz, 2014; Tan *et al.*, 2014). The final product concentration or titre influences the cost of downstream processing for product recovery. By-product formation or selectivity also impacts the titre and yield, and is therefore also an important parameter to consider in selecting the fermentation strain when selectivity data is available (Cheng *et al.,* 2012). By-products include ethanol, acetate, malate, pyruvate, formate, nucleic acids and salts. These by-products and the constituents of the fermentation broth will affect the downstream processing.

The downstream process has three main process steps: the separation of microbial cells, the separation of impurities from the product, and the purification of the product (Cheng *et al.,* 2012). The fermentation and downstream production steps (separation and purification) will be discussed in detail under each product respectively since they are very specific to each product. The first process to be investigated is the production of succinic acid, followed by those of itaconic acid and PHA's.

2.4 Succinic Acid Production

Succinic acid production follows the general process overview: pretreatment, enzymatic hydrolysis, fermentation and downstream process steps.

2.4.1 Pretreatment and enzymatic hydrolysis

The synergy between pretreatment and enzymatic hydrolysis is important, because factors such as adsorption rates, substrate-, enzyme- and inhibitor concentration and surfactants, determine the degradation of the biomass (Akhtar, Idris and Abd. Aziz, 2014). Lignocellulosic biomass that is used for succinic acid production can be treated in separate hydrolysis and fermentation steps (SHF), as well as simultaneous saccharification and co-fermentation (SSCF) steps. SSCF that is performed under the right process conditions (i.e. temperature and pH) results in steady,

controlled release of sugar and simultaneous conversion of sugar into succinic acid (Akhtar, Idris and Abd. Aziz, 2014). This results in a reduced reaction time, lower energy consumption, reduced production and capital costs and increased productivity (Akhtar, Idris and Abd. Aziz, 2014; Tan *et al.*, 2014). Another advantage of SSCF, is that the enzymes and organisms are not inhibited by high initial sugar concentrations, since the microorganism utilises the sugar once it becomes available (Akhtar, Idris and Abd. Aziz, 2014). However, the information on succinic acid production from lignocellulosic biomass using SSCF is limited.

A succinic acid concentration of 83 $g.L^{-1}$ and yield of 0.87 $g.g^{-1}$ (Liang *et al.*, 2013) were obtained after DAT and enzymatic hydrolysis for separate hydrolysis and fermentation (SHF). The yield on available sugar is similar to when only DAT treatment is used, with no enzymatic hydrolysis, as seen in Table 2-7. Although the yields are similar, the titres vary. A titre of 83 $g.L^{-1}$ is achieved when enzymatic hydrolysis is included, which is much higher than the titres obtained (23.7, 15.7, 19.6 and 52 $g.L^{-1}$) without enzymatic hydrolysis (Borges and Pereira, 2011; Liang *et al.*, 2013; Liu *et al.*, 2013; Xi, Dai *et al.*, 2013).

Table 2-7: Succinic acid production from sugarcane bagasse (without enzymatic hydrolysis)

REFERENCES	Borges and Pereira, 2011	Liu *et al.*, 2013	Xi *et al.*, 2013
FEEDSTOCK	Sugarcane bagasse	Sugarcane bagasse	Sugarcane bagasse
ADDITIONAL PRETREATMENT	n/a	n/a	Milled (<1mm) bagasse ultrasound for 40min
DAT (H_2SO_4)	1% (v/v) 1:2 solid : liquid ratio 121˚C for 40min	2% (v/v) sulphuric acid 1:5 (w/v) 121˚C for 150min	2% 1:5 solid : liquid ratio (w/w) 125˚C for 150min
NEUTRALISATION	To pH 6 with $Ca(OH)_2$ Filtered to remove precipitate.	To pH 6 with $Ca(OH)_2$ at 50˚C Filtered to remove precipitate.	n/a
DETOXIFICATION Activated carbon	n/a	2% (w/v) at 50˚C for 120min. Filtered to remove carbon.	2% (w/v) at 30˚C. Hydrolysate was concentrated by vacuum evaporations to 30% of original volume.
SUGARS	52 $g.L^{-1}$	40.3 $g.L^{-1}$	153 $g.L^{-1}$.(dry kg of bagasse)$^{-1}$ Used 30 $g.L^{-1}$ non-detoxified Used 30 $g.L^{-1}$ detoxified
Xylose	52 $g.L^{-1}$	32.6 $g.L^{-1}$	126 $g.L^{-1}$
Glucose	-	3.3 $g.L^{-1}$	17.5 $g.L^{-1}$
Arabinose	-	2.9 $g.L^{-1}$	9.5 $g.L^{-1}$
Phenolic compounds	-	5.2 $g.L^{-1}$	-

RESULTS			
Non-detoxified			
Succinic acid (SA) titre, productivity and yield	22.5 $g.L^{-1}$ SA 1.01 $g.L^{-1}h^{-1}$ 0.62 $g.g^{-1}$ (55.4%)	- - -	23.7 $g.L^{-1}$ SA 0.99 $g.L^{-1}$ h^{-1} 0.87 $g.g^{-1}$ (77.3%)
Detoxified			
Succinic acid (SA) titre, productivity and yield	- - -	15.7 $g.L^{-1}$ SA n/a 0.84 $g.g^{-1}$ (75%)	19.6 $g.L^{-1}$ 0.82 $g.L^{-1}$ h^{-1} 0.73 $g.g^{-1}$ (65.7%)

2.4.2 Succinic acid fermentation

The most investigated succinic acid producers are *Anaerobiospirillum succiniciproducens, Actinobacillus succinogenes, Mannheimia succiniciproducens, Escherichia coli, Corynebacterium glutamicum, Saccharomyces cerevisiae, Bacteroides fragilis* and *Lactobacillus plantarum* (Cheng *et al.*, 2012; Brink and Nicol, 2014; Tan *et al.*, 2014; Pandey *et al.*, 2015).

Numerous researchers have investigated the genetic manipulation or genetic modification of bacterial strains for succinic acid production, striving to improve yield, productivity and robustness for industrial application. *A. succinogenes, E. coli* and *S. cerevisiae* are popular strains used for genetic modification (Beauprez, De Mey and Soetaert, 2010; Morales *et al.*, 2016). *C. glutamicum, S. cerevisiae, B. fragilis* and *L. plantarum* have recently been investigated for succinic acid production (Tan *et al.*, 2014). The majority of the strains are anaerobic, while some have the environmental benefit of using CO_2 during fermentation, contributing to the reduction of greenhouse gases.

The maximum theoretical yield of succinic acid from natural producers is 1.12 g of succinic acid per gram glucose (1.71 $mol.mol^{-1}$), if redox requirements are considered and biomass formation is ignored (van Heerden and Nicol, 2013; Brink and Nicol, 2014). Yields of 1 $g.g^{-1}$ are possible for some modified *E. coli* strains and 0.94 $g.g^{-1}$ for *A. succinogenes* (Brink and Nicol, 2014). However, the majority of experimental work was based on glucose and CO_2 as the feedstock for fermentation (Brink and Nicol, 2014).

This cannot be directly applied to the fermentation of pretreated sugarcane biomass, since the sugar mixture contains inhibitors such as weak acids, furans and phenolic compounds. These compounds negatively influence fermentation efficiency and product yield (Cheng *et al.*, 2012). The inhibitors can be removed by a detoxification process step, after which the information available for pure and mixed sugar fermentation can be applied to pretreated

sugarcane fermentation. Natural succinic acid producing organisms (*A. succinogenes, A. succiniciproducens and M. succiniciproducens*), excrete succinic acid anaerobically as a major catabolic product, starting with the phosphoenolpyruvate (PEP) carboxylation step. All of these organisms produce succinic acid through the reverse tricarboxylic acid cycle (TCA) (van Heerden and Nicol, 2013).

For the industrial production of succinic acid, some of the most important characteristics of the microorganism for the use of biomass as substrate, are (Okino *et al.*, 2008; Jiang *et al.*, 2013; Tan *et al.*, 2014; Salvachúa *et al.*, 2016; Pandey *et al.*, 2015):

i. resistance to inhibitors present in sugar streams obtained by pretreatment hydrolysis of lignocelluloses,
ii. acid tolerance,
iii. sugar utilisation (C_5 and C_6 sugars),
iv. inexpensive nutrient requirements and
v. high titre for simple downstream processing.

A. succinogenes has been identified as one of the most promising strains for industrial succinic acid production. It resists acetic acid, which is an inhibitor, up to 40 $g.L^{-1}$ but can only tolerate very low furfural concentrations (Xi, Chen *et al.*, 2013; Shen *et al.*, 2015). *A. succinogenes* also utilises a wide range of sugars such as glycerol, sucrose, maltose, lactose, fructose, arabinose, galactose, mannose and xylose as substrates (Song and Lee, 2006; Borges and Pereira, 2011; Shen *et al.*, 2015). *A. succinogenes* also produces the highest titre (105.8 $g.L^{-1}$) (Guettler, 1996; Beauprez, De Mey and Soetaert, 2010) recorded to date. A high titre is required to decrease the downstream process capacity (Orjuela *et al.*, 2011; Cheng *et al.*, 2012), which will reduce capital costs.

A. succinogenes has been genetically modified to improve its productivity obtained from sugarcane bagasse and trash hydrolysate fermentation. The productivities of succinic acid from hydrolysate (such as 0.84 $g^{-1}.L^{-1}.hr^{-1}$ and 1.01 $g^{-1}.L^{-1}.hr^{-1}$) are comparable to those achieved from pure glucose (such as 0.8 $g^{-1}.L^{-1}.hr^{-1}$, 1.01 $g^{-1}.L^{-1}.hr^{-1}$ and 2.31 $g^{-1}.L^{-1}.hr^{-1}$) (Cheng *et al.*, 2012; Jiang *et al.*, 2013; Liu *et al.*, 2013; Akhtar, Idris and Abd. Aziz, 2014). High volumetric productivities have been achieved using continuous fermentation with *A. succinogenes*. The filamentous nature of the microorganism causes a biofilm to form for continuous production (van Heerden and Nicol, 2013; Bradfield and Nicol, 2014). This biofilm has an enhanced

tolerance to toxic reactants and long term activity. It also causes the cells to self-immobilise, causing cell retention without a cell separation step. In this manner high volumetric productivities can be achieved at low capital expenditure for the bioreactor (van Heerden and Nicol, 2013; Maharaj, Bradfield and Nicol, 2014). However, the disadvantages include a higher risk of contamination due to the prolonged residence time, increased downtime, possible strain mutations and inflexible operating conditions (van Heerden and Nicol, 2013). Batch fermentation is popular due to its ease in handling and low risk of contamination (Tan *et al.,* 2014). Another proposed setup is the fed-batch fermentation reactor with slow feeding of medium. However, the feed sugar concentration must be monitored.

High initial sugar concentrations inhibit *A. succinogenes*. It is recommended to keep the sugar concentration below 100 $g.L^{-1}$ for *A. succinogenes*, so that a succinic acid product concentration of 120 $g.L^{-1}$ can be achieved to minimise downstream recovery costs (Lin *et al.,* 2008, Salvachúa *et al.*, 2016). Together with the feedstock, the microorganism also requires a nutrient medium.

Lin *et al.* (2012) investigated the various liquid mediums that are used in the fermentation step, which even includes the use of wheat derived media and seawater as a way to decrease production costs. However, these did not prove successful enough to replace a more complex nutrient medium, such as 10 $g.L^{-1}$ $NaHCO_3$, 3 $g.L^{-1}$ yeast extract, 5 $g.L^{-1}$ K_2HPO_4 and 2 $g.L^{-1}$ $MgSO_4$ (Borges and Pereira, 2011), since seawater may cause downstream processing difficulties that are not accounted for.

2.4.3 Downstream Recovery

The downstream process has a large influence on the capital cost of a biorefinery. The recovery of succinic acid from the fermentation broth can contribute 50% - 80% of the total cost of microbial production (Orjuela *et al.,* 2011, Cheng *et al.,* 2012). Process steps which contribute to an efficient separation train include (Kurzrock and Weuster-Botz, 2011; Orjuela *et al.*, 2011; Cheng *et al.*, 2012):

i. selective precipitation,
ii. crystallization,
iii. extraction with solvents and/or amines,
iv. ion-exchange,
v. adsorption,

vi. esterification,
vii. membrane separation (e.g. nano-filtration and electrodialysis) and
viii. *in situ* removal of succinic acid.

Morales *et al.* (2016) compared the economic feasibility and environmental sustainability of reactive extraction, electrodialysis and ion-exchange for succinic acid production. It was found that the process configurations with reactive extraction were the most environmentally sustainable (if low pH fermentation is used) and economically viable (if sugar resistant microbial strains are used) DSP configuration. This scheme is shown in Figure 2-9.

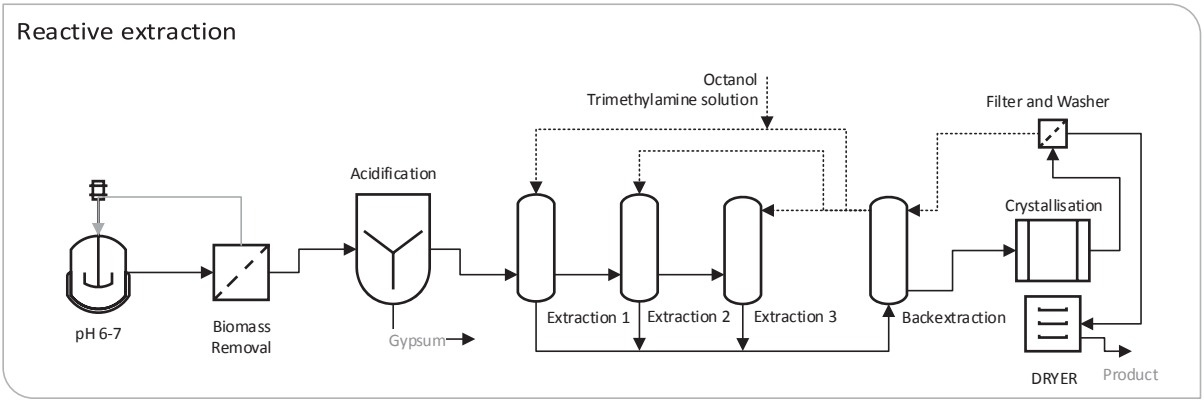

Figure 2-9: Reactive extraction process scheme for succinic acid downstream processing

A solvent is used to extract the succinic acid from the aqueous fermentation broth. The solvent is a mixture of 87% 1-octanol and 13% trioctylamine by weight. After three subsequent extraction columns, the succinic acid is back-extracted into the aqueous phase through a mixture of 25% trimethylamine and 75% water by weight. Some of the 1-octanol is lost into the aqueous phase, at 0.21% per extraction column.

The succinic acid recovered into the aqueous phase is then sent to a crystalliser at 20˚C. Here the succinic acid crystals are formed, washed and dried before the product is ready to be packaged and sold. The process has a very high succinic acid recovery rate of 99.7 %wt from the fermentation broth (Morales *et al.*, 2016). This is high when compared to the 95% recovery rate achieved by Kurzrock and Weuster-Botz (2011), who investigated the reactive extraction of succinic acid using 448 different amine-solvent mixtures. The yield from an *E. coli* fermentation broth was 78 – 85% due to the co-extraction of other organic acid by-products (lactic and acetic acid) as well as the ionic strength of the fermentation effluent (Kurzrock and Weuster-Botz, 2011).

2.5 Itaconic Acid Production

Itaconic acid production follows the general process overview as discussed in Chapter 2.3. The pretreatment, hydrolysis, fermentation and downstream processing steps will be discussed. Lignocellulosic feedstocks such as sugarcane molasses, wood, hydrolysates and beet have been used for itaconic acid production (Willke and Vorlop, 2001; Klement *et al.*, 2012; Mondala, 2015). The pretreatment for these feedstocks include ion-exchange, ferrocyanide (Okabe *et al.*, 2009), acid hydrolysis (Dwiarti *et al.*, 2007; Okabe *et al.*, 2009; Hu, 2012; Klement and Büchs, 2013; Mondala, 2015) and enzymatic hydrolysis (Okabe *et al.*, 2009; Mondala, 2015).

The final itaconic acid concentration, after fermentation from hydrolysed corn starch, is similar for acid and enzymatic hydrolysis. Itaconic acid titres of 28.5 $g.L^{-1}$ and 31.0 $g.L^{-1}$ were obtained using acid- or enzymatically hydrolysed corn starch, respectively (Okabe *et al.*, 2009). Similar results for acid and enzymatic hydrolysis are also seen for itaconic acid production from sago starch, using the microorganism *A. terreus* TN484-M1 (Dwiarti *et al.*, 2007).

The nutrient requirement of the microorganism for itaconic acid production is an important consideration when selecting a pretreatment method, since itaconic acid producing microorganisms are sensitive to chemical elements such as iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), phosphorus (P) and nickel (N) (Willke and Vorlop, 2001; Klement *et al.*, 2012). A high phosphorous content may result in an extended growth phase, which is disadvantageous, since itaconic acid is only produced during the stationary, maintenance phase (Klement and Büchs, 2013). The use of nitric acid for hydrolysis is also disadvantageous for organisms, such as *Utilago maydis,* that require a nitrogen limitation for itaconic acid production (Klement *et al.*, 2012; Klement and Büchs, 2013).

The microorganism *A. terreus* can secrete exo-enzymes, which enables the use of the SSCF (simultaneous saccharification and co-fermentation) process configuration for itaconic acid production during solid state fermentation (SsF) (Paranthaman, Kumaravel and Singaravadivel, 2014). Unfortunately the results for SsF is not sufficient for industrial application since the productivities, yield and titre are very low even when the feedstock surface area is large (i.e. the sugarcane bagasse is milled to a powder), which is not economically viable (Paranthaman, Kumaravel and Singaravadivel, 2014). The SHF process configuration is favoured for a feedstock that has different optimal pH conditions for hydrolysis and fermentation (Klement and Büchs, 2013).

2.5.1 Fermentation

The microorganism used for the majority of industrial itaconic acid production is a filamentous fungi *A. terreus* (Tevž, Benčina and Legiša, 2010). Other itaconic acid producers include *Ustilago zeae, Ustilago maydis*, *Candida* sp. and *Candida mutant, Rhodotorula* sp., *Pseudomonas antartica,* genetically modified *Escherichia coli,* genetically modified *Aspergillus niger, Aspergillus flavus, Aspergillus terreus* -SKR10 and -TN-484-M1 and *Aspergillus itaconicus* (Okabe *et al.*, 2009; Klement and Büchs, 2013; Huang *et al.*, 2014). Commercial yeast, *S. cerevisiae,* and yeast-like fungi, *Yarrowia lipolytica,* have been genetically modified to produce itaconic acid from glucose, but with very low titres of 0.17 $g.L^{-1}$ and 4.6 $g.L^{-1}$, respectively (Blazeck *et al.*, 2014, 2015).

The fungi *A. terreus* is widely used and can utilise various sugar sources such as glucose, starch, saccharose, lactose, glycerol and xylose. This is advantageous when using sugar cane bagasse as a feedstock, since the hemicellulose fraction can also be utilised. A recent study investigated the production of itaconic acid from wheat straw hydrolysate and identified the acceptable levels or fermentation inhibitors caused by dilute acid pretreatment as 0.4 g acetic acid, <0.1 g furfural and 100 mg HMF per L for combined sugar feed (Saha *et al.*, 2018). Therefore, detoxification of the feedstock is vital.

A. terreus is also the only strain that can achieve titres as high as 80 - 86 $g.L^{-1}$, when compared to titres in the range of 20 - 40 $g.L^{-1}$, typically obtained. However, compared to citric acid production where titres of >200 $g.L^{-1}$ is achieved, the titres for itaconic acid production can still be considered as low (Steiger *et al.*, 2013; Karaffa *et al.*, 2015).

A study conducted by Karaffa *et al.* (2015) investigated the effect of the initial sugar concentration and manganese deficiency on the production of itaconic acid. Very high titres of 95 - 100 $g.L^{-1}$ and a volumetric productivity of 0.3 $g.L^{-1}.h^{-1}$ were achieved for an initial sugar concentration of 150 $g.L^{-1}$ D-glucose when a manganese ion deficient culture was used. The culture also had an impact on the microorganism's morphology: manganese deficiency causes very small tight pellets. This is very advantageous for improved rheology of the fermentation broth as well as oxygen transfer and nutrient distribution (Karaffa *et al.*, 2015).

The biosynthesis route of itaconic acid production has not been confirmed and several potential pathways exist (Huang *et al.*, 2014). Itaconic acid production has a very similar pathway to citric acid, with the exclusion of an additional enzyme called *cis*-aconitate, which is a

tricarboxylic acid cycle (TCA) intermediate (Karaffa *et al.*, 2015). The general consensus is that the metabolic pathway occurs in the cytosol and mitochondria. Itaconic acid is produced from *cis*-aconitate through the action of the decarboxylase CadA (Tevž, Benčina and Legiša, 2010). According to the metabolic pathway, 1 mole glucose should be converted to 1 mole itaconic acid (IA), but the reported yields are in the order of 0.8 fractional conversion (i.e. 0.57 $g_{IA}.g_{GLU}^{-1}$). Theoretically, the maximum yield is 0.72 $g_{IA}.g_{GLU}^{-1}$ (100 %)(Klement and Buchs, 2013). *U. maydis* is more sensitive to itaconic acid through end-product inhibition than *A. terreus,* and is inhibited when the concentration is close to 50 $g.L^{-1}$ itaconic acid, whilst *A. terreus* is unaffected up to 80 $g.L^{-1}$ itaconic acid (Klement *et al.*, 2012).

2.5.1.1 pH control

Hevekerl *et al.* (2014) found that an initial pH of 2.9 – 4.9 is necessary to initiate organism growth, and that the pH drops to 2.1 after two days, when itaconic acid production starts. Thereafter, no pH control is required. If growth is started in a low pH environment (1.9 – 2.4), delayed growth and product formation occur. Therefore, an initial pH of 3.1, with no additional pH control, resulted in acceptable titres of 90 $g.L^{-1}$ for the experimental work by Kuenz *et al.* (2012).

Riscaldati *et al.* (2000) investigated pH control for itaconic acid production using *A. terreus* NRRL 1960 and a glucose substrate. pH control at 2.4 and 2.8 resulted in itaconic acid yields of 0.53 and 0.50 $g_{IA}.g_{GLU}^{-1}$ respectively, which are lower than the 0.62 $g_{IA}.g_{GLU}^{-1}$ yield achieved for Kuenz *et al.* (2012), where no pH control was executed during fermentation. The pH shift induced by NH_3 addition has a positive impact on the final titre, where the titre can increase from 80 $g.L^{-1}$ to 110 $g.L^{-1}$ (Hevekerl, Kuenz and Vorlop, 2014).

The pH also had an effect on the by-products formation. A decrease of 2.2%, 1.8% and 1.2% in by-products (from 3.3%) is experienced for a pH increase by NaOH, KOH and NH_3, respectively. However, the colour of the fermentation broth increases after the pH shift and the solubility of itaconic acid increases (Hevekerl, Kuenz and Vorlop, 2014), which might have a detrimental impact on the downstream process, where more intensive colour removal crystallisation might be required.

2.5.1.2 Fermentation strategies

Itaconic acid can be produced using submerged fermentation (SmF) (Karaffa *et al.*, 2015) or solid state fermentation (Mondala, 2015), which is similar to the natural fermentation state of

the fungi (Mondala, 2015). Pretreatment is not required, since the natural growth conditions of fungi uses solid substrates for support. Fungi also have the capability to excrete hydrolytic enzymes such as cellulase, which hydrolyse lignocellulose and breaks it down to fermentable sugars (Mondala, 2015). However, the productivity from the sugarcane bagasse SsF is at least a hundred times lower, at 3 x 10^{-4} $g.kg^{-1}.h^{-1}$, than productivities reported for submerged fermentations (SmF). The milling required for a powder feedstock is also energy intensive. Paranthaman, Kumaravel and Singaravadivel (2014) conducted experimental work on SsF with various strains, directly from sugarcane bagasse powder, and obtained a yield of 8.24 x 10^{-6} $gIA.gSB^{-1}$ (gram itaconic acid per gram of sugarcane bagasse) for *A. niger* at a pH of 3.5 and temperature of 35 ˚C (Mondala, 2015). Currently, all industrial production of itaconic acid occurs by using *A. terreus* in SmF only (Karaffa *et al.,* 2015).

2.5.1.3 Submerged Fermentation

SmF requires pretreatment and enzymatic hydrolysis of the lignocellulosic biomass to obtain a liquid media of sugar substrate(s). It includes batch, fed-batch and continuous fermentation reactors. Continuous production may be favourable, due to fewer cleaning cycles and smaller equipment sizes required for a reduced retention time, compared to batch (SmF) fermentation. The challenges for SmF production when using a fungi, such as efficient mass diffusion, oxygen transfer and high viscosity, are caused by its filamentous nature (Mondala, 2015).

Immobilised *A. terreus* TKK 200-5-3 on polyurethane foam cubes produced 15.8 – 26 $g.L^{-1}$ itaconic acid at a productivity of 0.145 $g.L^{-1}.hr^{-1}$, and was stable for four months after a growth period on the cubes for a week (Kautola, Vassilev and Linko, 1990). Studies were conducted with free submerged *A. terreus* cells to achieve a productivity of 0.48 $g.L^{-1}.h^{-1}$ (Klement and Buchs, 2013). The highest recorded productivity was 1.2 $g.L^{-1}.h^{-1}$ for immobilised *A. terreus* cells after 2.5 days (72 $g.L^{-1}$) with an overall productivity of 0.51 $g.L^{-1}.h^{-1}$ after 7 days (Kuenz *et al.,* 2012; Mondala, 2015). The volumetric productivity is a challenge since the US DOE stated that a minimum productivity of 2.5 $g.L^{-1}.h^{-1}$ is required for the process to be economically viable (Werpy *et al.,* 2004). However, no experiments were done to support the figure, which is much higher than the recorded productivities, including patented fermentation with relatively low productivities of 0.41 to 0.98 $g.L^{-1}.h^{-1}$ (Kuenz *et al.,* 2012). The volumetric productivity provides an indication of the time required and the size of the equipment, which will influence operational and capital costs, respectively.

The process parameters that require careful consideration in SmF are the oxygen supply, power input (agitation speed) and nutrient supply (Mondala, 2015). Itaconic acid fermentation is aerobic, and even an interruption in the oxygen supply as short as 5 minutes can lead to a production delay of 24 hr, the time required for the cells to return to the state prior to oxygen deficiency. Conversely, high aeration causes foaming leading to operational problems (Mondala, 2015). The fermentation bioreactor must be aerated and mixed thoroughly to maintain a sufficient concentration of dissolved oxygen. This must be balanced with the amount of shear stress experienced by the fungi cells due to agitation (Klement and Buchs, 2013; Mondala, 2015).

A degree of shear stress can be beneficial for the fungal morphology during growth. Fungal pellet formation can reduce viscosity and biofilm build-up. It also controls the pellet size which should not exceed 0.1 mm for ease of mass and oxygen diffusion to the centre of the fungal pellets (Klement and Buchs, 2013; Mondala, 2015). Experimental work showed that the fungi are more sensitive to shear stress at low pH conditions (pH = 1.85). Lower pH conditions are beneficial for the limitation of contaminant growth. The pH is also related to the formation of by-products. Fewer by-products are formed at a pH of 3 than at a lower pH (Mondala, 2015). A pH of 3 also allows better oxygen transfer (Klement and Buchs, 2013).

Nutrients (medium sources) such as ammonium salts and urea are preferred over yeast extract, since yeast extract promotes cell growth, which is detrimental to itaconic acid production (Mondala, 2015). For the fungi *A. terreus* the nutrient supply must be carefully added, since itaconic acid is only produced under growth-limited conditions (Klement and Buchs, 2013). This is one reason for the low overall productivities. Therefore, continuous production is desired, since it is possible to run optimal fermentation conditions for a long time period (Klement and Buchs, 2013).

2.5.2 Downstream Recovery

The traditional method of downstream recovery involves precipitation and acidification. This is undesirable as it generates a large gypsum waste stream. The downstream recovery of itaconic acid can contribute as much as 50% to the total production cost. This is a driver to consider more environmentally and economically viable downstream recovery processes (Kaur and Elst, 2014; Magalhães *et al.*, 2017).

Process optimisation was done in the study by Kaur and Elst (2014) to integrate the fermentation and separation steps. This is known as *in situ* process recovery (ISPR). *In situ* recovery of itaconic acid is attractive since the presence of itaconic acid (as low as 20 $g.L^{-1}$) has an inhibitory effect on the fermentation. However, *in situ* recovery has not been done for complex fermentation broths, such as itaconic acid fermentation from pretreated sugarcane bagasse and trash. Other separation methods include liquid-liquid extraction (i.e. reactive extraction), membrane separations and ion-exchange and adsorption (Okabe *et al.*, 2009).

A popular separation method is crystallisation, as shown in Figure 2-10. This is done using cooling or evaporation techniques. Since a single crystallisation and evaporation step may not remove all the by-products (Klement and Büchs, 2013; Magalhães *et al.*, 2015), the process is repeated. Alternatively, additional process steps such as reverse osmosis or electrodialysis can be implemented.

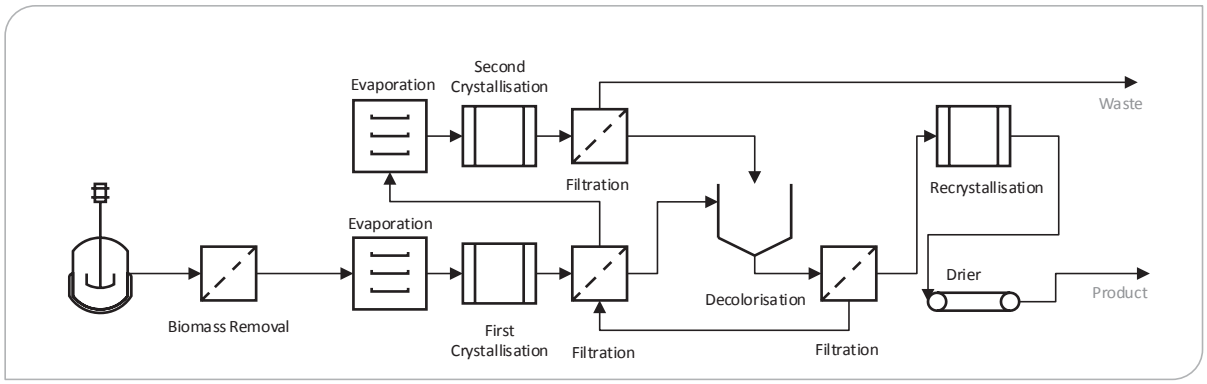

Figure 2-10: Downstream processing scheme for the separation and purification of itaconic acid (Okabe *et al.*, 2009)

Figure 2-10 shows the industrial itaconic acid separation and purification scheme, using consecutive evaporation, crystallisation and filtration. This is followed by discoloration, using activated carbon, filtration and final crystallisation purification. Although the 2-step process is more expensive due to additional equipment requirements, the yield and purity are higher than for a single step process.

2.6 Polyhydroxyalkanoates Production

Polyhydroxyalkanoates production follows the general process overview as discussed in Chapter 2.1. The pretreatment, enzymatic hydrolysis, fermentation and downstream processing steps will be discussed. The separation and purification is grouped together under downstream processing.

Polyhydroxyalkanoates are diverse biopolymers that are collected in the producing microorganism's cytoplasm as intracellular storage compartments of discrete granules, 0.2 - 0.7 µm in size (Khanna and Strivastava, 2004; Koller, Niebelschütz and Braunegg, 2013; Pandey *et al.* 2015). It can be produced from various feedstocks, such as molasses, whey, sucrose, starch, glycerol, palm oil, cellulose, fossil resources (CH_4, mineral oil and coal), chemicals and CO_2 (Khanna and Srivastava, 2004; Akaraonye, Keshavarz and Roy, 2010; Reddy *et al.*, 2013).

Yu and Stahl (2008) pretreated sugarcane bagasse (0.1-1 mm by 0.2-20 mm filaments as received by a sugar mill) with DAT (0.75 %wt, H_2SO_4, pH 1.1). Depending on the severity of the DAT process, different concentrations of inhibitors were produced [i.e. formic acid, acetic acid, furfural and hydroxymethylfurfural (HMF)]. Although inhibitors hinder the microorganism's growth and metabolism, there are a few strategies to overcome this effect. A larger inoculum (3 - 6 $g.L^{-1}$ compared to <1.2 $g.L^{-1}$) can be applied, enabling the microorganism to utilise the inhibitors, together with glucose, for PHA production. However, this will have an effect on the cost of fermentation (Yu and Stahl, 2008). Alternatively, as with succinic acid and itaconic acid production, the hydrolysate can be diluted, which will have an effect on the cost of downstream processing (Yu and Stahl, 2008; Lopes *et al.*, 2014), or a detoxification step can be introduced prior to fermentation (Silva *et al.*, 2014).

Silva *et al.* (2014) showed that *Bulkholderia sacchari* performed best for PHA production when three consecutive detoxification steps were used on the bagasse hydrolysate prior to fermentation: evaporation, neutralisation and adsorption using activated carbon. The increase in sugar concentration and decrease in by-products are shown in Table 2-8 for the evaporation, neutralisation and adsorption process steps. Evaporation removes the volatile compounds: acetic acid, furfural and HMF. Calcium oxide is used to neutralise the hydrolysate and activated carbon is used to remove the residual furfural and HMF via adsorption.

Table 2-8: Sugarcane hydrolysate compounds before and after detoxification (adapted from Silva *et al.,* 2004)

COMPOUND	INITIAL CONCENTRATION	FINAL CONC.	DIFFERENCE
Xylose ($g.L^{-1}$)	16.9	179.1	+960%
Glucose ($g.L^{-1}$)	9.7	129.5	+1235%
Arabinose ($g.L^{-1}$)	1.4	3.6	+157%
Furfural ($g.L^{-1}$)	244.7	12.6	-95%
HMF ($g.L^{-1}$)	103.3	59.0	-43%
Acetic Acid ($mg.L^{-1}$)	144.8	205.0	-42%

These detoxification steps result in increased yields: 156% increase in cell density and 180% increase in productivity (Lopes *et al.,* 2014). It was found that the microorganism growth is not sufficient if the activated carbon adsorption step was not applied, even though the hydrolysate had been subject to evaporation and neutralisation. If high volumes of HMF are produced, it can be converted into levulinic acid, which can be used as a feedstock for PHA production (Silva *et al.,* 2014). Together with levulinic acid, PHA producing organisms can use acetic and formic acid as carbon sources (Lopes *et al.*, 2014).

PHA producing microorganisms are not dependent on glucose sugars only and can also utilise other sugars present in the hydrolysate fraction of pretreated lignocellulose. Although *Bulkholderia* sp. F24 can utilise xylose in the production of PHB, it cannot utilise glucose (Lopes *et al.,* 2014). However, a higher titre is achieved when glucose is included in the fermentation feed stream (60 $g.L^{-1}$ PHA), compared to the titre (6 $g.L^{-1}$ PHA) obtained from hydrolysate (Silva *et al.,* 2004). Therefore, enzymatic hydrolysis will increase processing costs, but will reduce downstream processing costs due to the increased titre.

The type of microorganism selected for PHA production will influence the yield, residence time and titre (Pandey *et al.,* 2015). The choice of microorganism will also determine the growth conditions, the nutrient to limit for PHA production (if applicable), and the type of PHA polymer produced as well as the selection of downstream processing method.

2.6.1 Fermentation

As discussed in section 2.1.3 *Polyhydroxyalkanoates,* the microorganisms can be classified into two main groups. The first group requires a nutrient limitation under an available or excess carbon source for PHA production. The microorganisms that fall within this group include *Alcaligenes eutrophus, Ralstonia eutropha*, *Cupriavidus necator, Pseudomonas oleovorans*, *P. putida, Protomonas* sp. and *Bacillus* sp. (Khanne and Srivastava, 2004; Suriyamongkol *et al.*,

2007; Lopes *et al.,* 2014). For fed-batch fermentation using group 1 bacteria, a two-step "feast and famine" approach is used. The 'feast' step involves the growth of the bacteria in a nutritionally enriched medium with high dissolved oxygen content. The 'famine' step involves nutrient depletion and consequential organism stress, which produces PHA (Khanna and Strivastava, 2004; Verlinden *et al.*, 2007). The fed-batch fermentation process is the preferred fermentation route for PHA production (Akaraonye, Keshavarz and Roy, 2010).

The second group of microorganisms do not require a nutrient limitation for PHA production, and PHA can be produced during the growth phase. These include recombinant *E. coli* and *Alcaligenes latus* (Khanna and Strivastava, 2004). Other bacterial strains that have been investigated include *Aeromonas hydrophylia, Burkholderia sacchari, Burkholderia cepacia* and *Halomonas boliviensis,* although it is not certain in which group these bacterial strains fall (Verlinden *et al.,* 2007).

The type of co-polymer that is best described and most widely produced is PHB or P3HB (poly 3-hydroxybutyrate) (Khanna and Srivastava, 2004; Kapritchkoff *et al.*, 2006; Suriyamongkol *et al.*, 2007; Pandey *et al.* 2015). For the production of PHB, special attention has been given to A. *eutrophus, A. latus* and recombinant *E. coli* (Lee, 1996; Choi and Lee, 1999; Li, Zhang and Qi, 2007).

Recombinant *E. coli* in particular, is a favourable candidate for PHB production, since it falls within the second group of microorganisms (i.e. production of PHB during the growth phase), which will require fewer fermentation tanks and therefore a lower capital investment. Since *E. coli* does not require a nutrient limitation, sugarcane hydrolysate can also be used as substrate without nutrient limitation complications due to the complex nature of the hydrolysate. Other advantages include (van Wegen, Ling and Middelberg, 1998; Reddy *et al.*, 2003):

i. Ease of DSP and recovery due to large PHB granule size and weak cell walls
ii. High growth rates and PHB titres
iii. Ease of process control and simplified feeding strategy due to no nutrient limitation required
iv. *E. coli* does not produce the enzyme responsible for intracellular PHB degradation (Choi and Lee, 1997).

PHB is a short chain link PHA monomer and can reach 100 $g.L^{-1}$ CDW after 48-60 hours of fermentation, with about 80% intracellular PHA (Pandey *et al.,* 2015). PHA fermentation results are given for recombinant *E.coli* in Table 2-9, and it is shown that the % PHB and CDW titre achieved by *E. coli* is within this range and even slightly higher. Although a vast range of results exist for PHA production on glucose and other substrates, no information is available for *E. coli* fermentation on xylose or sugarcane bagasse hydrolysate.

Table 2-9: PHB production by fed-batch recombinant *E. coli*

CARBON SOURCE	YIELD $g.g^{-1}$ $Y_{PHB/C}$	PRODUCTIVITY $g.L^{-1}.h^{-1}$	TITRE $g.L^{-1}$ CDW	% PHA	TITRE $g.L^{-1}$ PHB	REFERENCES
GLUCOSE						
Glucose	0.28	3.2	204.3	77	157.3	(Choi and Lee, 1999; Lee, Choi and Wong, 1999; Wang and Lee, 1997)
Glucose	-	4.63	194.1	73	141.7	(Lee, Choi and Wong, 1999; Li, Zhang and Qi, 2007; Akaraonye, Keshavarz and Roy, 2010; Choi and Lee, 1998)
Glucose	-	2.8	153.1	65.9	100.9	(Lee *et al.,* 1999)
Glucose	0.43	1.43	86	99	85.2	(Il *et al.*, 2005)
Glucose	-	1.98	113	72	81.4	(van Wegen, Ling and Middelberg, 1998)
Glucose	0.29	1.98	112	72.3	81	(Choi and Lee, 1999)
XYLOSE						
Xylose	0.1	0.03	4.75	35.8	1.7	(Silva *et al.*, 2014)
Xylose + SH	0.23	0.07	5.95	73.9	4.4	(Lee, 1998; Silva *et al.*, 2004)

SH – soybean hydrolysate

The productivities and titres achieved for the pure substrates using *E. coli* are higher than those for the sugarcane bagasse hydrolysate using *B. sacchari* IPT 101 and *Bulkholderia* species (Silva *et al.*, 2004; Yu and Stahl, 2008; Lopes *et al.*, 2014). The titres for PHA production from glucose substrates are in the range of 100 - 200 $g.L^{-1}$ and productivities of 1 - 2.6 $g.L^{-1}.h^{-1}$. Conversely, the titres for PHA production from sugarcane bagasse hydrolysate were 4 - 20 $g.L^{-1}$ and productivities of 0.1 – 0.29 $g.L^{-1}.h^{-1}$ (Silva *et al.,* 2014). Therefore, PHB fermentation from glucose is preferred, which is obtained when the cellulignin is subjected to enzymatic hydrolysis. The hydrolysate can then be fermented separately using *B. sacchari IPT 101* to produce PHB, or the hydrolysate can be used for a different application. Such an integrated solution has been proven to be viable for a small commercial PHA production plant, where

PHA production is integrated with a sugar and ethanol production plant, and PHA is produced from sucrose (Silva *et al.,* 2014). An integrated biorefinery solution, for the products discussed, can be PHB production from glucose combined with succinic acid or itaconic acid production from xylose.

The type of microorganism selected for fermentation will also influence the downstream processing requirements. Unlike succinic or itaconic acid, the desired product is not within the fermentation broth, but within the microorganisms' cells. Therefore, the cell characteristics has an influence on the downstream recovery.

2.6.2 Downstream Recovery

The downstream separation process chosen depends on the bacterial strain used, type of PHA produced (SCL or MCL), intracellular load of PHA (% dry weight) and required product purity (Koller, Niebelschütz and Braunegg, 2013). After fermentation the biomass is separated from the broth using centrifugation, sedimentation, filtration or flocculation (Koller, Niebelschütz and Braunegg, 2013). Once the cells have been separated from the fermentation broth, the cell wall needs to be disrupted and the PHA polymer (PHB) can be recovered, with minimal or no degradation, and purified. The disruption and PHA recovery can be done in one step, i.e. extraction, or in two steps, i.e. cell preparation and extraction. The DSP steps are shown in Figure 2-11.

Cell wall disruption can be done through mechanical disruption, supercritical CO_2 or digestion. Digestion is done by either chemical digestion (using agents such as sodium hypochlorite, alkaline digestion and surfactants) or enzymatic digestion. The PHA granules within the bacterial cells are not crystalline, as would be expected from a thermoplastic, but rather in an amorphous state. It is only after exposure to shear forces, and after the release of PHA from the cells, that PHA crystallinity increases (Koller, Niebelschütz and Braunegg, 2013).
Mechanical disruption results in medium to high purity (95 %wt) and high recovery yields (98 %wt), with no chemical operational costs and negligible polymer degradation (Jacquel *et al.*, 2008). However, it requires the correct design to prevent blockages within the equipment (Jacquel *et al.*, 2008; Koller, Niebelschütz and Braunegg, 2013). The use of supercritical CO_2 for DSP, is beneficial for its low toxicity and high availability, but is not implemented due to its high cost (Jacquel *et al.,* 2008). Supercritical CO_2 also acts as a solvent with about 89 %wt recovery (Jacquel *et al.,* 2008).

Enzymatic digestion has high recovery yields (90 %wt), negligible polymer degradation, is suitable for industrial application, and has a low residence time, but has a high operating cost and requires additional purification, since it produces a purity of 86 %wt (Kapritchkoff *et al.*, 2006; Yasotha *et al.*, 2006; Yu and Chen, 2006; Suriyamongkol *et al.*, 2007; Jacquel *et al.*, 2008; Koller, Niebelschütz and Braunegg, 2013; Pandey *et al.* 2015).

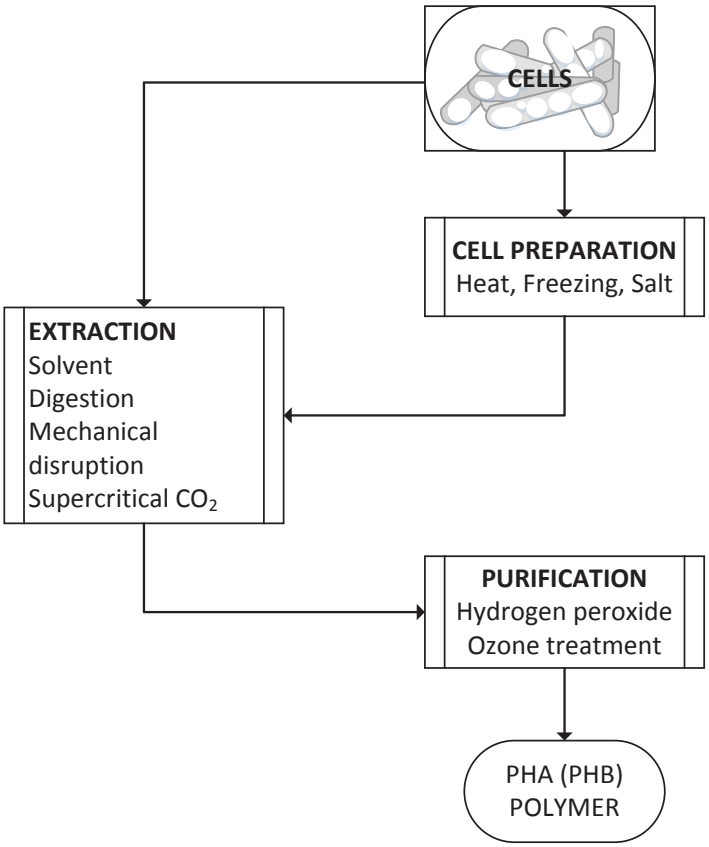

Figure 2-11: DSP steps required for PHA recovery (Redrawn from Jacquel *et al.*, 2010)

The use of digestion chemicals only, does not give satisfactory results due to high polymer degradation and is therefore not suitable for industrial application (Lee 1996, Jacquel *et al.*, 2008, Koller, Niebelschütz and Braunegg, 2013). Polymer degradation is measured by the reduction in the average- (M_w) and number (M_n) molecular weight, which is detrimental to the polymer's thermal and mechanical properties, such as tensile strength and melting temperature (Misra *et al.*, 2006; William and Callister, 2007). However, combinations of chemical digestion methods have proven to be successful. The two most popular methods, which are considered as the conventional DSP methods, are the chloroform-sodium hypochlorite (CSH) method and the surfactant-sodium hypochlorite (SSH) method (Jacquel *et al.*, 2008).

Sodium hypochlorite (NaOCl) is effective in removing the non-PHA cell mass (NPCM), but results in high polymer degradation. However, when combined with chloroform in the CSH-method, high purity (> 97 %wt), high recovery (91 %wt) and low polymer degradation is achieved (Hahn *et al.,* 1993). The NPCM is hydrophilic and the polymer is hydrophobic. Therefore, as the sodium hypochlorite lyses the cell, the polymer migrates to the chloroform phase and is shielded from degradation by the sodium hypochlorite (Hahn *et al.,* 1993). Although high yields can be achieved, the process has several disadvantages such as severe process conditions (i.e. high residence time and temperatures) and large volumes of hazardous solvent, which is a safety risk to plant personnel (van Wegen and Middelberg, 1998; Verlinden *et al.,* 2007; Jacquel *et al.,* 2008).

The surfactant (e.g. Triton-X100, SDS) in the SSH method also protects the polymer from degradation, has a low operating cost and results in high purity (98 %wt) (Jacquel *et al.,* 2008, Dacosta *et al.,* 2015). However, the recovery yield is mediocre (87 %wt), the surfactant is not environmentally friendly and results in high volumes of waste and waste water treatment (Yu and Chen, 2006; Jacquel *et al.,* 2008; Dacosta, Posada and Ramirez, 2015). The SSH process method and configuration is described elsewhere (Choi and Lee, 1997; Akiyama, Tsuge and Doi, 2003; Dacosta, Posada and Ramirez, 2015).

An alternative DSP method, the selective dissolution of NPCM by protons, was designed by Yu and Chen (2006) and resulted in high purity (98 %wt) and high recovery yields (98.7 %wt) at a 90% reduction in operating costs. Sulphuric acid is used to disrupt the NPCM, followed by an alkaline (10 N NaOH) and decolourisation step (6 %wt NaOCl). Although the authors claim a 90% reduction in operating costs, the chemical cost used in the economic analysis is 3.3 times higher, compared to the same chemical cost used in Dacosta, Posada and Ramirez (2015), when adjusted to the same currency and base year. If the chemical cost of Dacosta, Posada and Ramirez (2015) is used for the sodium hypochlorite and surfactant DSP, the method by Yu and Chen (2006) is only 24% cheaper at the expense of high polymer degradation (<50 %).

The alkaline DSP method is found to be 25% cheaper when compared with the SSH-method (Choi and Lee, 1998). The alkaline DSP has a low residence time (1 hr), low cost of chemicals and it is suitable for industrial application with a high PHB purity (98.5%) and recovery yield (91.3%) (Choi and Lee, 1998; Lee *et al.,* 1999). However, this method is only suitable for microorganisms with high PHB content (>60 %wt) and thin cell walls, such as recombinant *E.*

coli (Koller, Niebelschütz and Braunegg, 2013). Yu and Chen (2006) found that the PHB degraded into oligomers and monomers when *R. eutropha* was subject to alkaline cell preparation. This was due to the amorphous state of the PHB during cell lysis. However, once the PHB is crystalline, the polymer degradation is negligible (Yu and Chen, 2006), as with recombinant *E. coli* (Choi and Lee, 1998).

Since PHB is biodegradable and biocompatible, high purity PHB is used for medical applications such as sutures, conduits, carrier scaffold, stents, soft tissue repair, bone tissue scaffold, nerve repair, pericardial patch and artery augmentation (Valappil *et al.*, 2006). The PHB's mechanical properties can be manipulated by blending it with plasticizers or other degradable polymers, depending on the application requirement (Valappil *et al.,* 2006). However, if the PHB is produced from gram negative microorganisms (such as *E. coli*), it will contain endotoxins from the microorganisms' outer cell membranes (Valappil *et al.,* 2006).

These endotoxins cause an immunogenic reaction, which makes it unsuitable for biomedical applications. However, this can be remedied by additional purification steps such as hydrogen peroxide or benzoyl peroxide washing, which will add to the operating costs (Valappil *et al.,* 2016). Lee *et al.,* (1999) found that the alkaline method is suitable for the removal of endotoxins from recombinant *E. coli* produced PHB. If the residence time is increased from 1 to 5 hours at 30˚C and 0.2 N NaOH solution (Valappil *et al.,* 2006), the endotoxin level (EU) reduces from 10^7 EU per g PHB, to 1 EU per g PHB, which makes the PHB suitable for biomedical applications, at a high purity of 98 %wt (Lee, 1999).

2.7 Techno-economic evaluation

A biorefinery scenario must be economically viable and environmentally sustainable to generate industrial interest in the project. The techno-economic analysis is performed on the fixed capital investment (FCI) and operational expenses, also called the total cost of production (TCOP) of the biorefinery (Fernández-Dacosta *et al.*, 2015). These costs are provided by the process design's mass and energy balance (Gnansounou, Vaskan and Pachon, 2015). The conceptual process design is based on literature, heuristics or previous work from laboratory and pilot scale plants (Gnansounou, Vaskan and Pachon, 2015). The information for the process design is then used to model the simulation, using a tool such as Aspen Plus® for the mass and energy balances and kinetic simulations (Moncada *et al.,* 2013; Fernández-Dacosta

et al., 2015; Gnansounou, Vaskan and Pachon, 2015). A capital cost estimate can be classified according to the level of project definition, the expected accuracy and aim of the estimate.

2.7.1 Capital and operational expenditures

The mass and energy balances are used to size the major equipment. Equipment can be sized by hand or through the use of software such as Aspen Economic Analyzer in Aspen Plus® (Vlysidis *et al.*, 2011). Aspen Economic Analyzer® can also be used to calculate the capital cost and cost of production, which includes the cost of raw materials, waste disposal costs, utilities and some miscellaneous costs (Vlysidis *et al.,* 2011). The raw materials cost, income tax, annual interest rate and salaries should be specified so that it is relevant to the country of interest (Moncada *et al.,* 2013). Alternatively, the module costing technique (Turton *et al.,* 2013) can also be used to determine the equipment purchased and installed cost.

The installed cost is the purchased cost C_p^o, multiplied by the installation factor F, which takes the material of construction and system pressure into account. The purchased cost price should be adjusted to the desired capacity and relevant time of study, using the Chemical Engineering Plant Cost Index (CEPCI) (Vlysidis *et al.*, 2011; Efe and van der Wielen, 2013; Fernández-Dacosta *et al.*, 2015; Gnansounou, Vaskan and Pachon, 2015). Once the installed equipment cost is determined, the fixed capital investment (FCI) can be determined by adding direct and indirect costs (Humbird *et al.,* 2011; Görgens *et al.,* 2016).

Operational costs are defined as variable operating costs, fixed operating costs and general expenses (Turton *et al.* 2013; Coulson and Richardson, 2006). The total cost of production takes all three of these costs into account. The variable cost of production includes all the costs that vary with production volumes, such as the costs of raw materials and waste streams. The fixed cost of production takes the labour cost, maintenance, property taxes and insurance and plant overhead costs into account, and is independent of production volumes. General expenses include distribution and selling costs, research and development and administration costs (Turton *et al.,* 2013).

2.7.2 Economic indicators

The indicators or parameters utilised in a techno-economic analysis can include the discounted payback period (DPBP), net present value (NPV), discounted cash flow rate of return (DCFROR) (Turton *et al.,* 2013; Görgens *et al.,* 2016), minimum required selling price

(MRSP) and return on investment (ROI) (Apostolakou *et al.*, 2009; Humbird, 2011). The DCFROR is also termed the internal rate of return (IRR).

The capital and operating cost are used to determine the minimum selling price of the product through the discounted cash flow rate of return (DCFROR) analysis (Humbird *et al.*, 2011). A profitable investment must have an interest rate higher than other low risk investments such as property or bonds. The DCFROR analysis needs the specified discount rate, depreciation method used and the determined plant life, tax rates and construction start-up period parameters, as seen in Table 2-10. The plant life can vary from 10 to 30 years, the loan interest rate can vary from 6.3 to 20% and the construction period from one to three years, with a ramp-up period of a few months to two years.

Table 2-10: Biorefinery capital assumptions

REFERENCES	PLANT LIFETIME	LOAN INTEREST RATE	CONSTRUCTION PERIOD	RAMP-UP PERDIOD
Marchetti *et al.* 2008	15 years	-	1 years	4 months
Fernandez-Dacosta *et al.* 2015	20 years	15%	-	-
Apostolakou *et al.* 2009	10 years	Investigates both 10 and 20%	2 years	-
Humbird *et al.* 2011	30 years	10%	3 years 8% in year 1 60% in year 2 32% in year 3	4 months
Alimandegari *et al.* 2017	25 years	6.3%	1 years	2 years 50% in year 1 75% in year 2

In the techno-economic analysis of Vlysidis *et al.* (2011) for the production of succinic acid, the sensitivity analysis showed that assumptions related to the fermentation process had the most significant impact on the plant's profitability. These were the high cost of the fermenters, which are dependent on the fermenter volume, cycle time and water feed rate. This is also seen in Ali Mandegari, Farzad and Görgens (2017), where the fermenters constitute 10.52% of the total installed equipment cost. Therefore, it is important that these units are costed correctly. A comparison of purchased costs, scaled to a capacity of 946.35 m^3 total volume (250 000 gallons) and year (2016), is shown in Figure 2-12.

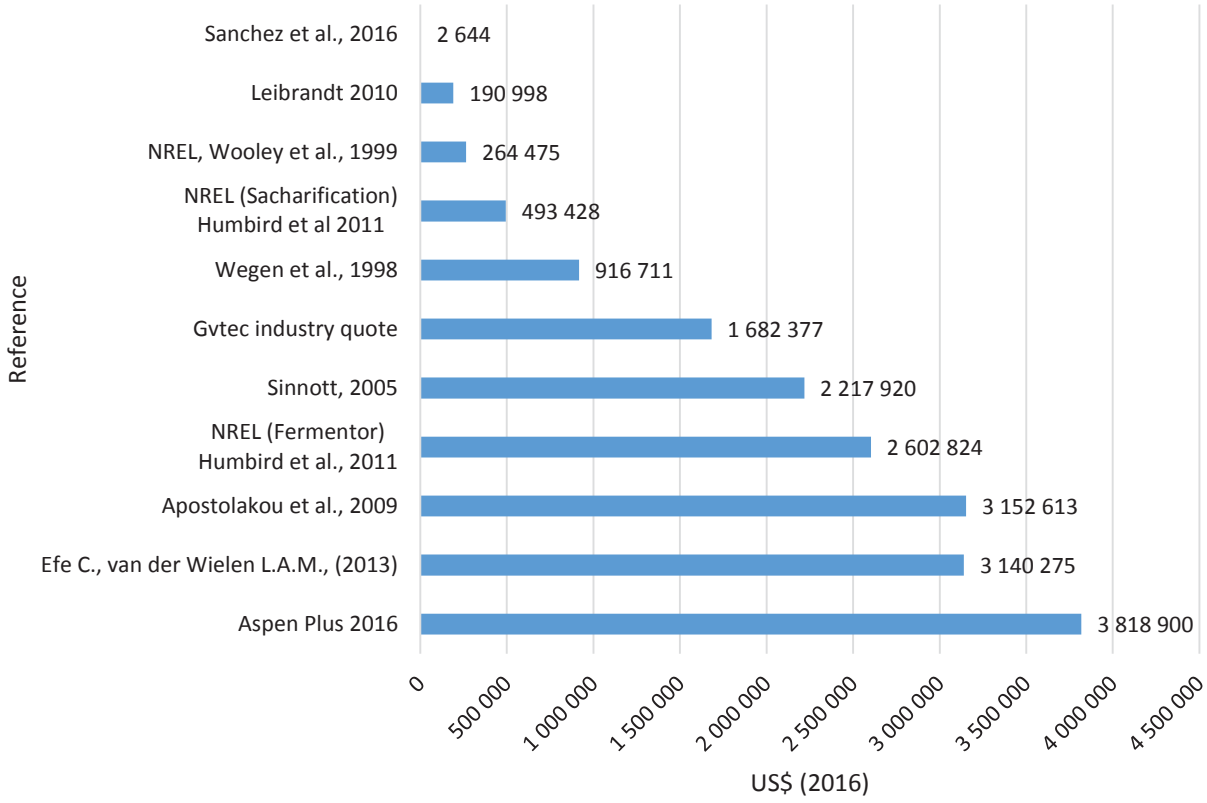

Figure 2-12: Purchased cost (US$) of a fermentation tank

The purchased cost of fermentation tanks vary in literature. The most expensive tank is provided by the Aspen Plus® economic analyser for a jacketed, agitated stainless steel (SS316) tank, followed by the tank cost scaled from Efe and van der Wielen (2013), which is three orders of magnitude larger than the lowest cost, quoted by Sanchez *et al.* (2016).

A South African industrial quote, obtained for a stainless steel fermentation beer tank, fitted with air sparging, cooling jacket and nozzles for pump-around mixing, falls well within the range of fermenter costs at US$1.6 million. However, to be conservative, the literature value obtained by the methodology provided for a jacketed, glass lined reactor is deemed suitable for use in the capital estimate (Sinnott, 2005), since it falls within the range of available costs. The tank cost suitable for enzymatic hydrolysis, is the NREL saccharification tank on the lower end of the range, at approximately US$580 000. This may be due to the tank design required, such as stirring and aeration (Humbird *et al.*, 2011).

2.7.3 Techno-economic biorefinery case studies

Fernandez-Dacosta *et al.* (2015) investigated PHA production from waste water and compared various downstream processing scenarios. Moncada *et al.* (2013) investigated PHB production

from glucose rich detoxified hydrolysates from cane juice, molasses or bagasse, depending on the scenario configuration. The cost of sugarcane presented the largest contribution to the cost of all the raw materials (consumables) in the biorefinery. Santos *et al.* (2016) compared six biorefinery scenarios and found that the scenario for the production of succinic acid was the only economically competitive scenario, compared to syn-gas production, ethanol production and energy generation. No case studies were found for the production of itaconic acid from lignocellulosic biomass.

Other case studies for biorefinery techno-economic analyses include biodiesel production from lower-cost feedstocks such as recycled cooking oils, and wastes from animal or vegetables oil processing operations (Marchetti, Miguel and Errazu, 2008; Apostolakou *et al.*, 2009). Gnansounou, Vaskan and Pachon (2015) conducted a techno-economic and environmental analysis for biorefinery scenarios for ethanol production from simple sugar substrates or first generation feedstocks (1G), lignocellulosic or second generation feedstocks (2G) and combined 1G/2G feedstock. The best economic results are achieved for the largest capacity ethanol plant and the best environmental results were found for an integrated sugar mill and 1G/2G ethanol production. This shows that the best economic scenario is not necessarily the best environmental scenario, and that a trade-off exits.

2.8 Environmental benefit of bio-based products

The environmental impact of a product can be estimated through a life cycle analysis (LCA). A life cycle analysis (LCA) is a tool that is used to quantify and qualify the environmental impact of a process within a set boundary or scope (Petersen, 2012). It is used to determine the environmental sustainability or impact of a process with regards to parameters such as global warming, fossil fuel depletion, human toxicity, acidification, particle matter formation and eutrophication (Petersen, 2012; Silalertruksa, Pongpat and Gheewala, 2017).

The following steps can be included within the LCA scope: raw materials supply, manufacturing process, packaging, distribution, usage by the customer and finally the disposal, recycling or reuse (Renó *et al.*, 2011). This life cycle is shown in Figure 2-13. The cycle starts with the raw materials, which are then turned into a product, packaged and distributed before it reaches the end user. The end user then either reuse, recycle or dispose of the product, reaching the end of its life. The biorefinery is the manufacturing step, which the project scope is limited to.

The first stage of a LCA is to define the goal, boundary scope and functional unit. The steps in a product's life cycle can be used to determine the scope of a LCA. The second stage is to compile a life cycle inventory (LCI), after which the LCI is used to evaluate the potential environmental impact of each item listed in the inventory. The final stage is to interpret the environmental impacts (Reno *et al.*, 2011).

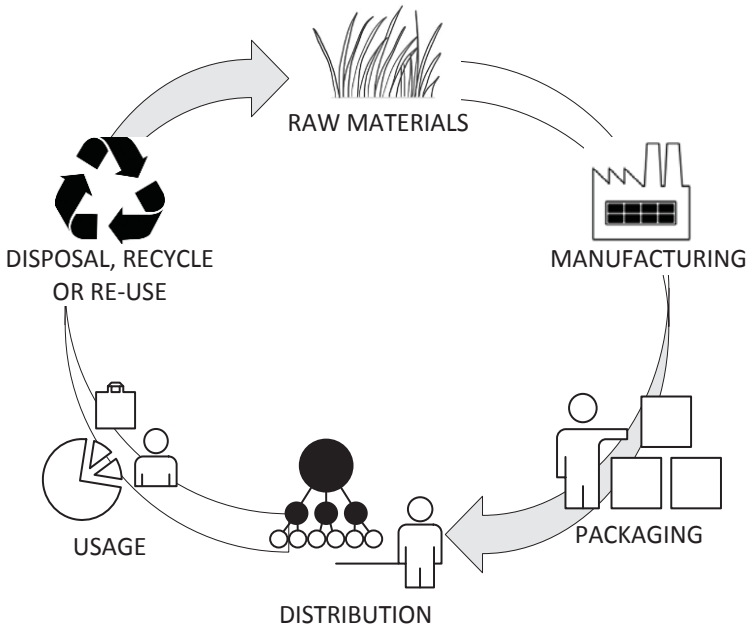

Figure 2-13: Generic product life cycle (self-drawn)

Lignocellulose is a good alternative to fossil based carbon sources since the CO_2 released during manufacturing or decomposition of the products, are fixated by the plant during its lifetime (Reno *et al.*, 2011). However, lignocellulose cannot be viewed in isolation from the agricultural practices required to grow and harvest the plant. Pryor *et al.* (2017) investigated the impact of South African sugarcane agricultural practices on energy use and greenhouse gases (GHGs). The total fossil energy inputs (gas, oil and coal) for non-irrigated plantations are between 2500-3000 MJ per tonne sucrose produced ($MJ.t_{SU}^{-1}$) and irrigated plantations are between 3000-4000 $MJ.t_{SU}^{-1}$ (Pryor *et al.*, 2017). Fertilisers contribute most to non-irrigated plantations (1450 $MJ.t_{SU}^{-1}$) and electricity contributes the most to the fossil fuel inputs of irrigated plantations (1550 $MJ.t_{SU}^{-1}$). Diesel, electricity, fertilisers, herbicides and pesticides are taken into consideration by evaluating the amount of fossil fuels, such as coal, oil and gas, required to manufacture the fuels and chemicals (Pryor *et al.*, 2017).

The cane burning harvesting method contributes 100 kg CO_2-$e.t_{SU}^{-1}$ and moving to green cane harvesting (manual or mechanised) can decrease net GHG emissions by 15 % (Pryor *et al.*, 2017). Although mechanisation itself will increase energy consumption and carbon emissions

(Pryor *et al.,* 2017), if the sugarcane residues are utilised in a CHP plant, the net fossil fuel impact will still decrease, since electricity from the CHP plant displaces electricity derived from coal (Silalertruksa, Pongpat and Gheewala, 2017).

The CHP also allows the biorefinery to strive towards being carbon neutral, since no additional electricity, generated from coal, is used in the production of sugar, energy or the co-product (i.e. succinic acid, itaconic acid or PHA's), which limits the amount of additional GHG's that are liberated from fossil fuels (Silalertruksa, Pongpat and Gheewala, 2017). Additional carbon fixing during the manufacturing of a bio-based product in a biorefinery is also advantageous for the reduction in GHG's (Pandey *et al.,* 2015). The fermentation route for the production of succinic acid uses CO_2, and results in a total of 30 – 50% less net fossil energy consumption than the petroleum based production route (Orjuela *et al.,* 2013; Tan *et al.,* 2014).

The final life cycle step of a product can have a significant impact on the environment, especially if the product cannot be recycled or reused. Conventional plastics take decades to decompose and produce toxins in the process, or end up in the marine environment and contribute to marine and aquatic eco-toxicity. Consequently, a need exists for bio-based and biodegradable plastics (Suriyamongkol *et al.*, 2007). PHA's, together with polylactic acid, polysaccharides and aliphatic polymers are completely biodegradable (Reddy *et al.,* 2003; Chen, 2009). Polybutylene succinate, which is produced from succinic acid and PHA's, can be degraded to water and carbon dioxide under microbial aerobic degradation and methane under anaerobic degradation in soil, oceans, lakes and sewage (Khanna and Srivastava, 2004).

The increased awareness of global warming and the detrimental impact of GHG's on the environment, has made society more aware of their consumer habits and fossil fuel dependency. It is vital that our fossil fuel dependency should decrease due to the environmental damage caused by the excess CO_2 and other GHGs released into the carbon cycle, as well as the fact that fossil reserves are diminishing and alternative feedstocks are required (Suriyamongkol *et al.*, 2007). This supports the drive for biorefinery implementation and the use and manufacture of bio-based products (Carole, Pellegrino and Paster, 2004; Akhtar, Idris and Abd. Aziz, 2014).

LCA's on bio-based products from sugarcane only included the raw materials and manufacturing life cycle steps in the LCA scope (Renó *et al.*, 2011; Amores *et al.*, 2013). A LCA has been done on South African sugarcane production, using the GHG emissions and

energy use parameters to determine the environmental impact (Pryor *et al.*, 2017). However, the GHG emissions and energy use have not been investigated for the manufacturing (i.e. the biorefinery) step of the bio-based products succinic acid, itaconic acid and PHA's from sugarcane.

Chapter 3

3. Process design and economic analysis of a biorefinery co-producing itaconic acid and electricity from sugarcane bagasse and trash lignocelluloses

From the literature study, it is seen that the annual production of itaconic acid is approximately half of the global production capacity. Due to its high cost relative to fossil based equivalents, itaconic acid is seen as a niche building block chemical with a limited current market, even though it has a large potential markets for detergent builders, thermoplastics, absorbent polymers, and polyester resins. To this end, this chapter focuses on determining *why* this specific bioproduct biorefinery has such a high selling price and poor techno-economic results.

The first aim was to determine if a lignocellulosic feed provides a better financial outcome than glucose, considering the capital and operational expenditures associated with pretreating the lignocellulose to obtain fermentable sugars. The second aim was to compare an energy self-sufficient itaconic acid biorefinery with a coal-supplemented biorefinery. Finally, it was also determined which key bioconversion parameter should be improved, on an experimental level within the context of a biorefinery, to further decrease the cost of itaconic acid production. The results of this study contributed to Objective 1 and 2 as stated in section 1.2.

The key outcomes of this chapter are the two itaconic acid sugarcane biorefinery simulations, one with a coal supplemented CHP plant, and the other with a bioenergy self-sufficient energy system. The coal supplemented biorefinery is profitable due to the economies of scale benefit obtained for a large processing capacity for the total biomass feedstock. The bioenergy self-sufficient scenario is not profitable, and therefore an assessment of the key process parameters was done to determine which process parameter could be optimised to progress towards a commercially viable itaconic acid biorefinery. Contrary to current research efforts, the focus should be directed away from improved product concentration (i.e. improved titres) to an increased itaconic yield on fermentable sugars through improved pentose sugar utilization.

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Declaration by the candidate:

With regard to Chapter 3, pg. 56-84, the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Scope definition, biorefinery design and simulation work, economic costings, interpretation of results and writing of manuscript.	80

The following co-authors have contributed to Chapter 3, pg. 56-84:

Name	e-mail address	Nature of contribution	Extent of contribution (%)
J.F. Görgens	jgorgens@sun.ac.za	Biorefinery concept project definition, providing writing assistance through review and proof reading of final manuscript and general discussion.	10
K. Haigh	khaigh@sun.ac.za	Provided writing assistance through suggestions, continual review and proof reading of article and general discussion.	10

Signature of candidate: ……

Date: 28/01/2019……..

Declaration by co-authors:

The undersigned hereby confirm that

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 3, pg. 56-84,
2. no other authors contributed to Chapter 3, pg. 56-84, besides those specified above, and
3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 3, pg. 56-84, of this dissertation.

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	Stellenbosch University	
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Process design and economic analysis of a biorefinery co-producing itaconic acid and electricity from sugarcane bagasse and trash lignocelluloses

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Abstract

Itaconic acid has economic potential as a commodity biochemical for the sugar industry, but its production is limited due to high production costs. Using cheaper and alternative lignocellulosic feedstocks together with achieving higher product titres have been identified as potential strategies for viable IA production. Consequently the use of sugarcane bagasse and trash for the production of itaconic acid (IA) and electricity have been investigated for an integrated biorefinery, where the production facility is annexed to an existing sugar mill and new combined heat and power (CHP) plant. Three IA biorefinery scenarios were designed and simulated in Aspen Plus®. Subsequent economic analyses indicated that cheaper feedstocks reduced the IA production cost from 1565.5 US$/t for glucose to 616.5 US$/t, but coal supplementation was required to sufficiently lower the production cost to 604.3 US$/t for a competitive IA selling price of 1740 US$/t, compared to the market price of 1800 US$/t.

Keywords: Biorefinery, lignocellulose, sugarcane bagasse, itaconic acid, combined heat and power (CHP) plant.

3.1 Introduction

The drive towards sustainable manufacturing and decreasing our fossil fuel dependency has led to the investigation of green- or biochemicals (Koutinas et al., 2014). These biochemicals are produced from renewable biomass and can replace their fossil based equivalents. One such a biochemical is itaconic acid. Itaconic acid (IA) has potential as a commodity biochemical due to its wide range of applications in the agricultural, pharmaceutical and medical fields (Kuenz et al., 2012; Okabe et al., 2009). This organic acid is used as a co-monomer for the production of detergent builders, thermoplastics, surfactants, polymers and polyester resins (Okabe et al., 2009; Weastra, 2011). It was first discovered by Baup in 1836 as a product of citric acid distillation (Klement and Büchs, 2013; Okabe et al., 2009; Weastra, 2011), but is commercially produced through submerged fermentation with the fungi *Aspergillus terreus* (Klement and

Büchs, 2013; Kuenz et al., 2012). However, it is currently seen as a niche chemical with low industrial relevance (Shekhawat et al., 2006) due to its high production cost and selling price (Okabe et al., 2009).

To promote IA from a niche chemical to a commercially produced biochemical, the price of IA should be competitive with end-use fossil based equivalent chemicals such as acrylic acid and maleic anhydride (Weastra, 2011). This could lead to an almost ten-fold expansion of the current IA market from 41 400 tonnes (2011) to 407 790 tonnes in 2020 (Weastra, 2011), ultimately building towards sustainable development and environmental conservation (Huang et al., 2014; Okabe et al., 2009; Werpy and Petersen, 2004). Factors contributing to the high cost of production are high feedstock costs of glucose and molasses, and the fermentation challenges of low titre and productivity seen for *A. terreus* (Klement and Büchs, 2013; Krull et al., 2017; Shekhawat et al., 2006).

Early fermentation improvements focused on achieving a higher IA yield from glucose (Yahiro K et al., 1995), with recent studies focusing more on improving productivity and titre, aiming to achieve titres similar to that of citric acid production at 360 g/L (Hevekerl et al., 2014; Klement and Büchs, 2013; Krull et al., 2017; Kuenz et al., 2012). A reproducible and consistent titre of 86.2 g/L, though at a low productivity of 0.51 g/L/hr, was obtained by Kuenz *et al.,* (2012) using optimised nutrient media. The nutrient media conditions were further improved for the highest reported productivity to date of 1.15 g/L/hr (Hevekerl et al., 2014). Furthermore, the IA titre was improved to 129 g/L (Hevekerl et al., 2014) and 160 g/L using a pH shift and control during fermentation (Krull et al., 2017). Although the IA titres obtained to date are not as high as that of citric acid, the improved IA titres together with a cheaper, alternative feedstock, could result in a viable commercial IA process.

Alternative feedstocks such as hydrolysate (wheat bran, wood or corn syrup), corn starch (Okabe et al., 2009; Wu et al., 2017), and horticulture waste (Reddy and Singh, 2002) can replace glucose and molasses as feedstock (Mondala, 2015; Willke and Vorlop, 2001). Molasses is cheaper than glucose, at 100 US$/t compared to 580 US$/t (Humbird, 2011; Vieira et al., 2016), but no significant advances have been made for the fermentation parameters (Hashizume et al., 1966; Sumanjali et al., 2010). Sugarcane molasses contains 18.9 % water, 31.8 % sucrose, 17.11% invert sugars (i.e. glucose and fructose) with 32.3 % constituents such as minerals and ash (Hashizume et al., 1966). The IA yield on sucrose is low, at 38.7 % molar yield, compared to 80 % molar yield for glucose (Sumanjali et al., 2010). Consequently, the

titre achieved is also low at 27 g/L IA, compared to 160 g/L for glucose (Krull et al., 2017; Sumanjali et al., 2010). Glucose, together with other fermentable sugars can be obtained from cheaper lignocellulosic feedstocks (Benjamin, 2014).

Sugarcane bagasse is a cheap and abundant lignocellulosic feedstock. In 2013, 17.3 million tonnes of South African sugarcane were harvested, yielding 5.9 million tonnes of bagasse as by-product (Mbohwa, 2013). Sugarcane bagasse is the milled and crushed cane fibre residue after sugar juice extraction and contains 35-50% cellulose, 26.2-41% hemicellulose, 11.4-25.2% lignin and 2.9-1% other components, including 1.4% ash, which can be converted into simple sugars through pre-treatment and enzymatic hydrolysis (Benjamin, 2014; Borges and Pereira, 2011; Nanda et al., 2014; Xi et al., 2013). Currently the bagasse is burned in low efficiency boilers to produce steam and electricity for the sugar mill (Mbohwa, 2013). However, surplus bagasse and trash can be obtained by the introduction of green harvesting methods and high efficiency boilers (Ali Mandegari et al., 2017a; Venkatesh and Roy, 2011). This excess bagasse and trash can be valorised as feedstock for biochemical production and co-generation of steam and electricity in a combined heat and power (CHP) plant.

To this end, an IA facility can be integrated with a CHP plant and annexed to an existing sugar mill to form a biorefinery complex (Ali Mandegari et al., 2017b). The available lignocellulosic feedstock can therefore be split between the IA facility and CHP. If the IA facility is energy intensive, more of the available feedstock would have to be used in the CHP for energy generation. Alternatively the CHP can be supplemented with coal, allowing more of the biomass to be used as feedstock. However, this is not desirable due to the detrimental environmental impact of greenhouse gases and the contribution to human toxicity caused by burning coal (Ali Mandegari et al., 2017b). Using an energy efficient CHP can also result in the production of excess electricity. By selling the excess electricity together with the IA biochemical, additional revenue can be generated from a viable biorefinery. This can assist in extending the sugar industry's sustainability, which is vital to its 430 000 employees and approximately 1 million dependants (Sugar Milling Research Institute NPC, 2016).

The aim of this study is to investigate whether the recent IA production improvements, namely higher titres and the use of a cheaper carbon feedstock (Okabe et al., 2009; Willke and Vorlop, 2001), such as sugarcane bagasse, will sufficiently decrease the cost of IA production in order to result in a commercially viable IA biorefinery. Alternative substrates to glucose, such as xylose, starch, molasses and lignocellulosic feedstocks have been used for IA production at

laboratory scale (Klement and Büchs, 2013; Magalhães et al., 2017; Mondala, 2015; Willke and Vorlop, 2001). However, to the authors' knowledge, this study will be the first to design and simulate the process flow sheets for IA production from lignocellulosic biomass, followed by an economic analysis to determine and compare the viability of using an alternative substrate for IA production, to glucose.

To this end, the first objective is to develop and describe the process for producing IA from sugarcane lignocellulose. The second objective is to determine if a lignocellulosic feed provides a better financial outcome than glucose, considering the capital and operational expenditures associated with pre-treating the lignocellulose to obtain fermentable sugars. The final objective is to determine if titre is the best process parameter to improve within the context of a biorefinery to further decrease the cost of IA production.

3.2 Process Design and Economic Methods

3.2.1 Process Design

3.2.1.1 Process Simulation

Aspen Plus® version 8.8 was used to simulate the IA biorefinery. It was assumed that reported laboratory scale data will be applicable to an industrial process. Therefore the results are adequate for a conceptual level of study, and could be verified and optimised using a pilot plant prior to implementation. The waste water treatment (WWT) and combined heat and power (CHP) plant simulation and physical properties for the feedstock components are based on work developed previously (Ali Mandegari et al., 2017a; Humbird, 2011; Leibbrandt, 2010; van der Merwe, 2010). The base property method is the electrolyte Non-Random Two Liquid (ELECNRTL) property method (Ali Mandegari et al., 2017a; Gorgens et al., 2016). However, the equation of state (EOS) is adapted for single units, where required, such as the NRTL-HOC (Hayden-O'Connell) property method for IA recovery in the downstream process (DSP), or steam property IAPWS-95 for the boiler and condensing extraction turbine (CEST).

Stoichiometric reactor blocks are used for the pretreatment reactor, enzymatic hydrolysis reactor, and fermentation tanks. A separator block is used for the granular activated carbon (GAC) adsorption column, based on the furfural and hydroxymethylfurfural (HMF) removal rates reported (Hodge et al., 2009) and a water recovery yield of 90 wt % for the reverse osmosis (RO) membrane (McFall et al., 2008). The aerobic digestion, clarifier and dewatering steps are modelled as a single centrifuge block with a 10 % solids loss to the liquid fraction

(Görgens *et al.*, 2016) and all centrifuge blocks solid outlet streams are specified for a moisture content of 50 %. Pumps are specified for an assumed discharge pressure of 2 atm with a pump efficiency of 75 % and a mechanical efficiency of 95 %. For cellulignin washing, a water to solid ratio of 2:1 is used.

3.2.1.2 Process Scenarios

The IA production facility and CHP is annexed to an existing sugar mill, and together these facilities are grouped together as a biorefinery. The IA production facility is termed the new products plant (NPP). The sugar mill produces 135 $t.hr^{-1}$ wet bagasse and trash (50 % moisture) and requires 120 t/hr steam at 28 atm and 340 °C (Gorgens et al., 2016). The steam is provided by the CHP, and used by the sugar mill's existing back pressure steam turbines to produce electricity for the sugar mill. Therefore, the CHP only produces electricity for the NPP. It is assumed that any excess electricity produced is sold back into the network (Ali Mandegari et al., 2017b).

The IA process was simulated for three scenarios: lignocellulosic feed for both the NPP and CHP (scenario A1), glucose feed for the NPP and lignocellulosic feed for the CHP (scenario A2) and lignocellulosic feed for the NPP and a coal supplemented CHP (scenario A3). The glucose feed stream in scenario A2 was sterilised at 121 °C for 20 min prior to fermentation (Sumanjali et al., 2010). Only the process description for the base case (scenario A1) is provided.

3.2.1.3 Process Description

A mixture of 70 % bagasse and 30 % trash (by weight) is used as a combined feedstock to the CHP and NPP, resulting in a feedstock with 40.7 % cellulose, 27.1 % hemicellulose, 21.9 % lignin, 6.7 % extractives and 3.5 % ash (Ali Mandegari et al., 2017a). The block flow diagram (BFD) of the IA biorefinery is shown in Figure 1. Since the sugars found in lignocellulosic biomass are in the form of complex carbohydrates, pretreatment and enzymatic hydrolysis are required to hydrolyse the carbohydrates to simple sugars. The biomass is soaked with 0.65 % H_2SO_4 and heated to 180 ˚C for 10 minutes (Benjamin 2014). Due to the mass transfer limitations met for an experimental setup reactor design, Benjamin (2014) used a 1:20 solid to liquid ratio and a fluidised sand bath to heat up the experimental tubular reactor, which is an unrealistic approach for a commercial application. Since the solids loading does not influence the pretreatment severity, it is assumed that the mass transfer limitations between the acid catalyst and lignocellulosic biomass can be addressed with the proper reactor design. To this end, the feedstock is diluted and heated by directly injecting steam at 320 ˚C and 9.5 atm into

a screw reactor with a 1:2 w/v solid to liquid ratio (Humbird, 2011). The pretreatment reactions and conversion efficiencies are provided in Table 1. A fraction of the cellulose is converted to glucose and cellobiose and most of the hemicellulose is converted to xylose, and some inhibitors furfural and HMF. Acetic acid is produced as a by-product during pretreatment and inhibits IA production.

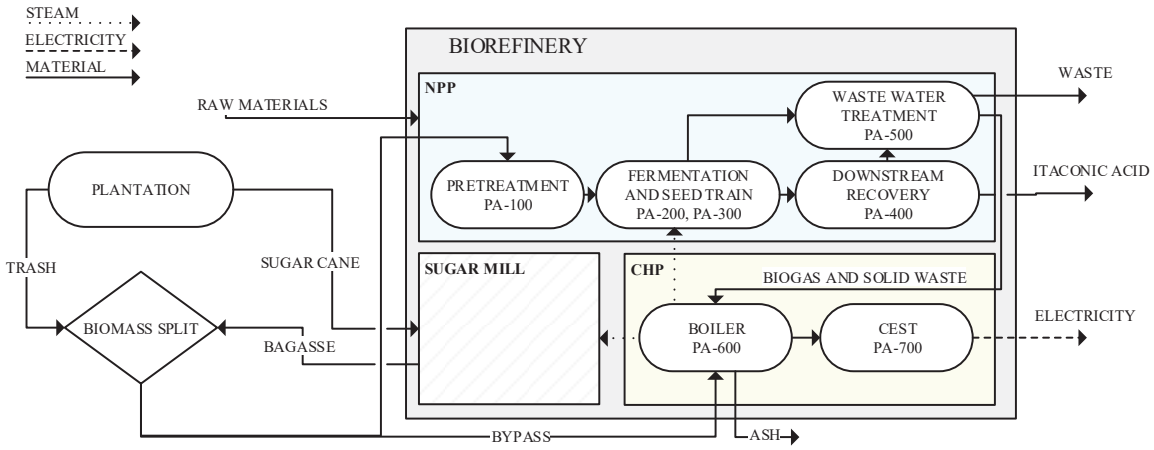

Figure 3-1: Biorefinery Block Flow Diagram (CEST – condensing extraction turbine, CHP – combined heat and power plant, DSP – downstream process, NPP – new product plant, WWT – waste water treatment plant)

The pretreated material is separated and the cellulignin (solid fraction) is washed to remove residual hydrolysate and inhibitors prior to enzymatic hydrolysis. An enzyme dosage of 20 mg/g (enzyme protein per gram dry mass cellulignin) is used for a solids loading of 20 wt % and feed temperature of 50 ˚C (Humbird, 2011) for 72 hours (Benjamin, 2014). The hemicellulose hydrolysate and diluted process water stream from the washing step is detoxified using a granular activated carbon (GAC) column, removing HMF and furfural (Hodge et al., 2009). The enzymatic hydrolysis reactions and conversion efficiencies are provided in Table 1. The combined sugar stream from pretreatment and enzymatic hydrolysis is concentrated to 180 g/L glucose (Hevekerl et al., 2014) in a single effect evaporator, which further detoxifies the sugar by removing 92 wt % of the acetic acid. This step also sterilises the sugar prior to fermentation. The added nutrients (Hevekerl et al., 2014) are sterilised at 120 ˚C for 15 min, where 3 g/L NH_3 is used in the reactor stoichiometry replacing ammonium nitrate (Leibbrandt, 2010).

Table 3-1: Dilute acid pretreatment reactions and fractional conversions used in combined sugar yield pretreatment (Benjamin, 2014) and enzymatic hydrolysis (Leibbrandt, 2010; Humbird, 2011)

PRETREATMENT REACTIONS	FRACTIONAL CONVERSION (g/g)	(mole basis)
Cellulose + H_2O → Glucose	0.048	0.043
2*Cellulose + H_2O → Cellobiose	0.006	**0.003**
Hemicellulose + H_2O → Xylose	0.831	0.731
Hemicellulose → Furfural + 2*H_2O	0.058	0.080
Arabinan + H_2O → Arabinose	0.923	0.812
Cellulose + H_2O → 3*Acetic Acid	0.081	0.219
Cellulose → HMF + 2*H_2O	0.002	**0.003**
ENZYMATIC HYDROLYSIS REACTIONS	**FRACTIONAL CONVERSION (g/g)**	**(mole basis)**
Cellulose + H_2O → Glucose	0.760	0.684
Cellulose + H_2O → Cellobiose	0.012	**0.006**
Cellobiose + H_2O → 2*Glucose	1.000	**0.474**

The fermentation is carried out in a batch stirred fermentor using *Aspergillus terreus* DSM 23081. For the base case a glucose molar yield of 80 %, xylose molar yield of 46.2 %, productivity of 1.15 g/L/hr and an initial glucose concentration of 180 g/L (Hevekerl et al., 2014; Kautola et al., 1990) is simulated. The IA conversion from arabinose, cellobiose and xylose are found in Larsen and Eimhjellen (1954). Stoichiometrically, one mole of glucose produces a mole of IA, shown in Table 2 (Klement and Büchs, 2013; Kuenz et al., 2012; Steiger et al., 2013) followed by the stoichiometric reactions for IA production from xylose, arabinose and cellobiose, respectively. By-products include malic acid ($C_4H_6O_5$), succinic acid ($C_4H_6O_4$) and α-ketoglucaric acid ($C_5H_6O_5$). The fumaric acid ($C_4H_4O_4$) production is negligible (Huang et al., 2014). The fractional conversions of these by-products and the assumed growth and maintenance reactions are calculated from the initial glucose concentration and balanced with oxygen and water molecules, based on 1 mole of sugar (Huang et al., 2014; Leibbrandt, 2010; van der Merwe, 2010). The fermentation temperature is 35 ˚C and although an initial pH control of between 2.9 – 4.9 is required (Hevekerl et al., 2014; Kuenz et al., 2012), it is adjusted and controlled at 3 using an ammonia solution after 2.1 days when product formation starts (Hevekerl et al., 2014).

Table 3-2: Itaconic acid, by-product (Larsen and Eimhjellen, 1954; Klement and Buchs, 2013; Steiger, 2013; Hevekerl, Kuenz and Vorlop, 2014; Huang et al., 2014), growth and maintenance (Leibbrandt, 2010; van der Merwe, 2010) stoichiometric reactions and conversions

FERMENTATION MICRO-ORGANISM REACTIONS	FRACTIONAL CONVERSION (molar yield)
IA PRODUCTION	
Glucose + 1.5*O_2 → Itaconic acid + CO_2 + 3*H_2O	0.800
Xylose + 0.5*O_2 → Itaconic acid + 2*H_2O	0.462
Arabinose + 0.5*O_2 → Itaconic acid + 2*H_2O	0.156
Cellobiose + 1.2*O_2 → 2.4*Itaconic acid + 3.8*H_2O	1.000
BY-PRODUCT FORMATION	
Glucose + 1.5*O_2 → 1.5*Malic acid + 1.5*H_2O	0.00167
Xylose + 1.25*O_2 → 1.25*Malic acid + 1.25*H_2O	0.0011
Glucose + 1.2*O_2 → 1.2*α·keto Glucaric acid + 2.4*H_2O	0.0084
Xylose + O_2 → α·keto Glucaric acid + 2*H_2O	0.0055
Glucose + 0.75*O_2 → 1.5*Succinic acid + 1.5*H_2O	0.0115
Xylose + 0.625*O_2 → 1.25*Succinic acid + 1.25*H_2O	0.0076
GROWTH AND MAINTENANCE REACTIONS[a]	
Glucose + 1.1429*NH_3 → 5.7143*Micro-organism + 2.5714*H_2O + 0.2857*CO_2	0.085
Xylose + 0.9524*NH_3 → 4.7619*Micro-organism + 2.1429*H_2O + 0.2381*CO_2	0.043
Glucose + 6*O_2 → 6*H_2O + 6*CO_2	1.000
Xylose + 5*O_2 → 5*H_2O + 5*CO_2	1.000

a) Reactions occur in series

The DSP scheme is based on the industrial process, using two evaporation and crystallisation steps, followed by discolouration, final crystallisation and drying (Chenyu Du, 2014; Okabe et al., 2009; Pfeifer et al., 1952). The DSP for IA is shown in Figure 2. The first evaporator is a triple effect evaporator and reduces the volume of the stream by 75 % (Chenyu Du, 2014; Pfeifer et al., 1952), to a concentration above 350 $g.L^{-1}$ IA (Okabe et al., 2009). The second step is batch crystallisation at 15 ˚C for 16 hours (Okabe et al., 2009; Pfeifer et al., 1952). IA has a solubility of 95 $g.L^{-1}$ at 295 K (Hogle et al., 2002). After the first crystallisation step the crystals are separated from the liquid using a basket filter and sent to the decolourisation step. The permeate fraction undergoes another single-effect evaporation and crystallisation step (Pfeifer et al., 1952). The second separation step permeate is discarded to the WWT plant. The crystal particle size distribution determined for water-soluble crystals in aqueous slurries is used (Miller, 1978). The crystals are decoloured using 2 % (w/v) activated carbon at 80 ˚C for 30 min (Okabe et al., 2009; Pfeifer et al., 1952). The overall recovery of IA from the fermentation product stream to the dried crystals is 82.3 % with 95 % recovery in the filtration

step, 98 % recovery in the evaporation steps and 95 % in the crystallisation and drying steps (Okabe et al., 2009). The final crystal product purity was > 99 % (Pfeifer et al., 1952). The filtered *A. terreus* cells and vapour streams from the DSP evaporation steps are combined in the WWT area. The design and process flow diagram is based on the cellulosic ethanol model WWT by Steinwinder *et al.*, (2011).

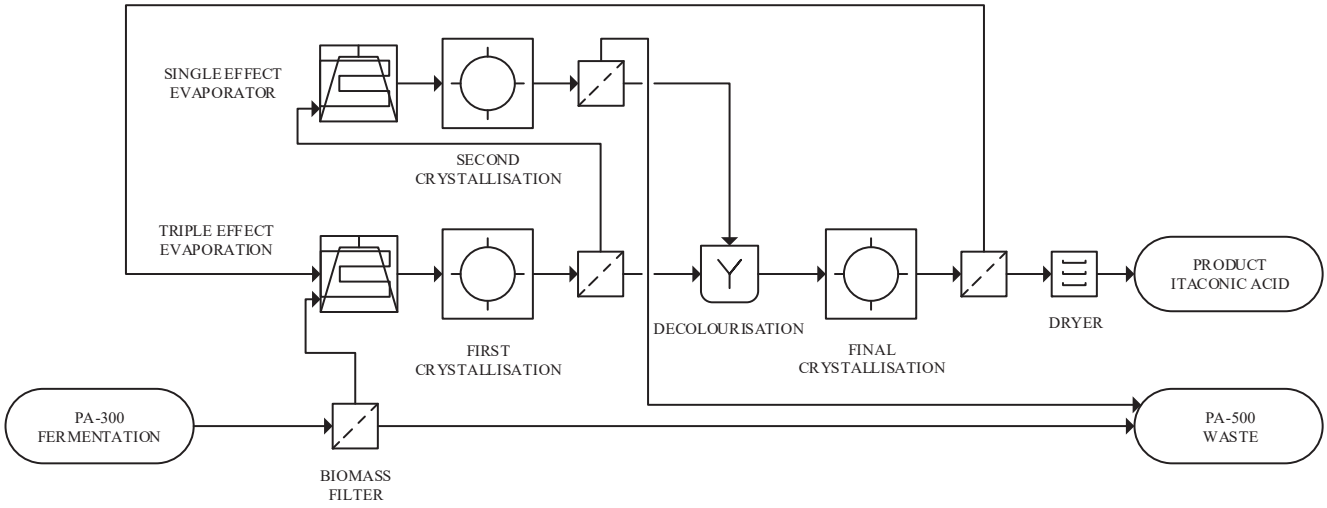

Figure 3-2: Downstream processing scheme for the separation and purification of IA (Okabe et al., 2009)

All the NPP waste streams are collected and fed to a mesophilic anaerobic biodigester (AD). The set of AD stoichiometric reactions are based on the work by Peris, (2011) and Tenneti, (2015). The product streams from the AD are biogas and sludge. The biogas (methane, carbon dioxide and small traces of hydrogen sulphide and nitrogen) is combined with the boiler feed and sent to the CHP, equipped with a limestone absorbent to reduce sulphur oxide emissions (Steinwinder et al., 2011). The biogas line is also equipped with a flare. The AD sludge is treated by aerobic digestion and fed to a clarifier where the sludge is pumped to a centrifuge, concentrated, and conveyed to the CHP section (Humbird, 2011). The centrifuge liquid stream is pumped to a RO membrane modular plant (Gorgens et al., 2016; Humbird, 2011). The brine retentate from the RO plant is treated by a multiple effect evaporator (Ali Mandegari et al., 2017a), increasing the dry matter concentration to 50 wt % for combustion, since the selected boiler can treat a brine feed stock (Gorgens et al., 2016; Steinwinder et al., 2011). The RO permeate is recycled back to the NPP as process water. A fire water tank and pump is included for safety purposes. Vapour from the evaporation unit is condensed in a flash drum and the brine liquid fraction is disposed of. Since a black box approach to the sugar mill is used, the simulation only includes the high pressure steam that is provided to the sugar mill for the existing primary movers and back pressure steam turbines (BPST). The low efficiency (28 atm)

boiler is removed and replaced by a high efficiency and pressure (63 atm) boiler included in the CHP plant. The BPST remain in the sugar mill and condensing extraction steam turbines (CEST) are added in the CHP. Cooling and chilled water utilities are used at 28 °C and 4 °C at 2 atm, respectively.

3.2.2 Economic evaluation

The Aspen Plus Economic Analyser® was used to determine the purchased and installed cost for the majority of equipment in the process flowsheet (Vlysidis et al., 2011). Purchased costs not available in the Aspen Plus Economic Analyser® database include the boiler, fermentation tanks, cellulase plant, clarifier, reverse osmosis plant, flare, and CEST, and were sourced from literature, (Humbird, 2011; Wooley et al., 1999). The purchased cost of equipment was adjusted to the desired capacity and relevant time of study (Vlysidis et al., 2011) using the Chemical Engineering Plant Cost Index (CEPCI) of 536.5 for the year 2016. Cooling and chilled water are not included as operational costs, but as a installed cost, contributing 6.5 % of the total NPP installed cost (Ali Mandegari et al., 2017a).

Once the installed costs are calculated, the fixed capital investment (FCI) is determined by adding direct and indirect costs. Direct costs are associated with a Greenfields installation such as the NPP and calculated as a fraction of the NPP purchased equipment cost: for the warehouse (4 %), site development (9 %) and additional infrastructure (4.5 %). Indirect costs (pro-rateable expenses, 10 % field expenses, 20 % home office and construction fees, 10 % contingency and 10 % other costs for smaller items such as travel for site visits, permits and accommodation) are calculated as a fraction of the total direct costs (TDC). These fractions are guidelines, based on previous studies of the same level estimate (Ali Mandegari et al., 2017a; Humbird, 2011).

The TDC and indirect costs are added to provide the fixed capital investment (FCI). For South Africa, a location factor of 1 is applied for the corrected FCI, together with an allowance for working capital (5 % of the corrected FCI), resulting in the total capital investment (TCI) (Ali Mandegari et al., 2017a). The total cost of production (TCOP) includes variable operating costs, fixed operating costs and general expenses. The variable operating costs include raw materials, waste streams and labour. Raw material costs include the feedstock, sulphuric acid for pretreatment, boiler chemicals and nutrients for fermentation.

The fixed operating costs include labour, labour overheads, plant maintenance, property taxes and insurance. The labour rates are based on an ethanol biorefinery (Gorgens et al., 2016). The

maintenance is taken as 3 % of the NPP installed cost and the property taxes and insurance is taken as 0.7 % of the total FCI (Ali Mandegari et al., 2017a). The annual capital charge consists of items that are purchased at intervals during the year, such as the GAC used for hydrolysate detoxification and the enzyme nutrients. The cost of raw materials are shown in Table 6.

The minimum required selling price (MRSP) is used as an indication of the biorefinery profitability for a discount rate of 9.7 %, based on a real term discounted cash flow (DCF) rate of return analysis. The MRSP indicates the selling price required for a net present value (NPV) of 0 US$ during the plant life. Economic assessment assumptions used for the DCF are shown in Table 3. An economic sensitivity analysis will investigate the impact of the economic parameters on the plant profitability to identify which parameters are most sensitive to change thus representing the greatest investment risk.

Table 3-3: Economic parameters (Alimandegari, Farzad and Görgens, 2016)

PARAMETERS	VALUE
Annual operating hours	6480 h
Project life	25 years
Discount rate	9.7 % for real term DCF analysis
Income tax rate	28 %
Inflation rate	5.7 %
Depreciation	Straight line method applied over 5 years (i.e. 20 %)
Salvage value	0
Construction period	2 years
% Spend in year -2	10 %
% Spend in year -1	60 %
% Spend in year 0	30 %
Working capital	5% of fixed capital investment, C_{TM}
Start-up time	2 years
First year NPP capacity (% design)	50 %
Second year NPP capacity (% design)	75 %
Selling price: Electricity	0.08 US$/kWh

3.3 Results and Discussions

Three scenarios were compared:

- Scenario A1: IA produced from 46 % of the sugarcane lignocelluloses, with energy co-generation in the CHP from 56 % of the bypassed lignocelluloses and NPP residues for energy self-sufficiency of the biorefinery and sugar mill.
- Scenario A2: IA produced from glucose, with energy co-generation in the CHP from 100 % of the lignocelluloses and NPP residues for energy self-sufficiency of the biorefinery and sugar mill, as well as sellable electricity production.
- Scenario A3: IA produced from 100 % of the lignocelluloses, with energy co-generation in the CHP from the NPP residues and coal.

The fermentable sugars produced in PA-100 for scenario A1 and A3 were concentrated to 180 g/L glucose, and fed at 180 g/L glucose in Scenario A2, as basis for comparison. Furthermore, the amount of glucose fed was selected in order to obtain the IA product rate similar to scenario A1. The base case scenario A1 is the desired process configuration, resulting in an energy self-sufficient biorefinery. Scenario A2 was included to show and compare the economic outcome of using different feedstocks, while scenario A3 was included to show the economic potential of utilising all the sugarcane lignocelluloses for IA production in an energy reliant scenario by supplementing the CHP with coal. The three scenarios have the same process flow sheet design, with the exception of different bypass ratios and the exclusion of the pretreatment and enzymatic hydrolysis plant area (PA-100) for scenario A2.

3.3.1 Mass and energy balance

The most important mass and energy balance results are provided in Table 4, which includes the bypass ratio of lignocellulose from the NPP to the CHP, IA titre, IA produced, excess (sellable) electricity produced, total power and steam consumption and the amount of coal required for scenario A3. For the lignocellulosic fed NPP's, scenario A1 resulted in a production rate of 5.6 t/hr IA from 29.9 t/hr dry mass (DM), and scenario A3 resulted in 12.2 t/hr IA from 65 t/hr DM, whereby the CHP was supplemented with 25.1 t/hr coal.

Table 3-4: Mass and Energy balance results per scenario

PARAMETERS	UNIT	A1 Base case	A2 Glucose	A3 Coal fed
Mass balance				
Bypass ratio	%	54	100	0
Biomass[a] feedstock to NPP	t/hr	29.9	0	65
Glucose feedstock to NPP	t/hr	0	10.0	0
IA titre[b]	g/L	148	88.4	146
IA produced	t/hr	5.6	5.6	12.2
Energy balance				
Sellable electricity	MWh	5.8	42.1	5.1
Total power consumption	MWh	1.5	1.5	2.2
Coal	t/hr	0	0	25.1
HPU[c] required	t/hr	133.5	79.5	288.9

a) dry basis, b) Measured after fermentation and cell removal, c) HPU: high pressure utility, high pressure steam at 320 °C and 9 atm.

On the other hand, GHG emissions generated by burning bagasse and trash in the CHP are biogenic and therefore considered carbon neutral. An added environmental advantage of using a lignocellulosic feedstock is that scenario A1 and A3 did not require additional process water, since the biomass has a 42.5-50 %wt inherent moisture content and water was formed during the anaerobic digestion of the unfermented carbohydrates in the WWT. Washing and dilution process water, together with the inherent and produced water, were recycled in the WWT plant. For the glucose fed NPP in scenario A2, 5.8 t/hr process water was required to produce 5.6 t/hr IA.

Since all the sugarcane lignocelluloses were fed to the NPP in scenario A3, the NPP has the largest plant capacity and highest steam requirement (288.9 t/hr). Where the lignocellulosic feedstock was split between the NPP and CHP for scenario A1, a bypass rate of 54 % was required to generate the required HPU steam rate (133.5 t/hr). This is a high bypass rate compared to an annexed cellulosic ethanol plant, where 35 % of the available excess bagasse and trash was bypassed from the plant to the CHP (Ali Mandegari et al., 2017b).

The high bypass rate was due to an energy intensive IA production process, caused by the high pretreatment steam consumption, the DSP technology (i.e. evaporation and crystallisation) and pre-concentration of the sugar feed stream to fermentation. Concentration of the fermentation feed stream was only required for scenarios A1 and A3 since the pretreated solids were washed and diluted prior to enzymatic hydrolysis.

The wash water, containing pre-treatment inhibitors as well as valuable soluble sugars, was added to the hemicellulose hydrolysate stream prior to detoxification, which further diluted the final sugar stream used for fermentation. Therefore the fermentation feed stream for scenario A1 and A3 was concentrated to 180 g/L glucose using an evaporation unit. This unit is the most steam intensive piece of equipment, using 48 %wt of the total HPU steam required by the NPP in scenarios A1 and A3, followed by the steam consumption for the pretreatment using 30 %wt of the total HPU steam. Since the glucose fed scenario A2 did not require pre-treatment or a single effect evaporator, only 79.5 t/hr HPU steam was required.

The production rate of IA in scenario A1 can therefore be increased by lowering the NPP steam demand, which will lower the bypass rate of lignocellulosic feedstock from the NPP to the CHP, and subsequently increase the amount of feedstock available for the NPP. This may result in a more profitable plant due to economies of scale (Gorgens et al., 2016). Steam consumption can be reduced by using less steam intensive process technology and pretreatment methods. For example, downstream process (DSP) technology such as adsorption, membrane separation and reactive extraction separation technology could replace crystallisation and evaporation (Magalhães et al., 2017).

Due to the steam intensive nature of the process equipment required, excess electricity was produced in the CHP. The CHP's in scenarios A1 and A3 produced 5.8 MWh and 5.1 MWh, respectively. Since all the available feedstock was fed to the CHP in scenario A2, the maximum amount of sellable electricity was produced from the available lignocelluloses (42.1 MWh) and sold as co-product to IA and sucrose from the annexed sugar mill.

Viewing the steam consumption and electricity production in isolation, scenario A2 is preferred to scenario A1 or A3. However, for an initial glucose sugar concentration of 180 g/L, the lignocellulosic fed scenarios had higher IA titres (148 and 146 g/L for scenario A1 and A3, respectively) compared to scenario A2 (88.4 g/L) obtained after fermentation, due to a higher combined initial sugar concentration for glucose and pentose sugars obtained from the pretreated lignocellulosic feedstock.

Consequently, the IA yield on pentose sugars present in the feed stream increased the product titres for scenarios A1 and A3. Overall, the 88.4 g/L IA titre obtained for scenario A2 was comparable to 86.2 g/L reported by Kuenz *et al.*, (2012) for an initial glucose concentration of 180 g/L, after batch fermentation. However this was low when compared to the high IA titres

reported for the fed-batch fermentation strategy used by Hevekerl, Kuenz and Vorlop, (2014) with 129 g/L and Krull *et al.,* (2017) with 160 g/L, which were obtained by adding solid glucose to the fermentation broth. More sugar was added to the fermentation broth at intervals to prevent substrate inhibition, since the glucose concentration continually decreased as the micro-organism metabolised the sugars to biomass, IA and by-products, resulting in a total glucose concentration below 180 g/L.

Simultaneously, no additional water was added to the fermentation broth when feeding solid glucose (Hevekerl et al., 2014; Krull et al., 2017), resulting in IA titres higher than 86.2 g/L (Kuenz et al., 2012). Even with the additional IA produced from the pentose sugars present in the feed stream for scenario A1 and A3, the simulated IA titres (148 and 146 g/L, respectively), were still lower than the reported 160 g/L IA titre (Krull et al., 2017).

The solid glucose fed-batch fermentation strategy (used for the high titres reported) is problematic for commercial scale lignocellulose-fed IA biorefineries, since it is not realistic to concentrate the sugars obtained from pretreated lignocelluloses very high or to a solid stream, considering the high amount of energy that would be required. Consequently, it is not possible to add sugar to the fermentation broth without inherently adding water as well.

The literate data used for the Aspen Plus® process flow sheet designs were sufficient for this concept study. The mass and energy balances results were comparable to literature and could be used in the economic analysis to determine the IA production costs and minimum required selling prices for a lignocellulosic feedstock discussed in the next section.

3.3.2 Economic analysis

3.3.2.1 Results: Capital and Operation costs

The mass and energy balances were used to size each piece of equipment and determine the installed and operational costs for all the scenarios. The total installed equipment cost for each plant area are provided in Table 5, together with the total capital investment (TCI). The pretreatment (PA-100) area for scenario A1, which includes the dilute acid pretreatment, hemicellulose hydrolysate detoxification and cellulignin enzymatic hydrolysis, contributed 51.2 % of the total NPP installed cost. Of which the enzymatic hydrolysis tanks contributed 44 % of the total NPP installed equipment cost. The fermentation (PA-300) area was the second most expensive NPP area, where the fermenters' installed cost contributed 28 % of the total

NPP capital cost. The large cost contribution of the fermentation area was in line with that previously reported (Vlysidis et al., 2011).

The TCI for scenario A2 was 24.5 % lower than the TCI for scenario A1, since there is no pretreatment area or cellulase production module, a smaller waste water treatment (WWT) plant and less utilities required. The CHP installed cost for scenario A2 was slightly larger since all the lignocelluloses were burned for energy. The TCI for coal supplemented scenario A3 was 74.4 % higher than scenario A1, due to the higher plant capacity required to process 65 t/hr DM, compared to 29.9 t/hr DM for scenario A1.

The total operating cost, i.e. the cost of production (TCOP) break down is provided in Table 6. The variable cost of production (VCOP), which includes the raw materials, consumables and waste disposal, was higher for scenario A3 than scenario A2. This is primarily due to the cost of coal, which contributed 47.3 % of the VCOP for scenario A3. The VCOP for scenario A2 was the highest at 46.97 million US$ per annum (M$/yr) due to the high feedstock cost of glucose (21.7 M$/yr), compared to sugarcane lignocellulose (4.54 M$/yr).

Consequently, the 46.97 M$ VCOP for scenario A2 was 5.2 times more than the VCOP for scenario A1, at 9.01 M$/yr. Due to the low feedstock cost, the raw materials contribution of scenario A1 to the TCOP was only 38.5 %, which is comparable to the 45 % obtained for IA production from dimethyl succinate and formaldehyde reported by Shekhawat, Jackson and Miller, (2005). Since the maintenance, property taxes and insurance were calculated as a percentage of the NPP installed cost, the fixed cost of production (FCOP) reflects the TCI. Therefore a high FCOP (25.75 M$) was seen for a high TCI (662.9 M$) as per scenario A3.

Table 3-5: Total Capital Investment (TCI) and Minimum Required Selling Price (MRSP) per scenario

SCENARIOS	A1 Base case	A2 Glucose	A3 Coal supplemented
Bypass ratio	54 %	100 %	0 %
PROCESS AREA INSTALLED COST	**M$**	**M$**	**M$**
PA-100: Pretreatment and EH	66.7	0	137.6
PA-200: Seed train and cellulase production	11.1	5.8	16.7
PA-300: Fermentation	37.6	58.5	74.5
PA-400: Downstream processing	7.1	8.7	11.7
PA-500: Waste water treatment	7.8	6.0	10.8
Total NPP Installed cost	**130.3**	**79.0**	**251.4**
PA-600 & PA-700: CHP	58.3	68.9	70.3
PA-800: Utilities and Storage (11.1 % of NPP)	15.0	9.1	28.9
Total Installed cost	**203.5**	**157.0**	**350.6**
Warehouse (4 % of NPP)	5.2	3.2	10.1
Site Development (9 % of NPP)	11.7	7.1	22.6
Additional Piping (4.5 % of NPP)	5.9	3.6	11.3
Total Direct Costs (TDC)	**226.3**	**170.8**	**394.6**
Total Indirect costs (60 % of TDC)	**135.8**	**102.5**	**236.7**
Fixed Capital Investment (FCI)	**362.1**	**273.3**	**631.3**
Working capital (5 % of FCI)	18.1	13.7	31.6
Total Capital Investment (TCI)	**380.2**	**287.0**	**662.9**
MRSP (US$/t)	**2000**	**2157**	**1740**

CHP – Combined heat and power plant, EH – Enzymatic hydrolysis, MRSP – Minimum required selling price, NPP – New Products plant.

Table 3-6: Total Operating Cost (TOC) per annum and production cost per scenario

PARAMETERS	PRICE US$/kg	A1 Base case M$/yr	A2 Glucose M$/yr	A3 Coal supply M$/yr	Ref.
Bypass ratio	-	54 %	0 %	0 %	-
Operating hours per year	6480 hr				Gorgens et al., 2016
Total Feedstock cost (to NPP and CHP)	0.0108	4.54	4.54	4.54	Ali Mandegari, Farzad and Görgens 2016
Sulphuric acid	0.094	0.18	-	0.38	Tao *et al.*, 2011
Nutrient medium:	0.595	1.18	1.68	2.28	Tao *et al.*, 2011, Efe, van der Wielen and Straathof, 2013
Glucose	0.58	-	37.59	-	Humbird, 2011
Make-up water	0.21 US$/t	-	0.10	-	Gorgens et al., 2016
Inoculum, boiler and cooling tower chemicals	-	2.53	2.53	2.53	*Footnote a*
Coal[f]	0.057	-	-	9.27	Ali Mandegari, Farzad and Gorgens et al., 2016
Waste stream: disposal of ash	28.86	0.58	0.62	0.59	Gorgens et al., 2016
VCOP[e]	**-**	**9.01**	**46.97**	**19.60**	-
Total labour cost[c]	-	3.47	3.47	7.15	Gorgens et al., 2016
Maintenance (3 % of NPP installed cost)		7.35	4.46	14.18	
Property taxes and insurance (0.7 % of FCI)		2.53	1.91	4.42	
FCOP		**13.36**	**9.84**	**25.75**	**-**
Activated carbon charge	1.2[d]	0.05	0.00	0.10	Mussatto et al., 2013
Enzyme nutrients	0.53	0.67	0.00	2.32	Humbird, 2011
ACC	-	**0.72**	**0.00**	**2.42**	-
TCOP	(VCOP+FCOP+ACC)	**22.37**	**56.81**	**47.77**	
Production cost	(US$/t)	**616.5**	**1565.5**	**604.3**	

a) Hydrazine at 2.5 US$/kg, cooling tower chemicals 2679.6 US$/kg, and Boiler chemicals at 4519.1 US$/kg (Görgens *et al.,* 2016), b) industry quote, c) based on 35 % bypass cellulosic ethanol plant, and scaled to plant capacity., d) based on a purge cycle every 6 months, for 24 hours residence time, e) by-product electricity is included in the DCF, and utilities are included in TCI as % of NPP installed cost, f) Heating value of 23,25 MJ/kg coal.

3.3.2.2 Discussion: Economic assessment

The total capital investment (TCI) and total cost of production (TCOP) were used in a discounted cash flow (DCF) rate of return analysis for a real term discount rate of 9.7 %, to determine the minimum required selling price (MRSP). A biorefinery scenario is considered to be potentially viable if the MRSP is below 1800 US$/t, since the IA selling price is within the range 1800 – 2000 US$/t. This price range is applicable for the project life span of 25 years, since constant costs are used in a real term DCF analysis. Therefore the cost of consumables or IA and electricity selling prices are not adjusted for future trends or fluctuations (as for a nominal DCF), but are taken as constant. Consequently, a discount rate of 9.7 % was used and not 15.4 % as for the nominal DCF, which takes the expected inflation rate of 5.7 % into account.

As a result, scenario A3 had the lowest MRSP of 1740 US$/t, making it the most favourable scenario even though the capital and operational costs for scenario A3 were higher than scenario A1. The TCI was larger due to a larger plant capacity and the TCOP for scenario A3 included the cost of coal. Furthermore, the biorefinery in scenario A3 produced 2.2 times more IA than the biorefinery in scenario A1, thus generating more revenue per tonne of feedstock. Therefore, even with the cost of coal inflating the TCOP, the production cost (i.e. cost per tonne of IA produced) for scenario A3 was the lowest. However, this scenario is unfavourable from an environmental viewpoint, due to detrimental environmental impacts such as greenhouse gas (GHG) emissions and contribution to human toxicity caused by burning coal (Ali Mandegari et al., 2017a).

The desired strategy is to rather improve the MRSP of 2000 US$/t for the energy self-sufficient biorefinery in scenario A1 to result in an economically favourable scenario. The use of a cheap lignocellulosic feedstock results in a lower MRSP than using glucose as feedstock. The MRSP for the glucose fed scenario A2 was 2157 US$/t, which is more than the reported 2000 US$/t market price. Even though scenario A2 had the lowest TCI (287.0 M$) of all the scenarios, and produced 42.1 MWh sellable electricity, it did not generate sufficient revenue from its electricity sales to justify the high capital cost (68.9 M$) of co-generation required for the CHP plant.

Therefore, despite having the lowest TCI and generating the most revenue from electricity sales, the MRSP for scenario A2 (2157 US$/t) was higher than the MRSP for scenario A1 and

A3, at 2000 US$/t and 1740 US$/t, respectively, due to its high operating cost. To this end, the capital cost required for the pretreatment area equipment, to obtain fermentable sugars from the lignocellulosic feedstock, was justified by the low feedstock cost of sugarcane lignocelluloses bagasse and trash, compared to glucose.

The production cost for lignocellulosic scenarios A1 and A3 were 616.5 US$/t and 604.3 US$/t IA, respectively, compared to scenario A2's production cost of 1565.5 US$/t IA. The production cost of lignocellulosic scenarios A1 and A3 are comparable to other organic acids, such as lactic acid (LA). The production cost of LA, co-produced from brewer's spent grains in a biorefinery with xylitol, activated carbon and phenolic acids, is 860 US$/t (Mussatto et al., 2013). However, co-producing IA and electricity is not favourable compared to the co-production of LA and ethanol from sugarcane bagasse and trash. For a selling price of 2000 US$/t, an IRR of 9.7 % is achieved for scenario A1, compared 18.9 % achieved for the LA and ethanol biorefinery scenario (Gorgens et al., 2016).

Although advances have been made in terms of higher titres and cheaper feedstocks, which contribute to lower MRSPs, further developments to the technology will be required for a commercially viable, energy self-sufficient, itaconic acid biorefinery. In order to identify the required research endeavours, the impact of key process parameters on the MRSP were assessed.

3.3.3 Assessment of the impact of the key process parameters

The current research trend is to increase the IA titre value to that of citric acid (360 g/L) (Klement and Büchs, 2013). Product titre reduces the size and thereby capital and operational cost of the DSP. However, within the context of a biorefinery, the DSP only contributed 4 % of the total installed equipment cost and required no raw materials or general expenses for the operating costs for the base case scenario A1. Therefore, the key process parameters were investigated to confirm if increased titres are indeed the right approach, or whether improved yields or residence times are not perhaps more suitable to decrease the production costs for a viable IA biorefinery.

To this end, a 25 % increase and decrease (from the reported base case scenario A2 values) for IA yield on glucose, IA yield on xylose, the volumetric productivity (i.e. residence time) and the initial sugar concentration were investigated to determine which process parameter(s) could be improved for a viable biorefinery. To ensure an energy self-sufficient scenario the bypass

rate was adjusted for each change and the respective mass and energy balances were used to calculate the TCI and TCOP and used in a DCF analysis to determine the MRSP.

The 25 % increase in values are purely hypothetical, since the most favourable conditions reported to date were used in the simulation, and increased yields and titres might cause adverse fermentation effects, such as substrate and product inhibition. These adverse effects are not reflected by the simulation, since the molar yields are static input values. Therefore, the purpose of changing the process parameters is to evaluate the impact of each respective change on the MRSP. This will provide an indication of the specific research efforts that would be most beneficial in the development of commercially viable IA processes. The MRSP for each alternative scenario (-25%, base value, +25 %) are shown in Figure 3.

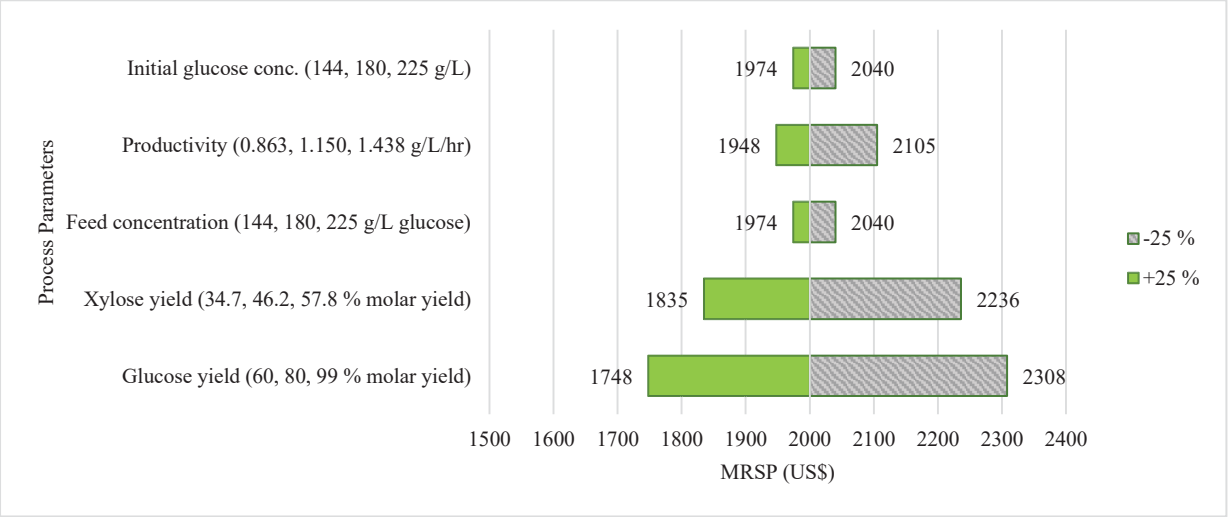

Figure 3-3: Alternative process scenarios (solid bar: 25 % increase and hatched bar: 25 % decrease in process parameter) for scenario A1

IA titre is a function of volumetric productivity, IA yield on fermentable sugars, and initial sugar feed concentration. Therefore if the productivity increases for a fixed residence time and feed concentration, the IA titre will increase. Likewise, if the initial sugar concentration increases, for a fixed residence time and IA yield, the titre will increase while the productivity decreases. The initial sugar feed concentration was varied by changing the energy input to the single effect evaporator in the pretreatment area, to remove excess water, and thus concentrating the sugar stream. A 25 % change in initial glucose concentration and productivity do not have a significant impact on the biorefinery's viability. Although increases in productivity and initial sugar concentration decreases the MRSP, changing these parameters was not the most effective method to achieve a viable biorefinery.

Conversely, if IA yield on fermentable sugars increase, for a fixed residence time and initial glucose concentration, the titre and productivity will both increase. The fermentable sugar yields were varied by increasing or decreasing the molar yield of the respective IA producing stoichiometric reactions. To this end, a 25 % change in the IA yield on glucose had the largest impact on the MRSP. For a 25 % increase in the IA yield on glucose, scenario A1 became favourable at a MRSP of 1748 US$/t. Although the 25 % increase was hypothetical, achieving a 99 % glucose (molar) yield may prove challenging due to the current genetic engineering techniques and fermentation strategies and since glucose is consumed for microbial cell maintenance.

Although unrealistic, it demonstrates that the IA yield on glucose should not be sacrificed in order to obtain a higher IA titre (using higher initial sugar feed concentrations) as done previously. Studies reported an IA titre increase from 129 g/L to 160 g/L, but the glucose yield decreased from 80.3 % (molar) yield to 63.7 % (Hevekerl et al., 2014; Krull et al., 2017). Furthermore, the biorefinery profitability was second most sensitive to the IA yield on xylose. A 25 % increase in the xylose molar yield from 46.2 % to 57.8 %, decreased the MRSP to 1835 US$/t, and may be more realistic to obtain experimentally (Borges and Pereira, 2011). Consequently, research efforts could be directed towards genetic engineering of IA producing micro-organisms to improve the IA yield on xylose or hemicellulose hydrolysate for reported feed concentrations and residence times. Improving IA yields on xylose is the preferred strategy, compared to improved product titres, for a viable IA lignocellulosic biorefinery.

3.3.4 Economic sensitivity analysis

For the economic sensitivity analysis the impact of change in economic parameters on plant profitability were investigated. The change in MRSP for a 25 % increase and decrease in each economic parameter was evaluated for the energy self-sufficient scenario A1. The MRSP was most sensitive to changes in the IA product rate, FCI and TCOP, as shown in Figure 4. The MRSP decreased from 2000 US$/t to 1597 US$/t for a 25 % decrease in FCI, and to 1600 US$/t for a 25 % increase in IA product rate, resulting in a favourable biorefinery scenario.

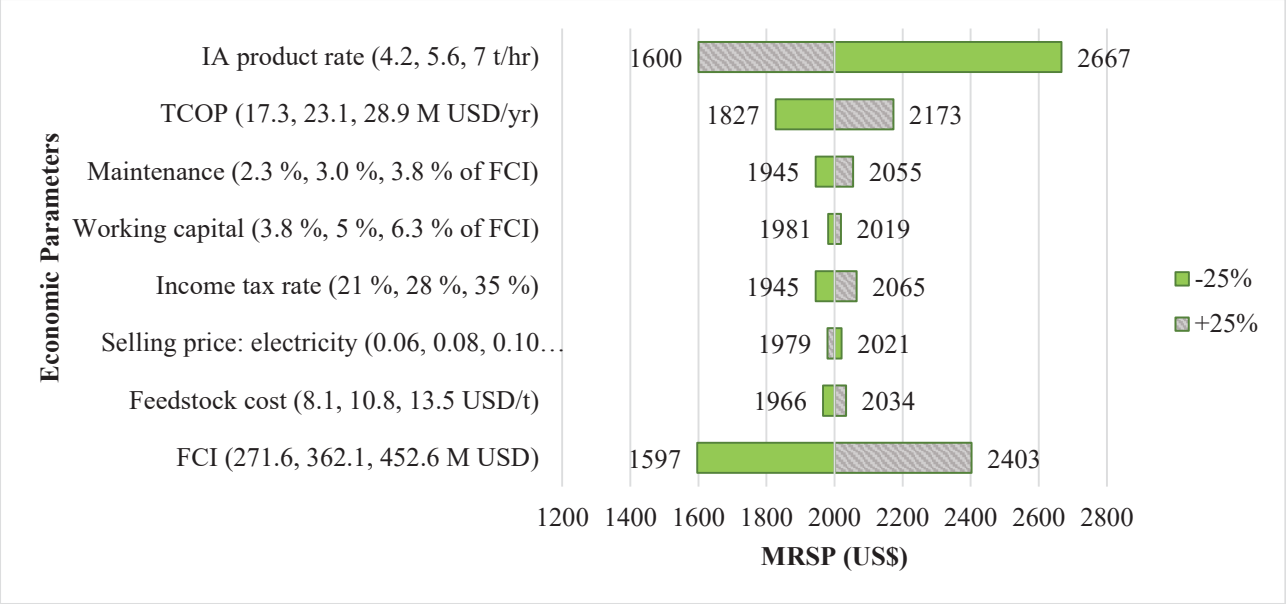

Figure 3-4: Economic sensitivity analysis (solid bar: 25 % decrease and hatched bar: 25 % increase in economic parameter) for scenario A1

It may be possible to decrease the FCI using the simultaneous saccharification and co-fermentation (SSCF) fermentation strategy (Ali Mandegari et al., 2017), since the enzymatic hydrolysis tanks and fermenters are the major installed cost contributors (i.e. 72 % of the total NPP installed cost). The SSCF strategy allows the enzymatic hydrolysis and fermentation steps to be combined. Although the pH required for enzymatic hydrolysis is 4.8 – 5 (Benjamin, 2014; Humbird, 2011) and the optimum pH for IA fermentation is 3.1 (Hevekerl et al., 2014), *A. terreus* can still produce IA at a higher pH, but with less favourable fermentation results (Sumanjali et al., 2010). Therefore it could be relevant to determine if the reduction in installed capital costs, using an SSCF scheme, would be sufficient to justify less favourable fermentation conditions. It is therefore recommended to conduct SSCF experiments with *A. terreus* to obtain the data for simulation.

Considering the impact of key fermentation process parameters in section 3.3, a less favourable IA titre and volumetric productivity might be acceptable, while a decrease in glucose yield would not. A change in product rate reflects a change in the IA MRSP, since a higher product rate causes a larger income generated from IA sales, but the product rate is also related to changes in the IA yields on glucose and xylose during fermentation, as well as the fermentable sugars yields on lignocellulose during pretreatment. Therefore the MRSP will decrease if these yields increase, as seen for the fermentation yields in the assessment of key fermentation process parameters in section 3.3.

The MRSP is least sensitive to changes in the working capital, the electricity selling price and the feedstock cost. The commercial viability will not be impacted significantly if less electricity is sold. Therefore alternative, electricity rather than steam intensive pretreatment methods and DSP technologies, such as AFEX™ or reactive extraction and adsorption, could be used to decrease the biorefinery's steam demand and increase the NPP capacity. These technologies will not impact the FCI significantly, since they are not necessarily more expensive than crystallisation and evaporation (Magalhães et al., 2017).

3.4 Conclusion

The IA production cost was decreased using a lignocellulosic feedstock and resulted in a favourable coal supplemented IA biorefinery with a MRSP of 1740 US$/t. However, the MRSP obtained (2000 US$/t) for an energy self-sufficient biorefinery was not favourable, since it was higher than the IA market price (1800 US$/t). Improved titres could be obtained by increasing the IA yield on pentose sugars. Overall the process improvements made in IA production cause an IA biorefinery, annexed to a CHP and existing sugar mill, to be a realistic endeavour with great market potential for the sugarcane industry.

Supplementary information

E-supplementary data for the pretreatment and DSP Aspen Plus® NPP process flowsheet diagrams, stream tables and the design and operating conditions for each Aspen Plus® model unit can be found in the e-version of this paper online.

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Chapter 4

4. Process design and economic evaluation of an integrated, multiproduct biorefinery for the production of bioenergy, succinic acid and polyhydroxybutyrate (PHB) from sugarcane bagasse and trash lignocelluloses

In the previous chapter it was investigated why the bioenergy self-sufficient itaconic acid biorefinery resulted in an unprofitable scenario, by comparing it to a coal supplemented and a glucose fed biorefinery, followed by an assessment of the key process parameters. The coal supplemented biorefinery resulted in favourable techno-economic results. Although coal supplemented PHB and succinic acid biorefineries could also have been investigated, it was not deemed necessary since the techno-economic results of the bioenergy self-sufficient scenarios were profitable at the current bioproduct selling prices.

However, some uncertainty exists regarding the selling price of PHB, which has a significant impact on the scenario's profitability. Therefore, the relationship between the production volume and selling price was taken into account for the techno-economic investigation in this study. Moreover, due to the utilisation of different sugars by the succinic acid microorganism, *Actinobacillus succinogenes*, and the PHB producing microorganism, recombinant *Escherichia coli*, it was investigated whether the biorefinery system could be optimised by combining the production of these two bioproducts into a multiproduct biorefinery. This resulted in a number of potential scenarios for the production of succinic acid and PHB from sugarcane lignocelluloses.

To this end, this study investigated the profitability of a biorefinery co-producing i) electricity and succinic acid, ii) electricity and PHB, iii) both succinic acid and PHB with electricity, and iv) electricity. The economic analysis considered the selling prices together with the production volumes and market shares to determine the economic viability of each scenario. The results of this study contributed to Objective 1 and 2 as stated in section 1.2.

The key outcomes of this chapter are the respective Aspen Plus® simulation models, together with the market perspective of how the production volumes can impact the economic outcome. As a result, economies of scale, which has previously been pursued for low value, high volume bioproducts such as biofuel, should not necessarily be pursued in the same manner for low volume, high value bioproducts. Instead, high value bioproducts could be combined in a multiproduct biorefinery, where the economies of scale benefit is seen for shared process areas, such as the pretreatment plant, CHP (combined heat and power) plant and WWT (waste water treatment) plant. Consequently, the multiproduct biorefinery scenario for the production of electricity, succinic acid and PHB was the most profitable with an IRR of 24.1% and a NPV of 447.2 million US$.

Although not considered in this study, it should be noted that there are 14 major sugar mills operating in South Africa, and that the relationship between the bio-product production volume and selling price was only taken into account for a biorefinery integrated into one typical South African sugar mill. If all the sugar mills are to implement the same biorefinery scenario, the impact of the total bioproduct production volume will have to be taken into consideration. Alternatively, other multiproduct plant biorefinery options could be implemented, such as the production of xylitol and glutamic acid, depending on the bioproduct market demand or potential of South Africa.

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Process design and economic evaluation of integrated, multi-product biorefineries for the co-production of bio-energy, succinic acid and polyhydroxybutyrate (PHB) from sugarcane bagasse and trash lignocelluloses

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Keywords:	Bio-energy, biorefinery, lignocelluloses, polyhydroxybutyrate (PHB), sugarcane bagasse, succinic acid

Declaration by the candidate:

With regard to Chapter 4, pg. 89-125, the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Scope definition, biorefinery design and simulation work, economic costings, interpretation of results and writing of manuscript.	80

The following co-authors have contributed to Chapter 4, pg. 89-125:

Name	e-mail address	Nature of contribution	Extent of contribution (%)
J.F. Görgens	jgorgens@sun.ac.za	Biorefinery concept project definition, providing writing assistance through review and proof reading of final manuscript and general discussion.	10
K. Haigh	khaigh@sun.ac.za	Provided writing assistance through suggestions, continual review and proof reading of article and general discussion.	10

Signature of candidate: MWH ……

Date: 28/01/2019 ……..

Declaration by co-authors:

The undersigned hereby confirm that

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 4, pg. 89-125
2. no other authors contributed to Chapter 4, pg. 89-125, besides those specified above, and
3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 3, pg. 88-123, of this dissertation.

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Process design and economic evaluation of integrated, multi-product biorefineries for the co-production of bio-energy, succinic acid and polyhydroxybutyrate (PHB) from sugarcane bagasse and trash lignocelluloses

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Abstract

This study investigates whether a biorefinery, annexed to an existing sugar mill and co-producing succinic acid, polyhydroxybutyrate (PHB) and electricity from sugarcane bagasse and trash lignocelluloses, will be a viable investment opportunity for existing sugar cane mills. Four scenarios were simulated in Aspen Plus® and included in the economic analysis: Scenario A for the production of PHB and electricity, Scenario B for the production of PHB, succinic acid and electricity, Scenario C for the production of succinic acid and electricity, and Scenario D for the production of electricity only. The most favourable configuration was found for Scenario B where PHB is produced from 25 % of the fermentable glucose stream, and succinic acid from the hemicellulose hydrolysate together with 75 % of the glucose, resulting in an internal rate of return (IRR) of 24.1 % with a net present value of 477.2 million US$. Alternatively, Scenario D could be selected if low capital (130.1 million US$) and operational costs (13.2 million US$) are desired, although weak returns (IRR 10.3 % and Net Present Value 6.08 million US$) were observed for an electricity price of 0.08 US$/kWh.

Keywords: Bio-energy, biorefinery, lignocelluloses, polyhydroxybutyrate (PHB), sugarcane bagasse, succinic acid

4.1 Introduction

4.1.1 Background information

Lignocellulosic biomass can be converted into bio-fuels, bio-products and bioenergy in a biorefinery. When a biorefinery that is integrated with an established production chain such as an existing sugar mill it has multiple advantages, some of which are reduced feedstock transport costs and constant seasonal availability. A single feedstock type, or blend of lignocelluloses such as bagasse and trash, also reduces the feed variability and eliminates the need to design for a range of biomass feedstocks within the same biorefinery (Giuliano *et al*., 2016b). When

the biorefinery is integrated with a combined heat and power (CHP) plant, it is possible to facilitate energy self-sufficiency for both the biorefinery and the existing sugar mill. Consequently, biorefineries could lead to sustainable production chains and if energy self-sufficient, it can eliminate or decrease the detrimental environmental impacts caused by the use of fossil fuels to meet energy demands (Cherubini and Jungmeier, 2010). A multi-product biorefinery co-producing bioproducts and bio-energy, while annexed to an existing sugar mill and integrated with a CHP plant, may cause new markets to open up and generate additional income revenue for the sugar industry.

Of the 14 cane growing countries in Africa, South Africa is the largest sugarcane producer (Pryoer *et al.,* 2017). The South African sugar industry is thus key to the agricultural economy, but is faced with decreasing profit margins due to challenges such as increasing operational costs, droughts and low international sugar prices (Myers *et al*., 2017). South Africa processes 19 million tonnes of sugar,(Pryor et al., 2017) and 8 million tonnes of bagasse each year.(Mashoko et al., 2013) The bagasse is currently being burnt in low efficiency boilers to produce steam and energy for the sugar mill (Mashoko et al., 2013). However, a portion of this bagasse could be made available for valorisation by replacing the existing boiler with a high pressure, high efficiency boiler unit (Ali Mandegari *et al*., 2017a; Venkatesh and Roy, 2011). Similarly, trash (sugar cane tops and leaves) can be made available by introducing green harvesting methods (Görgens *et al*., 2016). Bagasse, together with trash, can be valorised as a lignocellulosic feedstock for the production of biofuels, -products and -chemicals in a biorefinery (Ali Mandegari *et al*., 2017a, 2017b; Clauser *et al*., 2015; Görgens *et al*., 2016; Nieder-Heitmann *et al*., 2018). The implementation of profitable biorefinery to an existing sugar mill may revitalise the rural economy by contributing to the sugar industry's economic sustainability. This solution is supported by the Sugarcane Technology Enabling Programme for bio-energy (STEP-Bio), initiated by the SMRI (sugar milling research institute) and the DST (Department of Science and Technology) (SMRI, n.d.).

Multiproduct plant biorefineries have been investigated for the production of furfural, xylitol, medium-density fibreboard (MDF) and electricity from sugarcane lignocelluloses, of which the production of xylose syrup and furfural combined with MDF was profitable with reported internal rates of return (IRR) of 16% and 19%, respectively (Clauser *et al*., 2015). Other examples include the co-production of ethanol, lactic acid, furfural, butanol, methanol and electricity from sugarcane bagasse and trash in various scenarios, of which ethanol and lactic

acid co-production was the most profitable with a reported IRR of 25.4% (Farzad *et al*., 2017). More recently, succinic acid and the polyhydroxyalkanoate (PHA), polyhydroxybutyrate (PHB), have been identified as suitable to include in the range of multi-product biorefineries (Booysen *et al*., 2016). However the profitability thereof has not been investigated to date.

4.1.2 Bioproduct overview

4.1.2.1 Succinic acid

Succinic acid (SA) is a dicarboxylic acid ($C_4H_6O_4$), and has been listed in both the 2004 and 2010 United States Department of Energy's list of the top 12 value-added chemicals that can be derived from biomass (Cheng *et al*., 2012; Luo *et al*., 2010; Salvachúa *et al*., 2016; Werpy and Petersen, 2004). It has been investigated as a co-product for multi-product biorefineries in several case studies and can be co-produced with levulinic acid and ethanol from hardwood, eucalyptus residues, wheat straw and olive tree pruning (Giuliano *et al*., 2016a, 2016b). SA has also been produced with ethanol, acetic acid and electricity from corn-stover for different biorefinery configurations (Luo *et al*., 2010).

SA has a wide range of applications in the food and pharmaceutical industry (Akhtar *et al*., 2014). It is also used as a solvent and as an ion chelator (Akhtar *et al*., 2014). SA has the potential to replace industrial chemicals such as benzene-derived chemicals, tetrahydrofuran and maleic anhydride. The most investigated micro-organisms for the production of SA are *Anaerobiospirillum succiniciproducens*, *Actinobacillus succinogenes, Mannheimia succiniciproducens, Escherichia coli, Corynebacterium glutamicum, Saccharomyces cerevisiae, Bacteroides fragilis* and *Lactobacillus plantarum* (Brink and Nicol, 2014; K. K. Cheng *et al*., 2012; Tan *et al*., 2014).

From these, *A. succinogenes* has been identified as one of the most promising strains for industrial SA production (Li *et al*., 2010). Besides glucose, *A. succinogenes* can also utilise glycerol, sucrose, maltose, lactose, fructose, arabinose, galactose, mannose and xylose (Borges and Pereira, 2011; Shen *et al*., 2015; Song and Lee, 2006). This micro-organism has been genetically modified to improve its productivity obtained from sugarcane bagasse and trash hydrolysate fermentation. The productivities obtained for SA production from hydrolysate, such as 0.84 and 1.01 g/L/h, are comparable to those obtained from glucose at 0.8, 1.01 and 2.31 g/L/h (Akhtar *et al*., 2014; Cheng *et al*., 2012; Jiang *et al*., 2013; Liu *et al*., 2013). Since SA can be produced from pentose sugars (Borges and Pereira, 2011), its production can be

combined in a lignocellulose biorefinery with PHB, which is produced from glucose (Wang and Lee, 1997).

4.1.2.2 Polyhydroxybutyrate

PHB, also known as poly-3-hydroxybutyrate (P3HB), is the best described and most widely produced polyhydroxyalkanoate (PHA) (Kapritchkoff *et al*., 2006; Khanna and Strivastava, 2005; Suriyamongkol *et al*., 2007). PHB's mechanical properties are similar to polypropylene (Lopes *et al*., 2014) and polyethylene (Verlinden *et al*., 2007). The unique properties of PHB make it eligible for high-end market applications in the biomedical field, where PHB could be used for surgical sutures, blood vessel replacements and bone growth stimulation (Reddy *et al*., 2003).

For the production of PHB a wide range of micro-organisms can be used, but special attention has been given to *Alcaligenes eutrophus, Alcaligenes latus* and recombinant *E. coli* in literature (Choi and Lee, 1999a; Lee, 1996; Li *et al*., 2007). Recombinant *E. coli* is a favored candidate for PHB production, since it produces PHB during both the growth and synthesis phases, resulting in a low overall residence time and capital costs. Conversely, many micro-organisms only produce PHB during the synthesis phase, following a "feast and famine" fermentation strategy. This involves bacteria growth in a nutritionally and oxygen enriched environment followed by nutrient depletion in the presence of a carbon source for PHB production (Khanna and Strivastava, 2005; Verlinden *et al*., 2007). However recombinant *E. coli* does not require nutrient limitation to produce PHB, resulting in less complicated process control requirements (Van Wegen *et al*., 1998). Other advantages of using recombinant *E. coli* to produce PHB include the i) ease of DSP and recovery due to a large PHB granule size and weak cell walls, ii) high growth rates and PHB titres, iii) ease of process control and iv) the absence of the enzyme responsible for intracellular PHB degradation (Choi and Lee, 1997).

Since PHB is produced intracellularly the cell characteristics have an influence on the downstream recovery. Alkaline digestion is 25 % cheaper when compared with the conventional surfactant-sodium-method (Choi and Lee, 1999b). It has low chemical costs and it is suitable for industrial applications, thus resulting in a high PHB purity (98.5 %) and recovery yield (91.3 %) (Choi and Lee, 1999b; Lee *et al*., 1999). Moreover, the alkaline method is suitable for the removal of endotoxins from recombinant *E. coli* producing PHB (Lee *et al*., 1999). If the residence time is increased from 1 to 5 hours at 30˚C and includes a 0.2 M sodium

hydroxide (NaOH) solution (Valappil *et al*., 2006), the endotoxin level (EU) reduces from 10^7 EU per g PHB, to 1 EU per g PHB, making the PHB suitable for biomedical applications at a high purity of 98 %wt.[65] Therefore a higher selling price can be justified when producing high purity PHB for biomedical applications, rather than producing PHB to replace commodity plastics (Khanna and Strivastava, 2005).

4.1.2.3 Market Perspective

The selling price of SA has been reported for a range of 1 145 – 4 995 US$/t (Luo *et al*., 2010; Vlysidis *et al*., 2011). Currently, the bio-based market is at 38 000 tonnes per annum with a selling price of 2 900 US$/t, while the total SA market and selling price are 76,000 tonnes per annum (tpa) and 2 500 US$/t, respectively (CGEE, 2017). Bio-SA is produced commercially from glucose by Bioamber™, who opened their plant in 2015 with a capacity of 30 000 tonnes per annum. Companies such as BASF-Purac, Reverdia, Mitsubishi-PPT and Myriant technologies are actively developing and implementing industrial and pilot scale plants for the production of bio-SA (Van Heerden and Nicol, 2013).

On the other hand, PHB remains uncompetitive in comparison to conventional plastics due to its high production cost (Lopes *et al*., 2014; Silva *et al*., 2014). The current PHB selling price is 11 424 US$/t (Industry quote, 2016), which is high when compared to other plastics, such as biodegradable polylactic acid (PLA) at 2 600 US$/t (2.2 – 3 €/kg) and fossil based polyethylene terephthalate (PET) at below 1 000 US$/t (Wolf *et al*., 2005a). Although the market price has reduced from 28 250 US$/t (20 €/kg) in 2003 (Wolf *et al*., 2005b), the PHB production cost has not decreased significantly and the selling price of 11 424 US$/t is far from the forecasted price range of 2 200 - 3 300 US$/t (Dacosta *et al*., 2015; Naranjo *et al*., 2014). It may be possible to reduce the production cost of PHB by using cheaper, alternative feedstocks such as sugarcane bagasse and trash. Worldwide the PHA market volume varies from pilot scale to 100 tonnes per annum, up to a maximum of 50 000 tpa by ADM in the USA, with 30 000 tpa taken as the average market volume (Lunt, 2014).

4.1.3 Project aim

Therefore, the aim of the project was to determine the economic outcome of a biorefinery co-producing either succinic acid or PHB with electricity, or a combination of these bioproducts, as a potential solution to the sugar industry's financial needs. The biorefinery is annexed to an existing, typical South African sugar mill and integrated with a CHP plant. The first objective

was to optimise the bio-product production volumes taking the impact of each bio-product's production volume on its selling price into account. The second objective was to compare the economic outcome of the selected biorefinery scenarios to one another and a stand-alone CHP plant co-producing only electricity as sellable bioproduct.

4.2 Methodology

4.2.1 Process flow sheet design configuration

The typical South African sugar mill treats 300 t/h sugarcane and operates for 6480 hours per year (Ali Mandegari *et al.,* 2017a). The bagasse and trash feedstock fed to the biorefinery and CHP plant were based on a combined feedstock of 45 t/h bagasse and 20 t/h trash at a total dry mass feed rate of 65 tonnes per hour (t/h) (Ali Mandegari *et al*., 2017a). The feedstock composition used was 40.7 % cellulose, 27.1 % hemicellulose (67.8 % polysaccharides), 21.9 % lignin, 6.7 % extractives and 3.5 % ash.(Farzad et al., 2017; Nieder-Heitmann et al., 2018). Some of the feedstock was bypassed from the biorefinery to the CHP plant, indicated by variable *x* % in Figure 1, to ensure bio-energy self-sufficiency of the biorefinery and sugar mill for each individual scenario.

The biorefinery (Figure 1) was divided into four plant areas: i) lignocellulose pretreatment, enzymatic hydrolysis and detoxification ii) SA seed train, fermentation, and downstream processing, iii) cellulase plant, PHB fermentation and downstream processing, and iv) waste water treatment (WWT) plant. It is annexed to a new v) CHP plant, and existing (vi) sugar mill. The sugar mill is not simulated, but represented as a single value of steam demand (Görgens *et al.,* 2016).

The lignocellulose processing in plant area i) (discussed in section 4.3.1.1) produced two liquid sugar streams, i.e. a hemicellulose hydrolysate rich in pentose sugars and an enzymatic hydrolysis product stream rich in glucose. The PHB producing recombinant *E. coli* can only utilise glucose and not pentose sugars (Wang and Lee, 1997), while SA producing *A. succinogenes* can utilise both glucose and pentose sugars (Xi *et al*., 2013b). This difference in sugar utilisation provided design flexibility to develop a range of process options and thus alternative scenarios for the production of SA and PHB. An overview of the scenarios are provided in section 4.3.2.

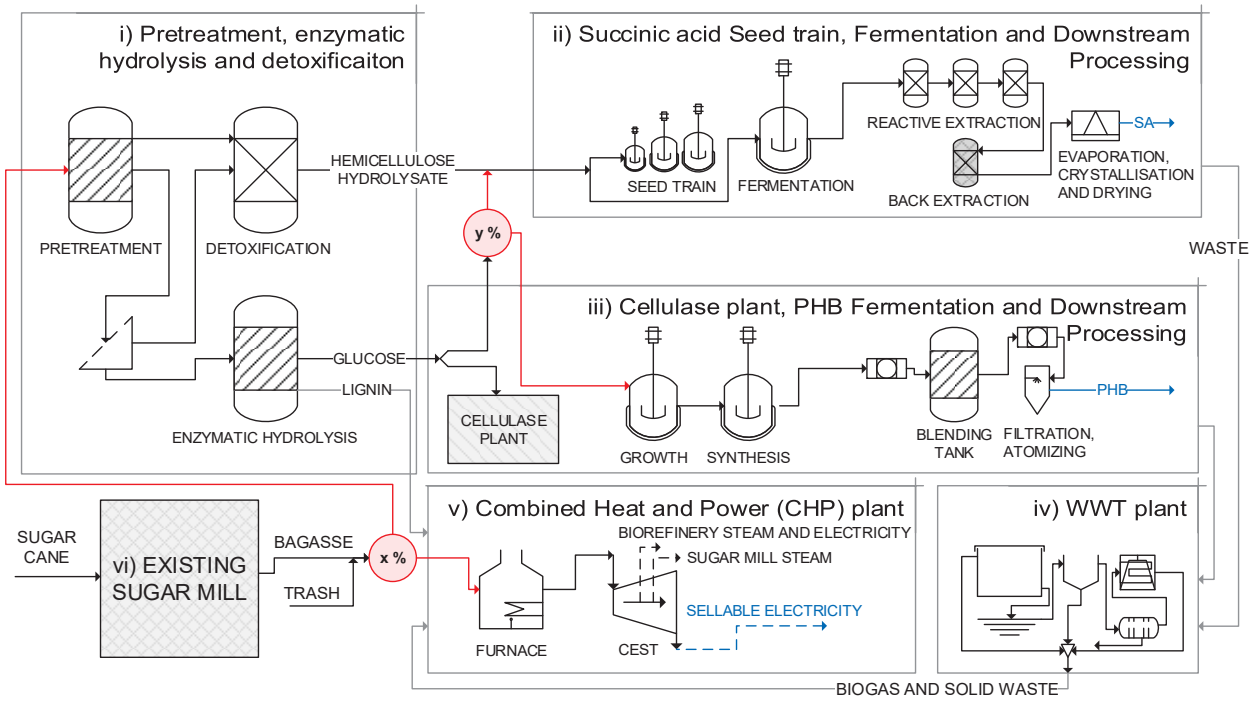


Figure 4-1: Multi-product plant block flow diagram (x % bypass ratio and a y % glucose rich split between PHB and SA production areas)Co-production of electricity

4.2.2 Simulation methodology

The physical properties for the lignocellulosic feedstock have been reported previously (Ali Mandegari *et al.*, 2017a). The scenarios were simulated in Aspen Plus® version 8.8 using literature data and the Electrolyte Non-Random Two Liquid (ELECNRTL) base property method (Ali Mandegari *et al.*, 2017a; Görgens *et al.*, 2016). ELECNRTL is a versatile electrolyte property method, which can calculate very high and low concentrations of aqueous and mixed solvent systems for polar electrolyte systems. It is consistent with the NRTL-RK property method, since the vapour phase properties are calculated using the Redlich-Kwong equation of state (EoS).

However, the NRTL-NTH (Nothnagel) EoS was used for the SA downstream vapour phase calculations, specifically the evaporation unit, since the RK EoS cannot model association behaviour in the vapour phase for carboxylic acids. In the WWT plant, the NRTL property method was used for the methane flash drum, which simulates the vapour-liquid separation within the biodigester. In the CHP plant, the Redlich-Kwong-Soave cubic EoS with Boston-Mathias alpha function (RKS-BM) was used for the furnace section of the boiler. The property method used for the boiler tubes section (simulated using a flash drum) and the CEST are based on the steam tables (i.e. IAPW-95 and/or STEAMNBS property methods).

The UNIFAC property method was used for a decanter Aspen Plus® model treating a simplified stream of SA and water with liquid-liquid separation to validate the reported separation efficiencies (Morales *et al.*, 2016), and energy requirements. The separation efficiencies were then used in the Aspen Plus® Sep models to simulate the extraction columns in the reactive extraction SA downstream recovery. The pretreatment, enzymatic hydrolysis, furnace and fermentation steps were simulated using RStoic Aspen Plus® stoichiometric reactor blocks. Pumps were included for an assumed discharge pressure range of 1.5 – 2 atm at a pump and mechanical efficiency of 75 % and 95 %, respectively, to account for the electricity consumption of minor equipment. No formal heat integration was performed by the means of a heat pinch analysis. For specific process areas the best opportunities for heat saving were identified allowing for the use of some heat recovery with heat exchangers. For these HeatX Aspen Plus® units, a temperature approach of 10°C were used. An overview of the equipment units' operating conditions, selection, sizing and costing are provided in the supplementary information.

4.2.3 Economic methodology

Aspen Process Economic Analyzer® was used to determine the installed cost for the majority of the equipment. The installed cost for the remaining units were sourced from literature and adjusted for the desired capacity and cost year using the relevant sizing exponent and the Chemical Engineering Plant Cost Index (CEPCI) indices, respectively (Vlysidis *et al.*, 2011). The equipment costs associated with cooling and chilled water utilities and storage were calculated as 6.5 % and 5.0 % of the biorefinery installed equipment cost, respectively (Görgens *et al.*, 2016). It should be noted that the capital cost estimate provided is classified as a preliminary estimate with an accuracy range of ±30%, typically used to decide between design choices, such as the different scenarios investigated (Sinnott, 2005; Towler and Sinnott, 2008).

The biorefinery installed equipment cost (plant areas i - iv) was added to the sum of the CHP plant installed equipment cost (plant area v), utilities and storage costs to calculate the total installed cost. The total installed cost was used to determine the fixed capital investment (FCI) by adding the direct and indirect costs (Görgens *et al.*, 2016; Humbird, 2011), based on previous studies of the same level estimate (Ali Mandegari *et al.*, 2017a; Humbird, 2011). The total capital investment (TCI) was calculated by adding a location factor of one and working

capital (5 % of FCI) to the FCI (Görgens *et al*., 2016). The total operating cost (TOC) is the sum of the variable costs, fixed costs and annual capital charge (Humbird, 2011).

The variable operating costs included the raw materials and consumables, waste, and by-products, namely the feedstock cost (10.79 US$/kg) (Ali Mandegari *et al*., 2017a), sulphuric acid cost (0.094 US$/kg) (Tao *et al*., 2011), PHB and SA growth media costs, ammonia (0.31 US$/kg) (Efe C., van der Wielen L.A.M., 2013), reactive extraction solvent make-up costs, boiler chemicals, and the cost of ash (Görgens *et al*., 2016) and purged solvent disposal. The fixed operating costs were based on the design capacity of the biorefinery and included the labour, labour overheads (90 % of the total operating labour cost), plant maintenance (3 % of the biorefinery installed equipment cost), property taxes and insurance (0.7 % of the total FCI) (Ali Mandegari *et al*., 2017a; Humbird, 2011). General expenses such as distribution and selling costs, R&D (research and development), and administration costs were excluded from the TOC (Ali Mandegari *et al*., 2017a).

The annual capital charge included items purchased at intervals during the year, such as the granular activated carbon (1.2 US$/kg) (Mussatto *et al*., 2013) for four total batch replacements during the year, SA recovery solvents, and enzyme nutrients (0.74 US$/kg) (Humbird, 2011). Since the SA DSP requires a recycle solvent stream for reactive extraction recovery, only the make-up solvent was included as a variable operating cost. The FCI and TOC were used as input values to a real term discounted cash flow (DCF) rate of return analysis (Towler and Sinnott, 2008). Economic indicators, such as the minimum required selling price (MRSP), the net present value (NPV) and internal rate of return (IRR) were used to compare the feasibility of different scenarios.

The project life was assumed to be 25 years with a two year construction period (10 % TCI spent in year -2, 60 % spent in year -1 and 30 % spent in year 0, and a two year production ramp-up period (50 % of design capacity in first year and 75 % in second year) (Nieder-Heitmann *et al*., 2018). Straight line depreciation over 5 years with zero salvage value was used for the real term DCF analysis with a discount rate of 9.7 %, income tax rate of 28 % and 5.7 % inflation rate (Nieder-Heitmann *et al*., 2018).

4.3 Biorefinery process design

4.3.1 Design Configuration of the Common Biorefinery areas

The same pretreatment and enzymatic hydrolysis, WWT plant and CHP plant design configurations were used for all the scenarios, while the fermentation (bioconversion) and downstream recovery steps were unique to each bioproduct. The polyhydroxybutyrate (PHB) and succinic acid (SA) fermentation and recovery plant areas are discussed under sections 4.4.2.1 and 4.4.2.3, respectively.

4.3.1.1 Pretreatment and Enzymatic hydrolysis

Dilute acid pretreatment was used to hydrolyse the hemicellulose fraction. The lignocellulosic feedstock was mixed with 0.65 % sulphuric acid (H_2SO_4) (Benjamin, 2014) , at a 1:2 solid to liquid ratio applicable to commercial dilute acid treatment (Humbird, 2011), and heated to 180 ˚C for 10 minutes by direct saturated steam injection (Benjamin, 2014). After pretreatment the hemicellulose hydrolysate and solid cellulose and lignin fraction (hereafter referred to as cellulignin) were separated using a centrifuge. The solid cellulignin fraction was then washed to remove fermentation inhibitors and residual soluble sugars, and diluted to a 20 % solids fraction prior to enzymatic hydrolysis (EH) (Humbird, 2011). EH was carried out at an operating temperature of 50 ˚C, 72 hours residence time and an enzyme dosage of 20 mg enzyme protein per gram dry mass (Benjamin, 2014).

The cellulignin wash water, containing inhibitors and soluble sugars, was combined with the hemicellulose hydrolysate and detoxified using a granular activated carbon column, at 2 % (w/v) and 30 - 50 ˚C for 120 min (Liu *et al*., 2013; Xi *et al*., 2013b), to remove HMF and furfural (Hodge *et al*., 2009). A fraction of the glucose rich EH product stream was diverted to the cellulase plant as substrate for cellulase production (Humbird, 2011). The dilute acid pretreatment and EH fractional conversions and stoichiometric reactions have been previously reported (Nieder-Heitmann *et al*., 2018). The simplified block flow diagram for the dilute acid pretreatment and EH is provided in Figure 2. The two major product streams are a xylose rich hemicellulose hydrolysate and a glucose rich EH (cellulose hydrolysate) product stream.

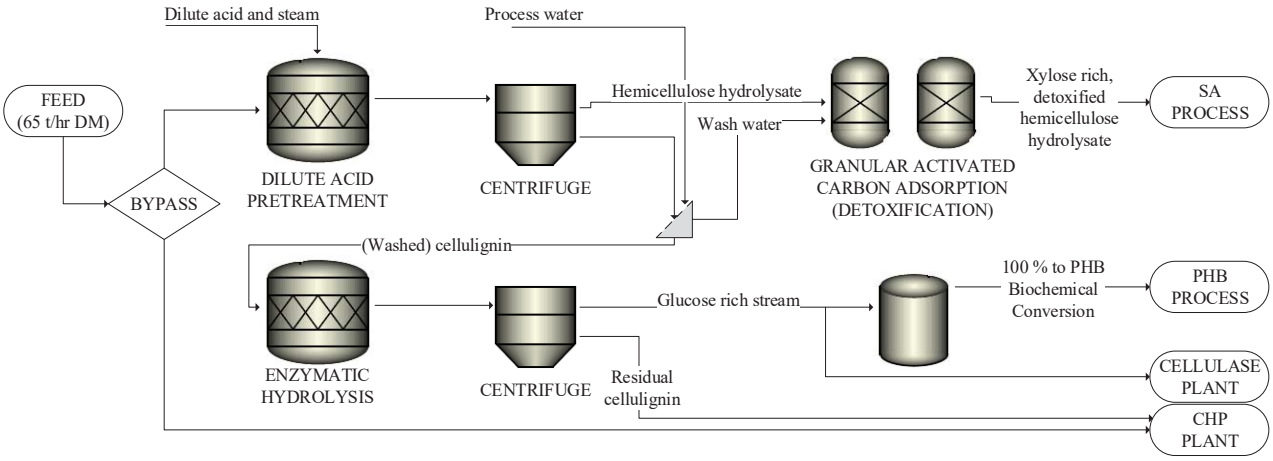


Figure 4-2: Simplified block flow diagram of the pretreatment, detoxification and enzymatic hydrolysis area

4.3.1.2 Waste Water treatment plant

The waste water treatment (WWT) plant process flow sheet design is based on the NREL report design configuration for the cellulosic ethanol model (Steinwinder *et al*., 2011), where all the biorefinery waste streams were collected and fed to a mesophilic anaerobic biodigester (AD) (Cheng *et al*., 2012; Peris, 2011; Rajendran *et al*., 2014; Tenneti, 2015), producing biogas and sludge. The biogas was combined with the boiler feed and combusted in the CHP plant. The AD sludge was pumped to an aerobic digestion step where a clarifier concentrated the sludge. The clarifier overflow was pumped to a reverse osmosis (RO) membrane modular plant (Görgens *et al*., 2016; Humbird, 2011)·(McFall *et al*., 2008; Watson, 1990).

The clarifier underflow was sent to a dewatering centrifuge. The concentrated solid stream was sent to the CHP plant and the centrifuge liquid stream was pumped back to the clarifier. The brine retentate from the RO plant was fed to a multiple effect evaporator to produce a 50 %wt concentrated stream, which was also combined with the boiler feed in the CHP plant (Ali Mandegari *et al*., 2017a; Steinwinder *et al*., 2011). Vapour from the evaporation unit was condensed and assumed to be re-used as irrigation water in the sugar cane plantation, since the stream has a theoretical chemical oxygen demand (COD) of less than 35 mg/L (data not shown), which adheres to the wine industry's legal requirement of <75 mg/L for the maximum irrigation of 2000 m^3/day (van Schoor, 2005). The RO permeate was recycled back to the biorefinery as process water.

4.3.1.3 Combined heat and power plant

The sugar mill's existing 28 atm (2.84 MPa) boiler was replaced by the high efficiency, high pressure (6.6 MPa) boiler (Mbohwa, 2013), together with a CEST operating at an isentropic

efficiency of 85 % in the CHP plant (Görgens *et al*., 2016). The boiler feed stream consisted of the bypassed feedstock (*x* %), residual cellulignin after enzymatic hydrolysis, biogas and solid waste produced from the WWT plant. The high pressure steam produced by the boiler was split into three streams: two streams were sent to de-superheating stations and one to the CEST, where it was used to generate electricity. From the CEST, two additional intermediate steam streams were removed.

High pressure steam was combined with saturated steam (104 °C and 0.12 MPa) to produce high pressure utility (HPU) steam (266 °C and 1.3 MPa) in the first de-superheating station, and saturated steam for direct steam injection in the pretreatment reactor in the second de-superheating station. The 6.6 MPa high pressure steam sent to the CEST was reduced to 3.04 MPa for the sugar mill (120 t/h at 400 °C) (Görgens *et al*., 2016) and then to 0.65 MPa for low pressure utility (LPU) steam used in the biorefinery (293 °C). It was assumed that the excess electricity was sold back into the network at 0.08 US$/kWh.(Ali Mandegari et al., 2017a; Nieder-Heitmann et al., 2018).

4.3.2 Biorefinery scenarios

4.3.2.1 Scenario A: PHB and electricity co-production

For a biorefinery co-producing PHB and electricity, 100 % of the available glucose was sent to PHB fermentation and downstream processing, while the hemicellulose hydrolysate was sent to the WWT plant for biogas production. No hemicellulose hydrolysate detoxification was required and plant area ii) is therefore omitted from the biorefinery process flow sheet design (Figure 1).

A fraction of the glucose rich stream (9.5 %) was sent to the growth reactor and mixed with process water to a glucose concentration of 20 g/L (Wang *et al*., 1997). The residual glucose rich stream (90.5 %) was sent to a triple effect evaporator and concentrated to 700 g/L glucose (Wang and Lee, 1997). After the first 12 h, the recombinant *E. coli* cells were sent to a centrifuge, removing the cells and the bulk of the fermentation broth. The cells and some of the growth reactor fermentation broth was placed in the synthesis reactor for the remaining 24 h. During this phase the concentrated glucose (700 g/L) was fed at intervals to ensure a final cell and PHB titre of 153.7 g/L and 101.3 g/L, respectively.

The PHB growth and synthesis operating conditions and nutrient composition has been reported previously (Wang and Lee, 1997). The growth and synthesis fermentation reactions

are provided in Table 1. PHB is accumulated intracellularly and recovered using alkaline digestion downstream processing (DSP) (Wang and Lee, 1997). The alkaline digestion DSP process flow diagram and stream results for Scenario A (PHB) is shown in Figure 3 (Lee, 1996; Wang and Lee, 1997). The boiler feed (i.e. bypassed feedstock (*x* %), residual cellulignin after enzymatic hydrolysis, biogas and solid waste produced from the WWT plant) was sent to the CHP plant for steam and electricity generation. The excess electricity was sold back into the network.

Table 4-1: PHB fermentation reactions for PHB growth and synthesis

PHB Fermentation Stoichiometric reactions	Fraction conversion efficiency	Reference
$Glucose + 1.5O_2 \rightarrow PHB^c + 2CO_2 + 3H_2O$	0.586	(Akiyama *et al*., 2003; Wang and Lee, 1997)
$Glucose + 2O_2 \rightarrow Acetic\ acid^d + 2CO_2 + 2H_2O$	0.022	(Wang and Lee, 1997)
$Glucose + NH_3 + 1.98O_2 \rightarrow 1.02\ BIO^c + 1.93CO_2 + 4.47\ H_2O$	0.705[a]	(Lopar *et al*., 2013)
$Glucose^e + 6O_2 \rightarrow 6H_2O + 6CO_2$	1.000[b]	(Leibbrandt, 2010)

a) Growth phase stoichiometry (Lopar *et al*., 2013). The molar fractional conversion is obtained from experimental results (Wang and Lee, 1997). b) Cell maintenance stoichiometry, followed in series to ensure no glucose in the product stream (Wang and Lee, 1997). c) PHB and BIO has the molecular structure $C_4H_{6.9}O_{1.64}N_{0.98}$ d) Acetic acid (CH_3COOH) e) Glucose ($C_6H_{12}O_6$)

Figure 4-3: Alkaline Downstream Process for PHB recovery (Choi and Lee, 1999b),(Lee *et al.*, 1999)

4.3.2.2 Scenario B: PHB, Succinic acid and electricity co-production

For a multiproduct biorefinery co-producing SA, PHB and electricity, the glucose rich stream (*y* %) was sent to plant area iii (Cellulase plant, PHB fermentation and downstream processing). The hemicellulose hydrolysate was sent to plant area ii (SA seed train, fermentation and downstream processing). The PHB and SA production volumes were adjusted by changing the amount of glucose sent to PHB production, *y* %, with (1-*y*) % sent to SA production shown in Figure 1. As a result, the multiproduct biorefineries have two fermentation and downstream recovery plant areas, with a shared cellulase, CHP, WWT and pretreatment and EH plant areas.

Different production volumes of SA and PHB were investigated for the multiproduct biorefinery in Scenario B to determine the process configuration that will provide the best economic outcome. This was achieved by varying the amount of glucose sent to the PHB and SA production areas in 25 % intervals (variable *y* in Figure 1). In Scenario B (100), 100 % of the glucose stream was sent to PHB production, (i.e. y = 100 % in Figure 1). In Scenario B (75), 75 % of the glucose rich stream was sent to PHB production, and the remaining 25 % was sent to SA production with the hemicellulose hydrolysate. This trend continued for Scenarios B (50) and (25) for 50 % and 25 % of glucose sent to the PHB fermentation area, respectively.

4.3.2.3 Scenario C: Succinic acid and electricity co-production

For a biorefinery co-producing SA and electricity, the glucose stream was combined with the hemicellulose hydrolysate and sent to plant area ii (SA seed train, fermentation and downstream processing). In this case hemicellulose hydrolysate detoxification was required and the cellulase plant formed part of plant area ii, since plant area iii was omitted (Figure 1).

An SA seed train provided inoculum to minimise the micro-organism's lag phase and promote exponential growth using the typical size of 10 % (v/v) of the fermentation volume (Pfeifer *et al*., 1952; van der Merwe, 2010). Therefore 90 % of the feed stream was pumped to the fermentation area and preheated prior to fermentation at 38 ˚C (Yan *et al*., 2014). The nutrient medium (Borges and Pereira, 2011) which was used in the seed train and fermentation area was sterilised at 120 ˚C for 15 min. The micro-organism fermentation reactions are shown in Table 2 (Leibbrandt, 2010; van der Merwe, 2010).

Table 4-2: Succinic acid biochemical conversion (fermentation) reactions

Succinic acid fermentation stoichiometric reactions	Fractional conversion efficiency	References
GROWTH REACTIONS		
Glucose[e] + $1.1429NH_3$ → $5.7143CELL^c$ + $2.5714H_2O$ + $0.2857CO_2$	0.085	(Leibbrandt, 2010; van der Merwe, 2010)
Xylose + $0.9524NH_3$ → $4.7619CELL$ + $2.1429H_2O$ + $0.2381CO_2$	0.043	(Leibbrandt, 2010; van der Merwe, 2010)
GLUCOSE REACTIONS		
Glucose + $0.8571\ CO_2$ → 1.7142 SA + $0.8571\ H_2O$	0.646[b]	(Yan *et al*., 2014)
Glucose + CO_2 → SA[a] + + CH_2O_2	0.003	(Cheng *et al*., 2012; Xi *et al*., 2013a)
3Glucose + $2CO_2$ → 4SA + 2Acetic acid[d] + $2H_2O$	0.162	(Xi *et al*., 2013a)
XYLOSE, ARABINOSE AND CELLOBIOSE REACTIONS		
7 Xylose + $5CO_2$ → 10SA + $5H_2O$	0.303	(Borges and Pereira, 2011)
7Arabinose + $5CO_2$ → 10SA + $5H_2O$	0.205	(Almqvist *et al*., 2016)
Cellobiose + CO_2 → 2SA + 2.5Acetic acid	0.971	(Jiang *et al*., 2013)
3Xylose + $2CO_2$ → 4SA + 0.5Acetic acid + $2H_2O$	0.266	(Borges and Pereira, 2011)
3Arabinose + $2CO_2$ → 4SA + 0.5Acetic acid + $2H_2O$	0.200	(Almqvist *et al*., 2016)

a) SA – succinic acid b) The fractional conversion has been selected to ensure an overall succinic acid yield of 0.87 g/g from glucose, for a replicable titre of 88.1 g/L, a productivity of 2.27 g/L/h and a yield of 0.87 g/g.(Yan *et al*., 2014) c) CELL represents the micro-organism $CH_{1.8}O_{0.5}N_{0.2}$ d) Acetic acid (CH_3COOH) e) Glucose ($C_6H_{12}O_6$) f) Xylose and Arabinose ($C_5H_{10}O_5$)

For a glucose only sugar stream an initial sugar concentration of ≤ 100 g/L is typically required for *A. succinogenes* (Li *et al*., 2011), and sugar concentrations of 50 – 80 g/L have been obtained and used for pretreated lignocelluloses reported previously (Chen *et al*., 2016; Li *et al*., 2011; Salvachúa *et al*., 2016). For Scenario C a fed-batch fermentation strategy was used whereby a portion of the sugar feed stream was sent to a triple effect evaporator to concentrate the combined glucose rich and hemicellulose hydrolysate stream to 200 g/L xylose and glucose (Yan *et al*., 2014).

Xi, Chen, *et al*., (2013) provided SA yields from hydrolysate fermentation experiments. However conversion efficiencies of individual sugars present in the hydrolysate, such as xylose, cellobiose and arabinose, were not specified, but could be found elsewhere (Almqvist *et al*., 2016; Borges and Pereira, 2011; Cheng *et al*., 2012; Jiang *et al*., 2014). Consequently, a low SA yield on glucose was selected to ensure that the overall SA yield (on the combined sugars present in the glucose rich and hemicellulose hydrolysate streams) was not more than

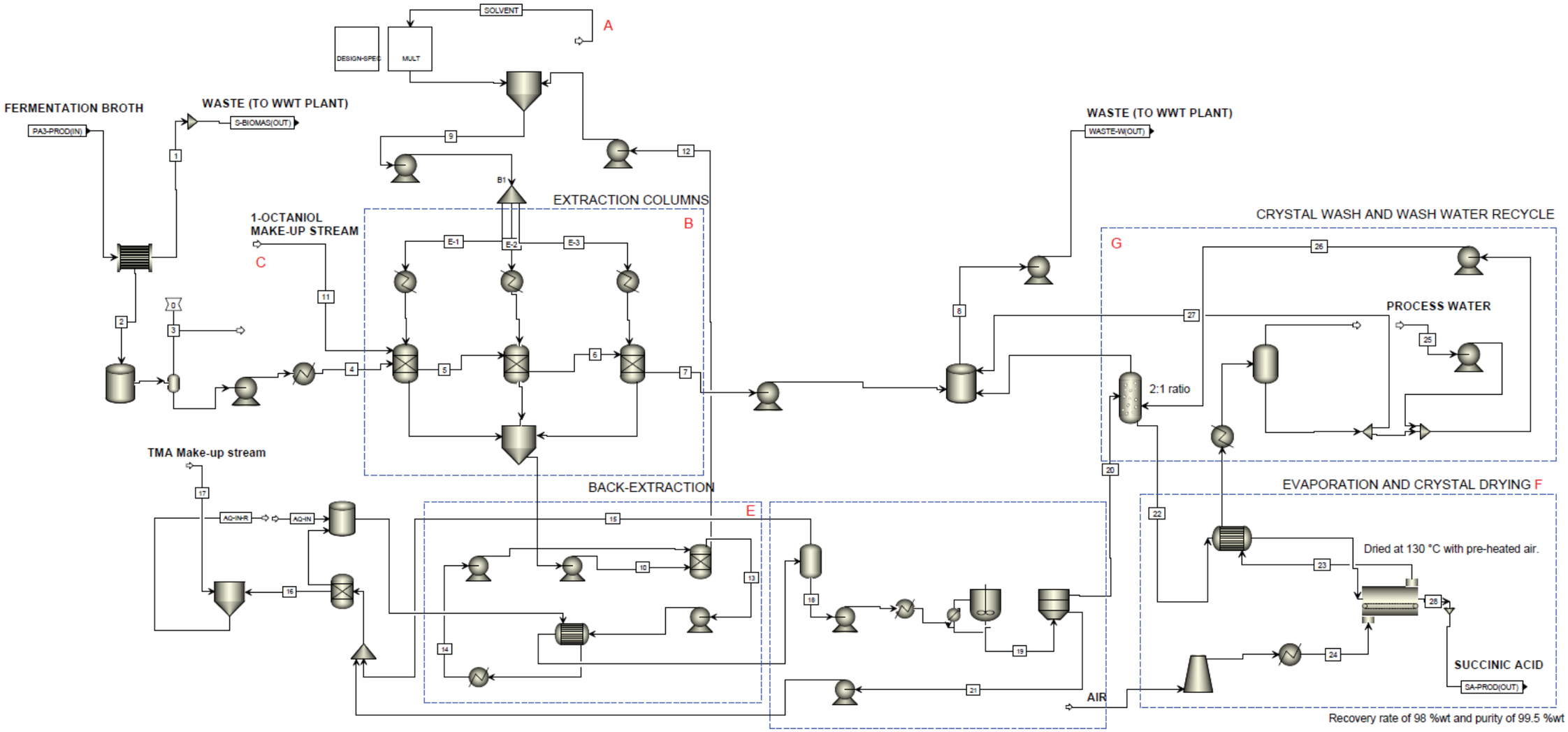
the reported yield for a combined sugar feedstock obtained from pretreated lignocelluloses.(Xi *et al*., 2013a).

The SA was recovered from the fermentation broth in the DSP using ultrafiltration (Wang *et al*., 2014), which removed the biomass cells, followed by reactive extraction, crystallisation and evaporation to recover and purify SA (Morales *et al*., 2016; Song *et al*., 2008). The SA DSP process flowsheet and stream results for Scenario C (SA) is provided in Figure 4.

4.3.2.4 Scenario D: Electricity only production

Lastly, for a stand-alone CHP plant co-producing only electricity, all of the bagasse and trash feedstock (65 t/h dry mass) were sent to the CHP plant (plant area v). Consequently, plant areas i), ii), iii), and iv) were excluded from the process flow sheet design (Figure 1).

The CHP plant in this scenario had no desuperheating stations since no SA or PHB was produced and therefore electricity production was a priority over steam production. All the high pressure steam from the boiler was sent directly to the CEST and only one intermediate steam stream, 120 t/h as required for the sugar mill, was removed. The rest of the high pressure steam from the boiler was used in the CEST to generate electricity. Excess electricity was sold back into the network. Since the majority of the steam exits the CEST through the last condensing extraction section, a heater was included for this Scenario D to ensure boiler feed pump saturated steam stream conditions of 104.78 ˚C and 120 kPa in the closed boiler water and steam cycle.



NOTES

A)Organic Solvent: 87 %wt 1-Octanol and 13 wt% trioctylamine. A volumetric ratio of 1:1 (w/w) solvent to feed stream is required used per extraction column.

B) The separation efficiencies reported for the extraction columns were validated using a decanter Aspen Plus® model with liquid-liquid separa and the UNIFAC property method. The separation efficiency of water was different to the reported values, but was found to be negligible. Therefore the separation efficiencies reported (Morales et al 2016) were used in the Aspen Plus® Sep model.

C) Succinic acid separation efficiency of 86 %wt, and a solvent loss of 0.21 %wt 1-octanol to the aqueous phase.

D) Aqueous solvent: 25 wt% trimethylamine (TMA) and 75 wt% water.

E) TMA is added to the back-extraction column in the ratio of two moles TMA per mole succinic acid in the organic phase with a loss of 0.46% 1-octanol to the aqueous phas in the back-extraction column.

F) 20 °C and ambient pressure. Succinic acid has a solubility of 77g/L at 22 °C. The crystals are centrifuged and 0.1 %wt TMA is lost.

G) 50 %wt of the dryer condensate is re-used in the washing step, together with a fresh wash water feed. The residual 50 %wt was disc to the WWT plant to prevent build-up of impurities.

Figure 4-4: Reactive extraction downstream process for succinic acid recovery (Operating conditions based on Morales *et al.*, 2016)

4.4 Results and Discussions

4.4.1 Mass and Energy balances

4.4.1.1 Bypass ratio

Part of the sugarcane bagasse and trash feedstock (x %) was bypassed from the biorefinery to the CHP plant to ensure bio-energy self-sufficiency of the biorefinery and sugar mill. The bypass ratio is dependent on the biorefinery's steam demand, resulting in excess electricity which is sold as a co-product. The bypass ratios and sellable electricity of each scenario are summarised in Table 3.

The bypass ratio could be lowered further by decreasing the biorefinery's steam demand, resulting in a larger biorefinery for the economies of scale benefit. This can be done by using a less energy intensive SA DSP such as ion exchange technology (Morales *et al*., 2016), since the SA DSP required more energy at 1.05 tonnes total steam per tonne DM fed to the biorefinery (Scenario B and C), compared to 0.20 for PHB production in Scenario A. Moreover, PHB is accumulated intracellularly and therefore a physical separation step such as centrifugation was sufficient to remove the bulk of the fermentation broth from the PHB containing micro-organism cells, contributing to the ease of recovery with regards to the low energy requirement and process complexity.

Although the PHB DSP was not energy intensive, more energy in the form of high pressure utility (HPU) steam was required for the triple effect evaporator during fed-batch fermentation of PHB in Scenario A, at 0.79 t_{HPU}/t_{DM} (tonnes HPU steam per tonne DM feedstock), compared to 0.18 t_{HPU}/t_{DM} for SA production in Scenario C. The required sugar stream concentration to be fed at intervals during fermentation was different, with 200 g/L glucose and xylose required for SA production and 700 g/L glucose required for PHB production. This has a direct effect on the bypass ratio, which decreased from 36 % to 31 % from Scenario B as more glucose was diverted from the energy intensive PHB to the SA fermentation area for the multiproduct plant scenarios (Figure 5). However, in contrast to this trend the SA stand-alone plant in Scenario C had a larger bypass ratio (28 %), compared to 13 % for the PHB stand-alone plant in Scenario A. This was due to the different uses of the hemicellulose hydrolysate: in Scenario C the hemicellulose hydrolysate was utilised for SA production, but in Scenario A the hemicellulose hydrolysate was sent to the WWT plant for biogas production where it was used as a fuel source in the CHP plant.

Overall, due to the high steam demand of the pretreatment and downstream processes, more steam than electricity was required for the biorefinery scenarios. The steam demand of plant area i) (dilute acid pretreatment, detoxification and EH) was the largest at 1.54 $t_{HPU}/t_{DM.}$ Since the bypass rate was determined by the biorefinery's steam requirement, excess electricity was produced for each scenario. The sellable electricity varied between 4.6 – 5.5 MWh for the biorefinery scenarios, while Scenario D produced the most electricity at 60.5 MWh (Figure 5). The bioproduct production volumes and rate of sellable electricity produced are summarised in Table 3.

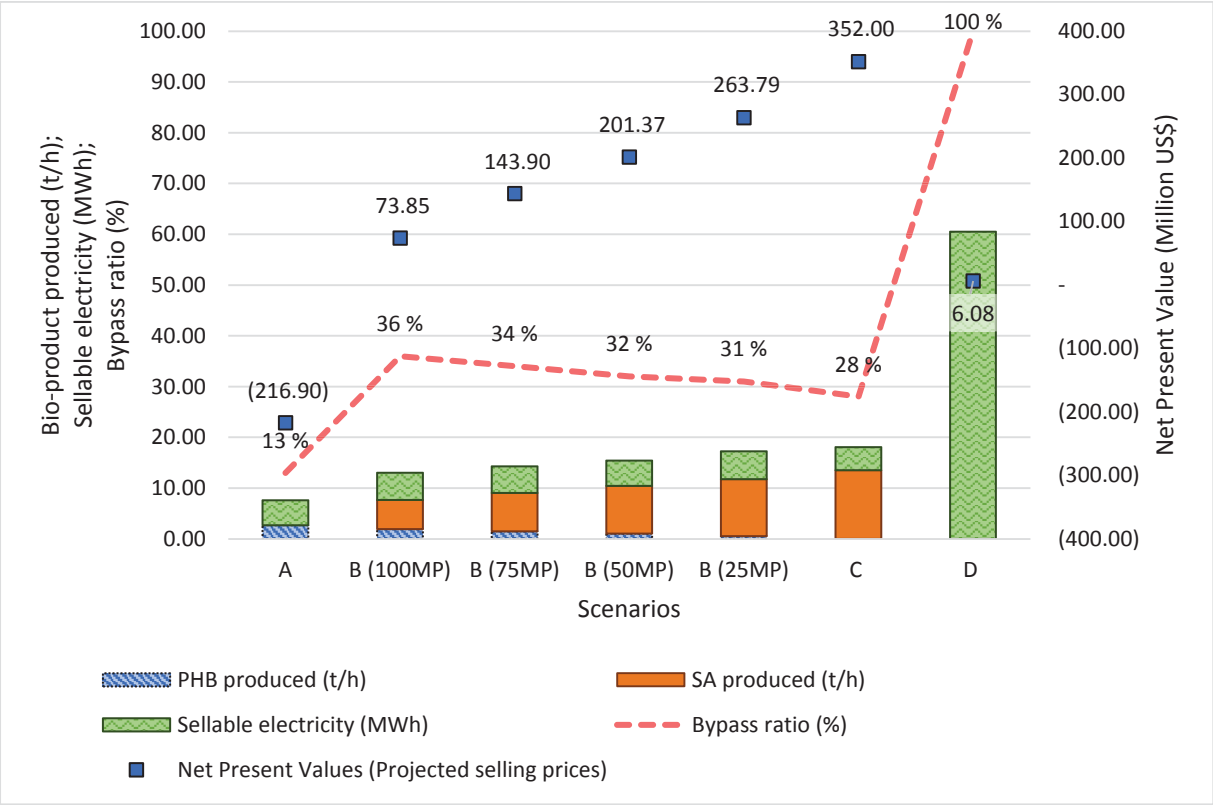


Figure 4-5: Total production rate and bypass ratio of feedstock from the biorefinery to the CHP plant for each scenario

Table 4-3: Bypass ratios, production rates and sellable electricity produced per scenario

DESCRIPTION	A (PHB)	B (100)	B (75)	B (50)	B (25)	C (SA)	D (CHP)
Bypass ratio (%)	13	36	34	32	31	28	100
SA produced (t/h)	-	5.8	7.6	9.4	11.3	13.5	-
PHB produced (t/h)	2.7	1.9	1.5	1.0	0.5	-	-
SA Production rate (t/t)	-	0.14	0.18	0.21	0.25	0.29	-
PHB Production rate (t/t)	0.05	0.05	0.03	0.02	0.01	-	-
Sellable electricity (MWh)	4.9	5.3	5.3	5.0	5.5	4.6	60.5

4.4.1.2 Product rate

A micro-organism's product yield is an indication of how efficiently the fermentable sugars are utilized. In addition, the product yields had an impact on the production cost (discussed in section 4.4.2.) and is therefore a vital parameter to consider when selecting a micro-organism and bio-product from a list of potential candidates to be included in a biorefinery. The SA production rate increased from 5.8 to 13.5 t/h and the PHB production rate decreased from 2.7 to 0.5 t/h as the glucose rich stream split between the SA and PHB fermentation areas for Scenarios A to C, shown in Figure 5.

The amount of SA produced per hour, i.e. the SA production rate, was higher than the PHB production rate for all the biorefinery scenarios, at 0.14 – 0.29 tonnes SA per tonne lignocellulosic DM, compared to 0.01 – 0.05 tonnes PHB was produced per tonne lignocellulosic DM (Table 3). Since the pretreatment and EH process were the same for all the scenarios and the DSP recovery efficiencies of SA and PHB were similar (>97 %wt), the variation in production rate may be due to the fermentation area performance. SA producing micro-organism *A. succinogenes* can utilise glucose, cellobiose and pentose sugars at higher yields, (0.63 - 0.74 g/g depending on sugar feed composition, shown in Table 2), than PHB producing micro-organism recombinant *E. coli* can utilise glucose (0.28 g/g for PHB, shown in Table 1) (Wang and Lee, 1997).

To utilise the monomeric sugars present in pretreated sugarcane bagasse and trash, such as pentose, arabinose and cellobiose more efficiently, the PHB bioproduct yield could be increased through additional genetic engineering and production development (i.e. fermentation) of recombinant *E. coli,* similarly to what has been done to the SA producing micro-organism *A. succinogenes*.(Yan et al., 2014).

4.4.2 Total capital and operational costs

The focus on the use of lignocelluloses has increased in recent years due to fossil dependency as well as environmental and food concerns (Petersen *et al*., 2017). The use of lignocelluloses instead of simple sugar feedstocks, such as glucose (Nieder-Heitmann *et al*., 2018), starch (Mcaloon *et al*., 2000) or corn grain (Ling *et al*., 2014), has shown to decrease the operating costs by 60.6 %, 40.7 % and 45.6 %, respectively. However, an additional pretreatment processing step is required to convert lignocellulose into fermentable sugars. The pretreatment, detoxification and EH total installed equipment costs increased the total capital cost and

contributed 26 % on average to the total biorefinery and CHP plant installed equipment costs for this study (Table 4).

The installed cost, total fixed capital investment and total capital investment costs are summarised in Table 4 for each scenario. The total operating costs (TOC) were calculated as a sum of the variable, as well as the fixed and annual capital charge expenses. The TOC are provided in Table 5.

Table 4-4: Installed cost, Fixed Capital Investment (FCI) and Total Capital Investment (TCI) costs for each scenario

SCENARIOS	A (PHB)	B				C (SA)	D (CHP)
		(100)	**(75)**	**(50)**	**(25)**		
Bypass ratio (%)	13	36	34	32	31	28	100
PLANT AREA INSTALLED COST (million US$)							
Pretreatment and EH	61.0	44.0	45.6	46.7	47.7	49.9	-
SA seed train and cellulase plant	-	13.0	13.2	13.5	13.6	13.3	-
SA fermentation	-	14.5	18.7	22.0	24.9	27.4	-
SA DSP	-	7.1	7.6	8.4	9.0	10.6	-
PHB Growth and Synthesis	20.8	15.9	13.8	9.8	6.0	-	-
PHB DSP	4.5	4.6	4.5	3.9	3.7	-	-
WWT plant	12.0	10.1	10.0	9.9	9.3	10.0	-
BIOREFINERY INSTALLED COSTS	**98.4**	**109.2**	**113.4**	**114.1**	**114.3**	**111.1**	**0.00**
CHP plant	63.7	63.1	62.7	63.5	62.2	61.7	73.6
Utilities	6.4	7.1	7.4	7.4	7.4	7.2	3.3
Storage	4.9	5.5	5.6	5.7	5.7	5.6	0.0
TOTAL INSTALLED COST	**173.4**	**184.9**	**189.1**	**190.8**	**189.6**	**185.6**	**77.4**
Direct costs	17.2	19.1	19.8	20.0	20.0	19.4	0
Indirect costs	114.3	122.4	125.4	126.4	125.8	123.0	46.5
FIXED CAPITAL INVESTMENT, FCI	304.9	326.4	334.3	337.2	335.4	328.1	123.9
Working Capital	15.3	16.3	16.7	16.9	16.8	16.4	6.2
TOTAL CAPITAL INVESTMENT, TCI	**320.2**	**342.7**	**351.1**	**354.0**	**352.2**	**344.5**	**130.1**

SA – succinic acid, EH – enzymatic hydrolysis, DSP – Downstream process (recovery), PHB – polyhydroxybutyrate, WWT – waste water treatment, CHP – combined heat and power

Scenario B (50) had the largest TCI at 354.0 million US$ compared to Scenario D with the lowest TCI of 130.1 million US$. The bypass ratios of the various multiproduct biorefineries B were larger (31 - 36 %), and the plant capacities smaller, than the single product biorefineries

in scenarios A, C and D. However the added process equipment required for the parallel SA and PHB processing streams in the multiproduct biorefineries resulted in larger TCI costs than for the single product biorefinery scenarios A, C and D.

The PHB fermentation and DSP areas installed equipment cost contribution of 15% (25.3 million US$ in Scenario A) was low when compared to the cost contribution of the SA fermentation and DSP at 28% (51.3 million US$ in Scenario C) shown in Figure 6. The large cost difference was primarily due to the micro-organisms' productivity and titre, as seen for the smaller installed equipment cost required for PHB production, since PHB producing micro-organism recombinant *E. coli* has a larger productivity and titre, at 2.8 g/L/h and 101.3 g/L PHB, compared to 2.3 g/L/h and 70.8 g/L SA for SA producing micro-organism *A. succinogenes* Z130 (Yan *et al*., 2014).

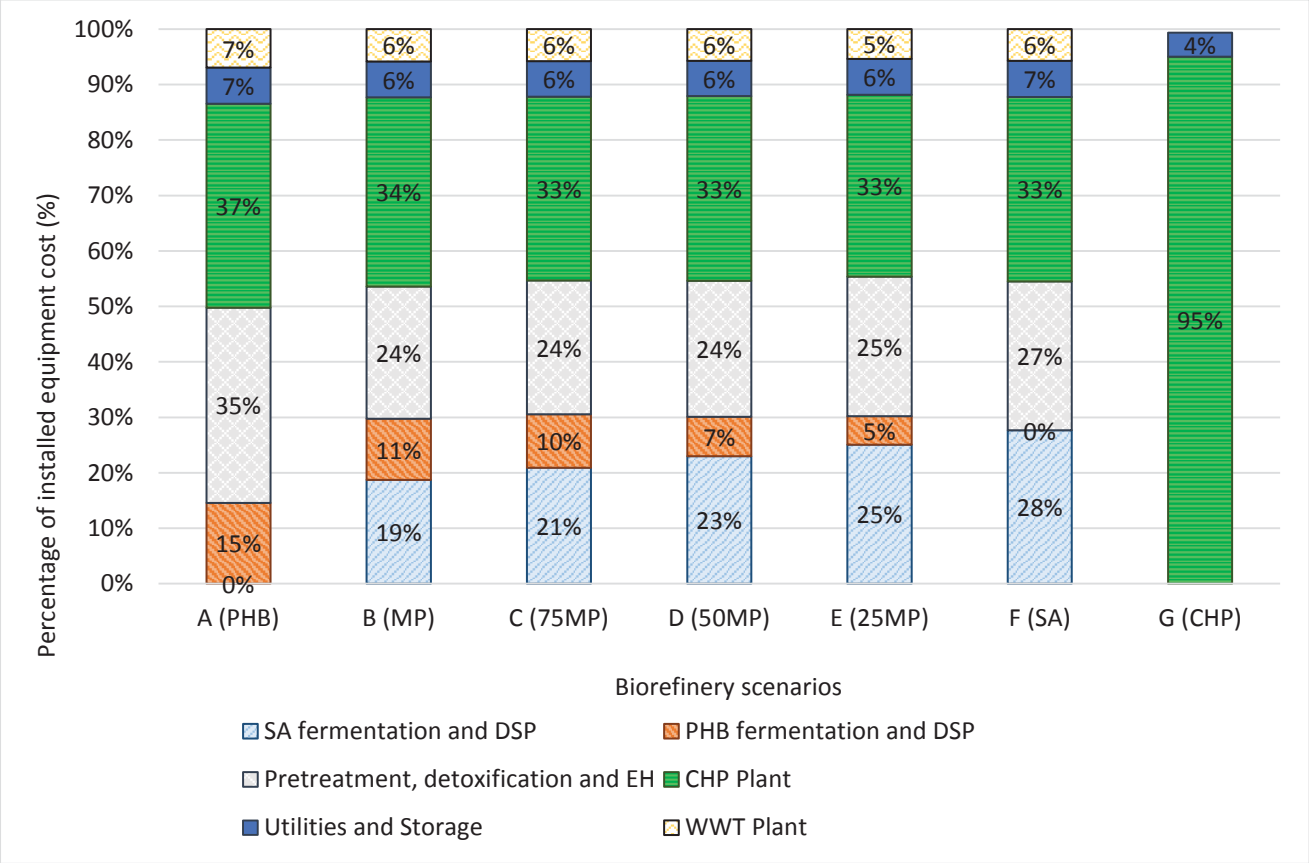


Figure 4-6: Installed capital costs per plant area shown as a percentage of the total installed equipment cost for each scenario (CHP – combined heat and power, DSP – downstream processing, EH – enzymatic hydrolysis, PHB – polyhydroxybutyrate, SA – succinic acid)

Since the production rate of PHB was low in Scenario A (2.7 t/h) compared to that of SA in Scenario C (13.5 t/h), the production cost (calculated by dividing the TOC by the annual production rate of SA, PHB and bio-energy, respectively) of PHB was five times higher than

SA, at 2.06 US$/t PHB compared to 0.37 US$/t SA for the respective scenarios A and C. The production rate will increase for a lower bypass ratio or a higher bioproduct yield on fermentable sugars.

Scenario D had the lowest TOC at 13.16 million US$ per annum, due to the low raw material and labour costs of the CHP plant compared to that of a biorefinery. The TOC for the biorefinery scenarios was 33.6 million US$ on average with a maximum of 35.7 million US$ for Scenario A (Table 5). The raw materials cost for PHB production was high since dipotassium phosphate (K_2HPO_4) is an expensive nutrient chemical (Tao *et al.*, 2011) contributing 35.4 % of the total raw materials cost for Scenario A.

4.4.3 Economic evaluation

The economic outcome in terms of investment viability was measured by the project internal rate of return (IRR) calculated for a real term discounted cash flow (DCF) rate of return analysis. The real term DCF does not take the rate of inflation (5.7 %) into account. Therefore the cost of raw materials, consumables and selling prices remain constant over the project life span, and the IRR was compared with a hurdle rate of 9.7 %. The minimum required selling price (MRSP) is the price at which the bioproduct must be sold in order to obtain a NPV of 0 US$ at a discount rate of 9.7 %.

4.4.3.1 Impact of production volumes on the selling price

It must be possible to sell bio-products at a competitive price or there will be no consumer or market demand even though they are produced from renewable feedstocks (Luo *et al.*, 2010). For example, the selling price of SA was 5 900 US$/t in 2005 (Luo *et al.*, 2009), but have since then decreased to the current price of 2 500 US$/t (CGEE, 2017) due to more bio-based SA that have been added to the market. Therefore an increasing supply may necessitate the selling price to decrease in order to ensure continued demand.

In Scenario C, 87 502 tonnes SA per annum were produced, contributing a significant 44 % of the current SA market volume (201 100 tpa), resulting in an oversupply. However, there are potentially other potential markets for SA, in particular fossil-based maleic anhydride, since both SA and maleic anhydride can be used as a precursor chemicals for the production of 1,4-butanediol (BDO), y-butyrolactone (GBL) and polybutylene succinate (PBS) shown in Figure 7 (Cheng *et al.*, 2012; Okino *et al.*, 2008; Orjuela *et al.*, 2013).

To this end, the selling price of 2 500 US$/t SA was lowered to the market price of fossil-based maleic anhydride at 1 500 US$/t (Lunt, 2014) thus broadening the market for SA. The biorefinery in Scenario C produces 10 % of the projected maleic anhydride market (910 000 tpa (Tan *et al*., 2014)), which should not results in an oversupply in this market segment and therefore will not affect the maleic anhydride selling price.

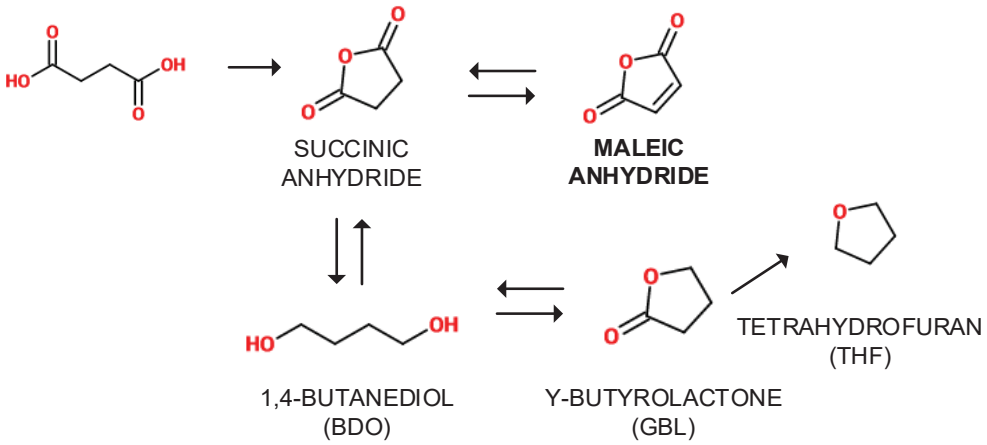


Figure 4-7: Succinic acid derivatives (partially redrawn(Delhomme *et al*., 2009))

Scenario A produced 17 364 tpa PHB, contributing 29 % of the total PHA market volume (60 050 tpa) (Chanprateep, 2010). Therefore the selling price of PHB was adjusted from the current selling price of 11 424 US$/t to that of biodegradable polylactic acid (PLA) at 2 600 US$/t (2.2 – 3 €/kg) (Chanprateep, 2010), where PHB can be sold as a bio-plastic and enter the larger bioplastics market. In the bioplastics market the PHB production only contributes 0.2 % of the potential bio-plastics market volume in 2020 (2 500 000 tpa) (Chanprateep, 2010).

4.4.3.2 Current and projected selling prices

If the impact of production volumes on the current selling prices are not taken into account, all the scenarios are profitable with a the highest NPV of 992.3 million US$ and IRR of 38.9 % for Scenario B (100), shown in Figure 8, for the current selling prices of 2 500 US$/t SA, 11 424 US$/t PHB and 0.08 US$/kWh. However, the only scenario where the current selling price can still be used is for Scenairo B (25). In Scenario B (25) it was assumed that the production volume of PHB was low enough (3 331 tpa, contributing 5.5 % to the PHA market) to have no impact on the current PHB market price due to oversupply, since it contributed less than 10 % of the total market supply (Table 3).

Consequently, Scenario B (25) proved to be the most profitable scenario with an IRR of 24.1 % and a NPV of 447.2 million US$ (Figure 8) for a combination of the expected SA selling

price (1 500 US$/t) and the current PHB selling price (11 424 US$/t). Therefore the PHB produced in Scenario B (25) could be made available to either the PHA market for pharmaceutical and biomedical applications at a selling price of 11 424 US$/t, (NPV of 447.25 million US$) or the bio-plastics market for packaging applications at a selling price of 2 600 US$/t for a NPV of 263.79 million US$ (Figure 8).

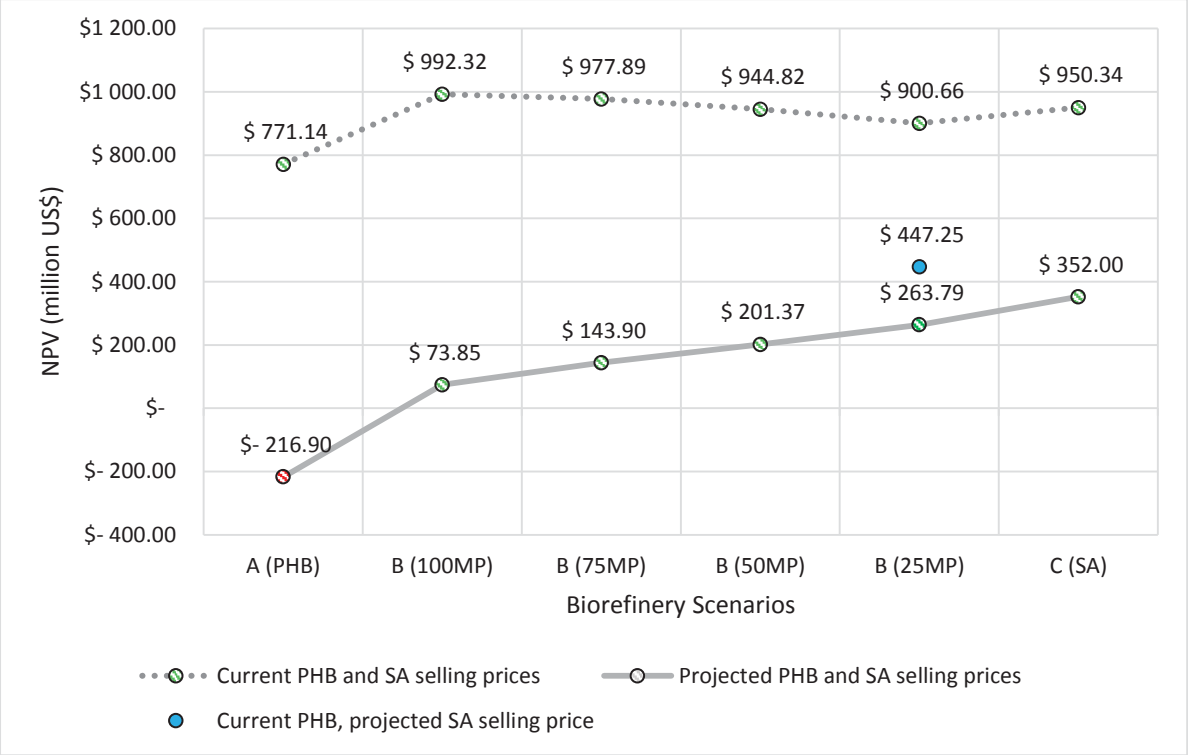


Figure 4-8: Economic results for the Net Present Value (NPV) of each biorefinery scenario for the current selling prices (2 500 US$/t SA and 11 424 US$/t PHB) and project selling prices (1 500 US$/t SA and 2 600 US$/t PHB)

For the selling prices of 1 500 US$/t SA, 2 600 US$/t PHB and 0.08 US$/kWh, all the scenarios except Scenario A were profitable (Table 5). Scenario C was the most profitable with an IRR of 21.6 % and a NPV of 352.0 million US$. The IRR values are summarised in Table 5. Scenario C was the most profitable with an IRR of 21.6 % and a NPV of 352.0 million US$, which compares well to an IRR of 20 % reported for a multiproduct plant producing levulinic acid (5 000 US$/t), succinic acid (3 750 US$/t) and ethanol (750 US$/t) from a hardwood biomass (66 % polysaccharides).(Giuliano et al., 2016a) However it was lower than the 46% IRR obtained for a multiproduct biorefinery producing acetic acid (700 US$/t), succinic acid (1000 US$/t), ethanol (357 US$/t), and electricity (0.11 US$/kWh) from corn stover.(Luo et al., 2010) This is most probably due to the economies of scale benefit obtained for the corn stover biorefinery processing 196.7 t/h feedstock,(Luo et al., 2010) compared to the 65 t/h bagasse and trash processed by the sugarcane biorefinery (current study).

Table 4-5: Total capital and operating costs, production costs and profitability indicators for each scenario

Scenarios	A (PHB)	B				C (SA)	D (CHP)
		(100)	(75)	(50)	(25)		
TCI [a] (million US$)	320.15	342.71	351.06	354.00	352.20	344.50	130.11
TOC[a] (million US$)	35.74	34.24	32.31	33.30	33.16	32.71	13.16
Production cost[b]							
PHB (US$/kg)	2.06	2.76	3.37	5.05	9.96	-	-
SA (US$/t)	-	0.92	0.66	0.54	0.45	0.37	
Electricity (US$/kWh)	1.12	0.99	0.95	1.04	0.93	1.10	0.03
Profitability: Expected selling prices (1 500 US$/t succinic acid, 2 600 US$/t PHB and 0.08 US$/kWh)							
IRR (%)	-	12.5 %	14.8 %	16.7 %	18.7 %	21.6 %	10.3 %
Profitability: Current selling prices (2 500 US$/t succinic acid, 11 424 US$/t PHB and 0.08 US$/kWh)							
IRR (%)	34.7%	38.9%	38.0%	37.0%	36.0%	36.4%	10.3 %
Profitability: Favourable outcome (1 500 US$/t succinic acid, 11 424 US$/t PHB and 0.08 US$/kWh)							
IRR (%)					24.1 %		

a) Based on 2016 values b) The production cost is calculated as the TOC divided by the annual production rate of SA, PHB and bio-energy, respectively. c) Net Present Value: 6.1 million US$.
Abbreviations: TCI – total capital investment, TOC – total operating costs, PHB – Polyhydroxybutyrate, SA – Succinic acid, IRR – internal rate of return, NPV – net present value

4.4.3.3 Economies of scale and investment viability

For economies of scale, high production volumes are desired to reduce the impact of capital costs on the economic outcome. However, high production volumes might have a negative impact on the projected selling prices of high value bio-products, if the market does not increase or is not developed accordingly, as discussed in section 4.4.3.1. Therefore large plant capacities may not be desired for low volume, high value bio-products such as PHB, as seen for the negative NPV of 216.9 million US$ in Figure 8.

In the case of PHB production where the global market size is limited, one strategy to alleviate this problem is to co-produce a low volume, high value bio-product with high volume, low value products such as electricity and SA. In doing so the economies of scale benefit is obtained for shared plant areas such as the pretreatment, detoxification, EH, WWT and CHP plant areas (Figure 1). This is seen for Scenario B (25), with a positive NPV of 447.25 million US$ in Figure 8.

The biorefinery plant capacities in Scenarios B and C could be further increased for the economies of scale benefit by lowering the bypass ratio. This can be done by lowering the plant's steam demand through the implementation of a different DSP for SA recovery such as membrane electrodialysis extraction (Fu *et al*., 2014) or by using an alternative pretreatment method, since the dilute acid pretreatment, detoxification and EH area required the most steam (section 4.4.1.1). Alternatively, formal heat integration such as heat pinch analysis could be implemented on a more detailed design, where the plant layout is taken into consideration, to lower the biorefinery's energy demand for a larger plant capacity and economies of scale benefit.

4.5 Conclusion

The economic outcome of a biorefinery co-producing SA (succinic acid), PHB (polyhydroxybutyrate) and electricity, or a combination of these bio-products, have been investigated. The biorefinery was annexed to a typical South African sugar mill, producing 65 t/h bagasse and trash (dry mass). The lignocellulosic feedstock was shared between the biorefinery and CHP plant. The split or bypass rate was determined by the biorefinery's energy demand. Therefore a lower energy demand, specifically steam, resulted in a larger plant capacity. In turn, the plant capacity had an influence on the biorefinery's profitability.

The plant capacity has an impact on both the bio-product selling prices (depending on the bio-products' production volumes) and total capital equipment cost (due to economies of scale). Consequently large plant capacities should be pursued for bioproducts with potential existing and emerging markets. Alternatively, low volume, high value bio-products can be co-produced with high volume, low value products such as biofuels and electricity. Therefore the question is not only how much you can produce, but how much you should produce for a required or sought after selling price.

At the expected selling prices of 1 500 US$/t SA, 2 600 US$/t PHB and 0.08 US$/kWh, all the scenarios except Scenario A were profitable. The current PHB selling price (11 424 US$/t) could be used in the economic analysis of Scenario B (25) due to the small PHB production volume (5.5 %), resulting in the most profitable scenario with an IRR of 24.1 % and a NPV of 447.2 million US$. If low process complexity, mature process technology, and the potential to increase future production capacities are desired; Scenario C (with an IRR of 21.6 % and NPV of 352.0 million US$) could be implemented in the South African sugar industry. Thereby

addressing declining profit margins, securing a vital sector of the national economy, and supporting the livelihood of nearly one million South Africans.

Supplementary information

More information is available on the variable, fixed and annual capital charge operating costs for each scenario in the supplementary information. The process flow diagram stream tables for Figures 3 and 4 are also provided. Detailed information of the equipment specification, operating conditions, sizing and costing are also provided for each plant area.

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Chapter 5

5. Economic evaluation and comparison of succinic acid and electricity co-production from sugarcane bagasse and trash lignocelluloses in a biorefinery, using different pretreatment methods: Dilute acid (H_2SO_4), Alkaline (NaOH), Organosolv, Ammonia Fibre Expansion (AFEX™), Steam explosion (STEX), and Wet oxidation

While the previous two studies in Chapters 4 and 5 were centered on the bioproducts, this study focuses on the sugarcane lignocellulosic feedstock. The bioproducts are produced through bioconversion from the fermentable sugars, which were liberated from the lignocelluloses. Therefore, this study investigated whether the techno-economic outcome could be improved further by selecting an appropriate pretreatment method for a specific bioproduct. Although any of the three bioproducts or biorefinery scenarios investigated thus far could have been selected for further investigation in this chapter, succinic acid was selected as it is the most widely reported in literature of the three bioproducts. Moreover, it was shown in Chapter 4 to be a profitable single product biorefinery at a market related succinic acid selling price.

Therefore, to select the most favourable pretreatment method for industrial application and maximise the valorisation of lignocelluloses in a biorefinery, the available pretreatment technologies for sugarcane bagasse and trash were screened and nine methods were identified. These methods were then simulated and their techno-economic results were compared for the co-production of succinic acid and electricity. The aim of this study was therefore to maximise the economic outcome of the succinic acid (bioenergy self-sufficient) biorefinery scenario. The results of this study contributed to Objective 3 as stated in section 1.2.

The key outcomes of this chapter are the respective Aspen Plus® simulation models for each pretreatment method. It is vital to select the pretreatment method while taking both the feedstock and bioproduct into consideration. Through this study the most favourable pretreatment method, steam explosion with autohydrolysis and enzymatic hydrolysis, was determined for a succinic acid biorefinery.

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Declaration by the candidate:
With regard to Chapter 5, pg. 128-167, the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Scope definition, biorefinery design and simulation development for pretreatment scenarios, economic costings, interpretation of results and writing of manuscript.	80

The following co-authors have contributed to Chapter 5, pg. 128-167:

Name	e-mail address	Nature of contribution	Extent of contribution (%)
J.F. Görgens	jgorgens@sun.ac.za	General discussion, proof reading and review of manuscript.	8
K. Haigh	khaigh@sun.ac.za	Provided writing assistance through suggestions, review and proof reading of manuscript and general discussion.	8
J. Louw	18655505@sun.ac.za	Developed AFEX™ Aspen Plus® simulation and equipment cost	4

Signature of candidate: MWH……

Date: 28/01/2019……..

Declaration by co-authors:
The undersigned hereby confirm that
1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 5, pg. 128-167,
2. no other authors contributed to Chapter 5, pg. 128-167, besides those specified above, and
3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 3, pg. 128-167, of this dissertation.

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Economic evaluation and comparison of succinic acid and electricity co-production from sugarcane bagasse and trash lignocelluloses in a biorefinery, using different pretreatment methods: Dilute acid (H_2SO_4), Alkaline (NaOH), Organosolv, Ammonia Fibre Expansion (AFEX™), Steam explosion (STEX), and Wet oxidation.

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Abstract

The sugar yield achieved during pretreatment of lignocelluloses has an impact on the economic outcome of a biorefinery. Consequently, chemical and Physio-chemical pretreatment methods were evaluated and compared to determine the most favourable pretreatment method for commercial co-production of succinic acid and electricity in a sugarcane lignocellulosic biorefinery. Nine methods were identified and simulated in Aspen Plus™. All the pretreatment methods were profitable except Organosolv and Wet oxidation. Steam explosion (STEX), sulphur dioxide (SO_2) catalysed STEX and Ammonia Fibre Expansion (AFEX™) resulted in the highest succinic acid yields, with 45.7, 43.5 and 33.4 kg succinic acid per 100 tonnes of dry feedstock, respectively, and are recommended for commercial application. However, due to the high cost of enzymatic hydrolysis (46.23 million US$), the AFEX™ pretreatment method was the most expensive scenario at 385.7 million US$ total capital cost with an IRR of 22.81 %. In comparison to 384.2 million US$ for STEX pretreatment, which resulted in the most profitable biorefinery scenario with an IRR of 28.04 %.

Key words: Biomass, Biorefinery, CHP plant, Succinic acid, Pretreatment

5.1 Introduction

5.1.1 Background information

Lignocellulosic biomass is a desirable renewable feedstock since it is abundant (Sathitsuksanoh *et al*., 2013), less expensive than conventional agricultural crops and in many cases does not compete with food sources (Alvira *et al*., 2010). It is composed of three major fractions: cellulose, hemicellulose and lignin (Benjamin, 2014; Gao *et al*., 2013). Simple sugars, such as D-glucose, xylose, arabinose, mannose and galactose, can be derived from the polysaccharide cellulose and hemicellulose fractions through pretreatment and hydrolysis processing steps.

These simple sugars can be used to produce biofuels, bioenergy and bioproducts in a biorefinery.

Due to the recalcitrant nature of lignocelluloses, pretreatment is required to modify the crystalline cellulose structure and its close association with hemicellulose and lignin in the plant cell wall, to such an extent that the polymer chains of cellulose and hemicellulose become accessible to enzymes in a subsequent enzymatic hydrolysis step (Benjamin, 2014). The factor with the most significant impact on the economic outcome of a cellulosic bioethanol biorefinery is the overall sugar yield achieved during pretreatment and hydrolysis (Tao *et al*., 2011). This supports the notion that efficient and cost-effective pretreatment and hydrolysis processing steps are required to maximise the valorisation of lignocelluloses in a biorefinery for the production of biochemicals and -products (Jorgensen *et al*., 2007).

Succinic acid has been identified as a promising bioproduct with a growing market and a forecasted selling price similar to fossil based maleic anhydride (1500 US$/t) (Orjuela *et al*., 2011; Vaswani, 2014). Although comparisons of the impact of pretreatment on bioethanol production costs have been reported for corn stover (Baral and Shah, 2017; Eggeman and Elander, 2005; Kazi *et al*., 2010), switch grass (Nlewem and Thrash, 2010; Tao *et al*., 2011), sugarcane and sweet sorghum bagasse (Cao *et al*., 2012; Dias *et al*., 2011), similar comparisons have not been reported for succinic acid production from sugarcane lignocelluloses.

5.1.2 Range of pretreatment methods considered

Pretreatment and hydrolysis methods available for sugarcane bagasse and trash include chemical-, physiochemical-, hydrothermal pretreatment, or a combination of these methods (Carvalheiro *et al*., 2008). Physical pretreatment methods such as thermal treatment, radiation, size reduction, and biological pretreatment methods such as white rot fungi, were not considered since they cannot compete with the process efficiency, time efficiency, selectivity and cost of chemical pretreatment methods (Wyman, 2013). Hydrothermal (steam explosion, supercritical carbon dioxide explosion, liquid hot water and wet oxidation) and physio-chemical methods (ammonia fibre expansion and oxidising agents) are included under chemical pretreatment methods, which also include alkaline, acid, solvent based, and oxidative hydrolysis (Nanda *et al*., 2014; Su *et al*., 2015; Tan *et al*., 2014). The pretreatment methods where experimental data are available for sugarcane bagasse or trash are summarised in Table 1.

When selecting the pretreatment and hydrolysis processing step, the feedstock selection is vital, since the physio-chemical properties of different lignocelluloses vary and should be taken into consideration together with the desired bioproduct and its process requirements (Alvira et al., 2010; Menon and Rao, 2012). From the pretreatment methods listed in Table 1, the oxidative hydrogen peroxide (H_2O_2) method is unfavourable, since it has been shown that it is not economically viable due to the high H_2O_2 catalyst price (Dias *et al.,* 2011). Likewise, ionic liquid (IL) pretreatment is not suitable for succinic acid production, since the presence thereof in IL pretreated pine wood significantly inhibited the microorganism responsible for succinic acid production, *Actinobacillus succinogenes* (Wang *et al.,* 2014). In addition, the liquid hot water method was excluded due to the extreme energy demand and sugar dilution, which could not be mitigated through integration with the succinic acid recovery process, as can be done with ethanol recovery in the distillation column(s) for the production of bioethanol (Archambault-Leger *et al.,* 2014).

The aim of this study is to select the most economically favourable pretreatment method for commercial co-production of succinic acid and electricity in a sugarcane lignocellulosic biorefinery. The first objective is to determine the most profitable pretreatment method, through an economic evaluation and comparison of the available pretreatment methods, for sugarcane lignocelluloses to produce succinic acid. The pretreatment methods selected for comparison are Dilute acid pretreatment, Acid hydrolysis, Alkaline pretreatment, Organosolv, Ammonia fibre expansion (AFEX™), Steam explosion (STEX), sulphur dioxide catalysed STEX (STEX with SO_2), STEX followed by alkaline delignification (STEX with NaOH), and Wet Oxidation (WO).

The second objective is to consider the commercial readiness of the profitable pretreatment methods for industrial application. This is addressed in respective case studies on the two major assumptions made for industrial application of the selected pretreatment method for succinic acid production from sugarcane lignocelluloses, namely the enzymatic hydrolysis solids loading and the solids to liquid ratio, where applicable. To this end, the available pretreatment technologies for sugarcane lignocelluloses were screened and nine methods were identified, simulated and compared for the co-production of succinic acid and electricity in a sugarcane bagasse and trash biorefinery.

Table 5-1: Pretreatment methods and combinations for sugarcane lignocelluloses reported in literature

PRETREATMENT METHODS	CHEMICAL					PHYSIO-CHEMICAL	HYDROTHERMAL			
Bioproduct	Dilute acid pretreatment (DAT)	Acid Hydrolysis	Alkaline method (NaOH)	Organosolv pretreatment (Ethanol)	Hydrogen Peroxide (H_2O_2)	Ammonia Fibre Expansion (AFEX™)	Steam Explosion (STEX)	STEX with SO_2	STEX with alkaline delignification (NaOH)	Other hydro- thermal pretreatments
Bioethanol	Archambault-Leger *et al.*, 2014; Canilha *et al.*, 2011; Gnansounou *et al.*, 2015; Neves *et al.*, 2016; Sindhu *et al.*, 2011	-	Maryana *et al.*, 2014	Dias *et al.*, 2011	Dias *et al.*, 2011	Krishnan *et al.*, 2010	Archambault-Leger *et al.*, 2014; Martín *et al.*, 2002; Neves *et al.*, 2016; Oliveira *et al.*, 2013	Carrasco *et al.*, 2010; Dias *et al.*, 2011; Laser *et al.*, 2002	Oliveira *et al.*, 2013; G. J.M. Rocha *et al.*, 2012; George J M Rocha *et al.*, 2012	*Archambault-Leger et al.*, 2014 (LHW)
Simple sugars	Harrison *et al.*, 2013; Leibbrandt, 2010; Yu *et al.*, 2013	Harrison *et al.*, 2013; Lavarack *et al.*, 2002; Moutta *et al.*, 2012	Guilherme *et al.*, 2015; Harrison *et al.*, 2013; Yu *et al.*, 2013	-	-	-	-	Leibbrandt, 2010	-	Biswas *et al.*, 2014 (WO)
Citric acid		Khosravi-Darani and Zoghi, 2008	Khosravi-Darani and Zoghi, 2008	-	-	-	-	-	-	-
PHA	Lopes *et al.*, 2014; Yu and Stahl, 2008	Lopes *et al.*, 2014	-	-	-	-	-	-	-	-
Succinic acid	Chen *et al.*, 2016; Jiang *et al.*, 2013	Liang *et al.*, 2013; Liu *et al.*, 2013	Chen *et al.*, 2016	-	-	Santos *et al.*, 2016	-	-	-	-

LHW: Liquid hot water; WO: Wet oxidation; PHA: Polyhydroxyalkanoates

5.2 Methodology

5.2.1 Feedstock basis of design

The feedstock and energy requirements were based on a typical South African sugar mill processing 300 tonnes of sugar cane per hour (Görgens *et al*., 2016; Nieder-Heitmann *et al*., 2018). It is estimated that 65 tonnes of bagasse and trash can be made available by upgrading the boiler to a high efficiency, high pressure boiler with a condensing extraction turbine in the CHP plant, coupled with the introduction of green harvesting methods (Nieder-Heitmann *et al*., 2018).

The compositional fractions of sugarcane bagasse and trash vary with crop variety, location, fertilisers and seasonal changes, but are within the ranges of 66.6 – 77.6% (dry mass) for the polysaccharides and 14.4 – 23.1% for lignin (Benjamin, 2014). The feedstock composition is based on a 60% bagasse (Benjamin, 2014) and 40% trash (Diedericks *et al*., 2012) ratio as shown in Table 2.

Table 5-2: Bagasse and trash feedstock composition as simulated in Aspen Plus® based on a normalised 60% bagasse(Benjamin, 2014) and 40% trash (Diedericks *et al*., 2012) feed ratio

Sugarcane Composition[a]	Solid fraction	Liquid fraction
Cellulose	40.6%	
Hemicellulose	23.5%	
Arabinan	1.2%	
Lignin (acid soluble)		2.2%
Lignin (acid insoluble)	20.7%	
Acetyl group and Extractives	10.5%	
Ash	3.5%	
Water		97.9%
Total	100%	100%
Mass flow rate	65 000 kg/h	48 000 kg/h

a) Reported values were normalised for a sum total of 100%

5.2.2 Biorefinery process overview

The scenarios were simulated in Aspen Plus® version 8.8 using literature data and the Electrolyte Non-Random Two Liquid (ELECNRTL) base property method *(*Ali Mandegari *et al.,* 2017; Görgens *et al.,* 2016). The vapour phase properties are calculated using the Redlich-Kwong (RK) equation of state (EoS). No formal heat integration, such as a heat pinch analysis, was done. However some heat saving was done within plant areas using heat exchangers. For these HeatX Aspen Plus® units, a temperature approach of 10°C were used. An overview of

the equipment units' operating conditions, selection, sizing and costing are provided in the supplementary information for each pretreatment method.

The biorefinery and CHP plant are annexed to an existing sugar mill. The sugarcane is fed to the sugar mill, where the sugar juice is pressed out and the remaining fibrous bagasse residue is made available for additional valorisation together with the trash, which is collected from the field. The bagasse and trash feedstock is then split between the CHP plant and biorefinery. The split or bypass ratio is determined to ensure sufficient energy generation in the CHP plant to sustain both the biorefinery (steam and electricity requirements) and existing sugar mill (120 t/h steam at 28 atm and 340°C (Görgens *et al.*, 2016)).

The biorefinery has four major plant areas as shown in Figure 1: a) the pretreatment and enzymatic hydrolysis, b) seed train, cellulase plant and fermentation area, c) downstream processing and d) waste water treatment plant area. The process description and economic evaluation of the seed train and fermentation, downstream processing, waste water treatment (WWT) plant and CHP plant have been reported previously (Chapter 4). The succinic acid biorefinery, CHP plant and existing sugar mill are shown in Figure 1. For the comparison of the various pretreatment options, the process flow configuration for plant area A in Figure 1 will vary and is discussed in section 5.2.3. for each of the nine pretreatment methods simulated.

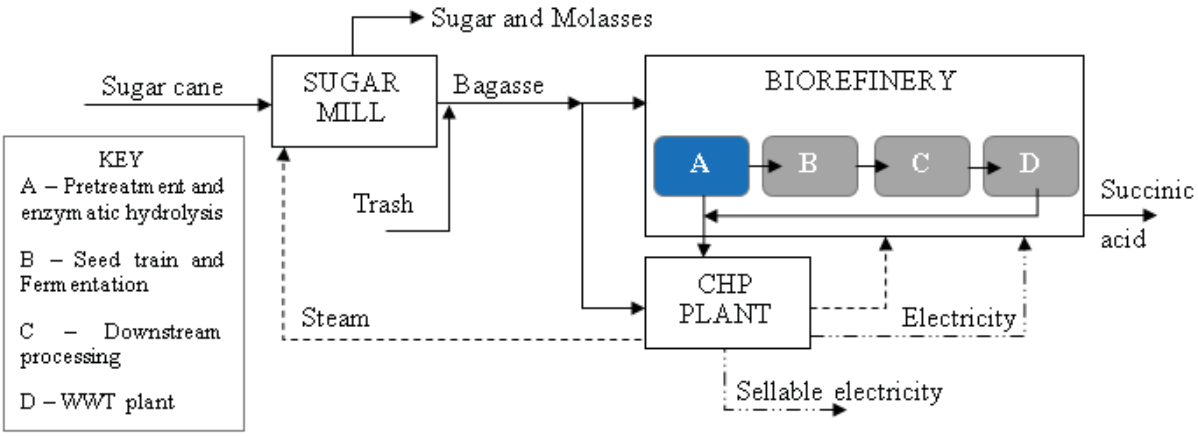

Figure 5-1: Biorefinery and Combined Heat and Power (CHP) plant annexed to an existing sugar mill (Plant area A (blue) is the pretreatment and enzymatic hydrolysis area)

5.2.3 Process descriptions of pretreatment hydrolysis methods

5.2.3.1 Dilute acid pretreatment and enzymatic hydrolysis (DAT)

The pretreatment method used for the base case scenario is dilute acid pretreatment followed by enzymatic hydrolysis (block A in Figure 1) (Benjamin, 2014). To ensure energy self-sufficiency, 28% of the available feedstock (18.2 t/h DM) was bypassed to the CHP plant, and

the remaining 46.8 t/h was mixed with 0.65% H_2SO_4 acid (Benjamin, 2014) and heated to 180°C for 10 minutes by direct steam injection (Benjamin, 2014). The 1:20 solid to liquid ratio was adjusted to a 1:2 ratio for industrial application, based on the 30 wt% solids loading design assumption used previously (i.e. 1:2.34 ratio) (Humbird, 2011), while assuming the same performance in terms of fermentable sugar yields and solids digestibility. This major design assumption is evaluated in section 5.4.3, *Case studies*.

The solid cellulose and lignin (cellulignin) fraction was washed and diluted to 20% solids by mass prior to enzymatic hydrolysis at 50°C for 72 h (Humbird, 2011). The liquid hemicellulose hydrolysate was combined with the cellulignin wash water and sent to granular activated carbon (GAC) detoxification to remove furfural and hydroxymethylfurfural (HMF) (Nieder-Heitmann *et al*., 2018). The process flow diagram is shown in Figure 2a.

5.2.3.2 Dilute acid hydrolysis (DAT without EH)

The dilute acid hydrolysis pretreatment hydrolyses the hemicellulose fraction at mild conditions for high pentose sugar yields and low sugar degradation to furfural and HMF (Benjamin, 2014). The pretreatment severity is therefore lower than the DAT with EH, since there is no subsequent enzymatic hydrolysis. As a result, the overall or combined sugar yield in the hydrolysates will be lower, but they will be rich in pentose sugars. Significant capital and operational costs associated with enzymatic hydrolysis, such as the hydrolysis fermentation tanks and cellulase modular production plant, will also be avoided.

The lignocellulose fed to the biorefinery was mixed with 0.5% H_2SO_4 acid at a 1:2 solid to liquid ratio (Humbird, 2011) at 165°C for 15 minutes (Koekemoer, 2018). After pretreatment, the treated lignocellulose slurry was centrifuged and the solid cellulignin (at 50 wt% moisture content) was removed, washed, and sent to the boiler for combustion in the CHP plant with the bypassed feedstock. Additional soluble and fermentable sugars are obtained by washing the solids while dilution caused by washing is not detrimental to the process performance. The succinic acid producing microorganism is inhibited by high sugar concentrations (more than 100 g/L) and therefore the concentrated fermentation feed stream is diluted by the sugar containing wash water. The combined cellulignin wash water and hemicellulose hydrolysate stream was detoxified using GAC adsorption to remove any furfural or HMF present (Nieder-Heitmann *et al*., 2018). The process flow diagram is shown in Figure 2b.

5.2.3.3 *Sodium Hydroxide Alkaline Pretreatment and enzymatic hydrolysis (NaOH)*

Alkaline pretreatment hydrolyses the hemicellulose and lignin fractions to increase enzymatic digestibility of the polysaccharides (Chen *et al*., 2016). The feedstock was mixed with 0.25 M NaOH at 121°C for 2 hours (Chen *et al*., 2016). The 1:15 solid to liquid ratio was adjusted to 1:2 for industrial application, based on the 30 wt% solids loading design assumption used previously (i.e. 1:2.34 ratio) (Humbird, 2011), as discussed in section 5.4.3. The slurry was centrifuged and the solid fraction washed prior to enzymatic hydrolysis at 50°C and 30 h (Chen *et al*., 2016). The cellulignin wash water and centrifuge liquid fraction were combined and sent to the lignin precipitation step, where H_2SO_4 was added to a pH of 2 and 48.2 %wt lignin was recovered from the original feedstock (Rocha *et al*., 2012), and sent to the boiler for combustion in the CHP plant with the bypassed feedstock. The process flow diagram is shown in Figure 2c.

5.2.3.4 *Organosolv and enzymatic hydrolysis*

Organosolv fractionates the lignocellulose by hydrolysing the lignin and increasing the enzymatic digestibility of the cellulose (Alvira *et al*., 2010). The feedstock is mixed with the aqueous ethanol solvent (50% v/v) at a solid to liquid ratio of 1:5 and 175°C for 60 minutes (Mesa *et al*., 2010). The reactor was heated using direct steam injection (208°C and 1.8 MPa), and 1.25 wt% H_2SO_4 acid was added as catalyst (Mesa *et al*., 2010). The pretreated slurry was centrifuged and the solid fraction was washed prior to enzymatic hydrolysis at 50°C for 24 h (Mesa *et al*., 2010). The liquid fraction, containing xylose, soluble lignin, sulphuric acid and ethanol, was pumped to a distillation column where the ethanol was recovered and recycled to the pretreatment reactor. A solvent make-up stream is added to account for the ethanol (4.9%) lost.

The bottoms stream from the distillation column was sent to a lignin precipitation step (Rocha *et al*., 2012). The product stream was then filtered and the recovered lignin was sent to the boiler in the CHP plant. The liquid fraction was neutralised from any remaining acid catalyst using calcium oxide ($Ca(OH)_2$) and sent directly to the triple effect evaporator in the fermentation area to ensure removal of any residual ethanol prior to fermentation. The process flow diagram is shown in Figure 2d.

5.2.3.5 *Ammonia Fibre Expansion (AFEX™) and enzymatic hydrolysis*

For the AFEX™ pretreatment method the feedstock is mixed with liquid ammonia and pressurised followed by a sudden pressure release causing expansion of the ammonia gas and

the consequent disruption of the lignocellulose, resulting in a dry feedstock with increased enzymatic digestibility (Alvira *et al*., 2010; Mokomele *et al*., 2018). The feedstock was pressurised to 1.7 MPa using compressed ammonia at a 1:1 solid to liquid ratio, and passed through a plug flow reactor with a 30 minutes residence time (Krishnan *et al*., 2010; Mokomele *et al*., 2018). No energy utility was required, since the heat of mixing between ammonia and water was sufficient to reach the required pretreatment temperature of 140°C (Krishnan *et al*., 2010; Mokomele *et al*., 2018). After pretreatment, ammonia was recovered from the biomass in a flash drum and stripping column using high pressure steam as the stripping agent (266°C and 1.3 MPa). The pretreated feedstock was then mixed with water to 20 wt% solids (Humbird, 2011) prior to enzymatic hydrolysis at 50°C and 72 h (Mokomele *et al*., 2018). The process flow diagram is shown in Figure 2e.

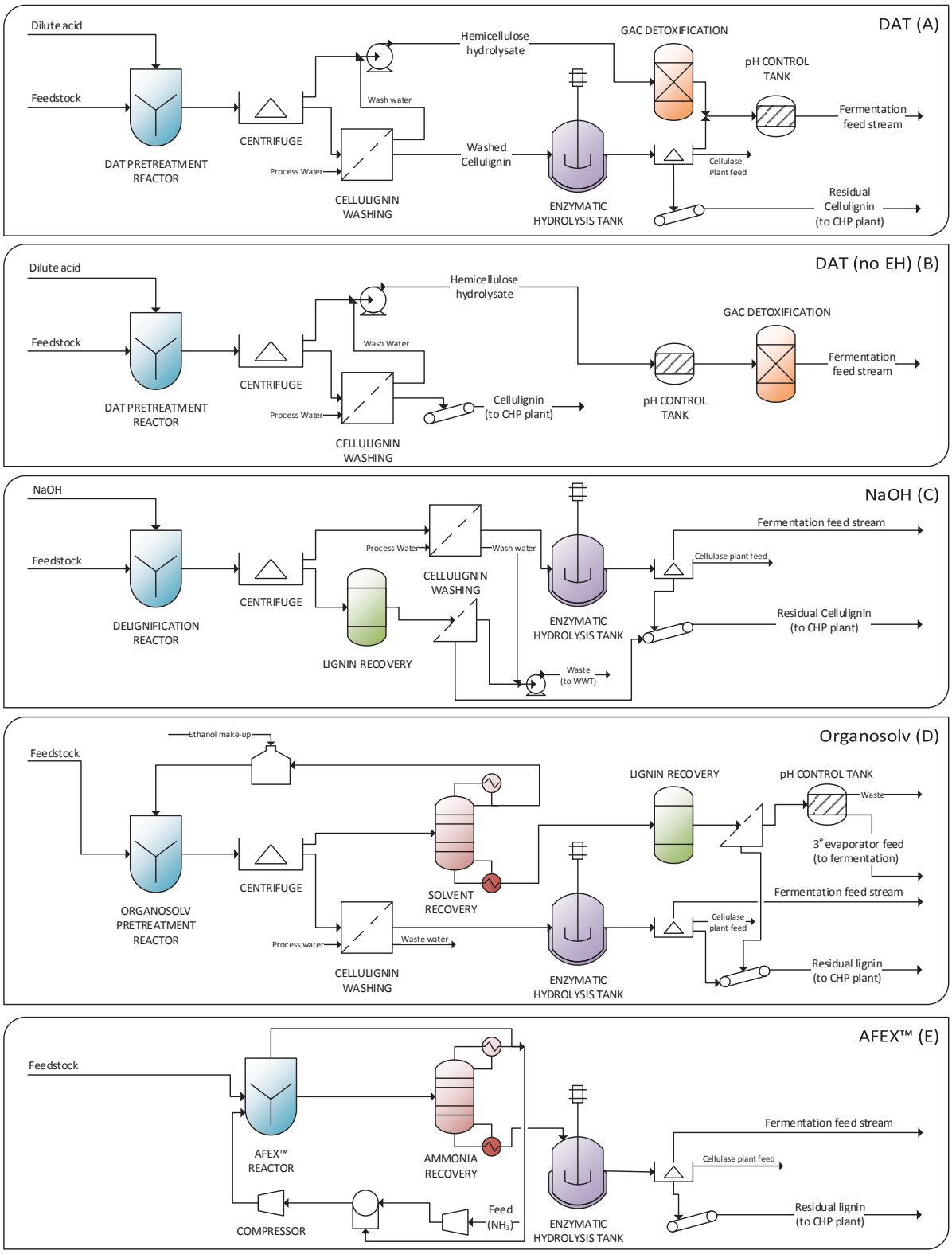

Figure 5-2: Process flow diagram for the chemical and physio-chemical pretreatment methods (a: Dilute acid without enzymatic hydrolysis (EH), b: Dilute acid with EH, c: Alkaline (NaOH) delignification with EH, d: Organosolv with EH and e: AFEX™ with EH)

5.2.3.6 Steam explosion with autohydrolysis (STEX) and enzymatic hydrolysis

During steam explosion without an acid catalyst, autohydrolysis occurs leading to biomass disruption, which is catalysed by the acetic acid produced by the breakdown of the acetyl in

hemicellulose (Neves *et al*., 2016) and by the protonation of water molecules under the high temperatures and pressures. The feedstock was loaded into the pretreatment reactor and heated to 195°C for 7.5 minutes using direct steam injection (Neves *et al*., 2016) (208°C and 1.8 MPa). During the sudden pressure release, mass losses occurred due to the volatilisation of components such as terpenes, aliphatic alcohols, aldehydes and derivatives, leading to a solids recovery of 74.6 wt % (simulated), which is close to the 73.8 wt% reported *(Neves et al*., 2016). The cellulignin solid was washed and diluted to 20 wt% solids concentration (Humbird, 2011) prior to enzymatic hydrolysis at 50°C and 96 h (Neves *et al*., 2016). The volatile components released during STEX were condensed and sent to the WWT plant together with the cellulignin wash water. The process flow diagram is shown in Figure 3a.

5.2.3.7 Steam explosion with sulphur dioxide infiltration (STEX with SO_2) and enzymatic hydrolysis

The pretreatment severity is increased from autohydrolysis in STEX pretreatment by the addition of an acid-generating compound such as sulphur dioxide (SO_2) (Neves *et al*., 2016). The feedstock moisture content was increased to 75 wt% and mixed with the SO_2 catalyst (produced on-site) at 2% mass per mass water content of the feedstock (Carrasco *et al*., 2010). The SO_2-enriched feedstock was then heated to 190°C using direct steam injection (208°C and 1.8 MPa) for 5 minutes (Carrasco *et al*., 2010). After the sudden pressure release the slurry was centrifuged and the solid cellulignin fraction was washed and diluted (20 wt%) (Humbird, 2011) prior to enzymatic hydrolysis at 40°C for 72 h (Carrasco *et al*., 2010).

The combined cellulignin wash water and hemicellulose hydrolysate stream was detoxified using GAC adsorption to remove furfural and HMF (Nieder-Heitmann *et al*., 2018). The detoxified hemicellulose hydrolysate, wash water and enzymatic hydrolysis product streams were combined and sent to the fermentation area. The process flow diagram is shown in Figure 3b.

5.2.3.8 Steam explosion with alkaline delignification (STEX with NaOH) and enzymatic hydrolysis

After autohydrolysis steam explosion at 190°C for 15 minutes (Rocha *et al*., 2012), 72.7 wt% solids were recovered, washed and sent to an alkaline delignification step to remove the residual lignin. The wash water was collected and sent to a post-hydrolysis step where the oligomers were treated with H_2SO_4 at 121°C for 30 minutes and converted to monosaccharides (Rocha *et al*., 2012). The cellulignin obtained after washing was mixed with 1% w/v sodium hydroxide (NaOH) at a solid to liquid ratio of 1:10 at 100°C for 60 minutes (Rocha *et al*., 2012).

The 1:10 solid to liquid ratio was adjusted to 1:2 for industrial application, based on the 30 wt% solids loading design assumption used previously (i.e. 1:2.34 ratio) (Humbird, 2011), as discussed in section 5.3.3. After delignification, the slurry was sent to a centrifuge where the solid cellulose fraction was recovered and washed through seven washing cycles. The washing cycles were simulated as washing step with a process water to solid cellulose ratio of 7:1. After washing, the slurry was diluted to 20 wt% solids prior to enzymatic hydrolysis at 50°C for 72 h (Humbird, 2011) with an assumed cellulose conversion of 1.0 (Neves *et al.*, 2016) and hemicellulose conversion of 0.35 (Carrasco *et al.*, 2010; Mesa *et al.*, 2010). The centrifuge liquid stream was sent to a lignin precipitation step (Rocha *et al.*, 2012). The recovered lignin was sent to the CHP plant and the residual liquid was sent to the WWT plant with the condensed volatile stream after STEX pressure release. The enzymatic hydrolysis and post-hydrolysis product streams were mixed together and sent to the fermentation area. The process flow diagram is shown in Figure 3c.

5.2.3.9 Wet oxidation (WO) and enzymatic hydrolysis

The wet oxidation pretreatment method uses oxygen as a catalyst to reduce temperatures and pretreatment time required to solubilise the hemicellulose and lignin fractions for increased enzymatic digestibility (Alvira *et al.*, 2010). The feedstock is diluted (16.6 wt% solid) and charged with oxygen (O_2) at 0.6 MPa (Biswas *et al.*, 2014) (at calculated flow rate of 77.45 kg per 10 L), whereafter the reactor was heated to 185°C for 10 minutes, followed by a sudden pressure release (Biswas *et al.*, 2014). The cellulignin and hemicellulose hydrolysate were separated using vacuum filtration, whereafter the cellulignin was washed and diluted (20 wt%) (Humbird, 2011) prior to enzymatic hydrolysis at 50°C and 96 h (Biswas *et al.*, 2014). The post-hydrolysis step, where the oligomers present in the hemicellulose hydrolysate are treated with H_2SO_4, was not included for the simulation since the amount of monosaccharides recovered did not justify the energy required for post-hydrolysis nor the significant dilution of the sugar stream sent to fermentation. The process flow diagram is shown in Figure 3d.

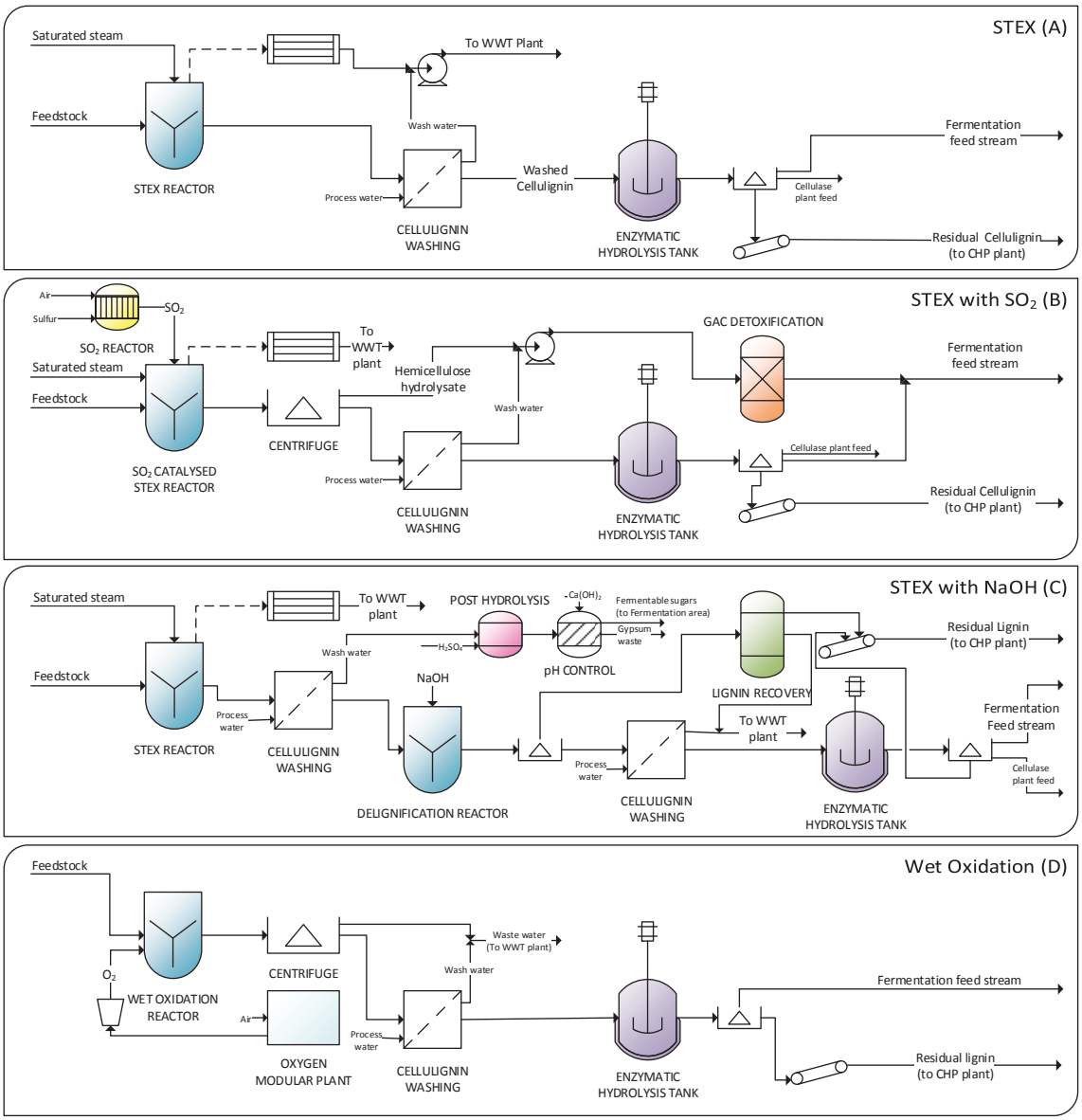

Figure 5-3: Process flow diagram for the hydrothermal pretreatment methods, followed by enzymatic hydrolysis (a: Steam Explosion (STEX), b: STEX with SO_2 catalyst, c: STEX with alkaline (NaOH) delignification and d: Wet Oxidation)

5.2.4 Economic methodology

5.2.4.1 Capital cost estimate

The capital cost estimate was done at a study estimate or concept design level, with an accuracy range of ±30% typically used to decide between design choices, such as the different pretreatment methods investigated (Turton, Bailie and Whiting, 2013). The mass and energy balances of each biorefinery scenario were used to size and finalise the major equipment list. The installed capital cost for the majority of the equipment units was determined using the Aspen Economic Analyser®.

The purchased cost of the dust suppression system, clarifier, reverse osmosis modular plant, CEST, boiler and cellulase plant were adjusted from literature (Humbird, 2011; Wooley *et al.*,

1999) for the capacity and time of study (2016) using equations E-1 and E-2, (Medina *et al.*, 2018; Turton, Bailie and Whiting, 2013) and multiplied with an installation factor *F* for the installed equipment cost C_I (equation E-3) (Medina *et al.*, 2018; Turton, Bailie and Whiting, 2013). The installation factor *F* was determined for the specific material of construction and operating conditions such as temperature and pressure, and is available elsewhere (Humbird, 2011; Turton, Bailie and Whiting, 2013).

$$\frac{C_1}{C_2} = \left(\frac{A_1}{A_2}\right)^n \qquad \text{Equation E-1}$$

In Equation E-1, C_1 is the new purchased cost, C_2 is the base purchased cost, A_1 is the new or required capacity, A_2 is the base capacity and *n* is the scaling exponent (Humbird, 2011; Turton, Bailie and Whiting, 2013).

$$C_P = C_b\left(\frac{I_r}{I_b}\right) \qquad \text{Equation E-2}$$

$$C_I = C_P * F \qquad \text{Equation E-3}$$

In Equation E-2, C_P is the purchased cost for the required year, C_b is the purchased cost for the base year (or C_1 from E-1 if the value has been adjusted for capacity), I_r is the Chemical Engineering Plant Index (CEPCI) value for the required year (536.5 in 2016) and I_b is the CEPCI value of the base year.

The installed equipment cost for the GAC absorption columns used for detoxification was determined using the CAPCOST method (Turton, Bailie and Whiting, 2013), and the installed equipment cost for the enzymatic hydrolysis and fermentation tanks were determined as for jacketed, glass lined reactors (Sinnott, 2005). The installed equipment cost for the milling unit and pretreatment reactors were adjusted for capacity (E-1) and time (E-2) from a similar pretreatment study (Magalhães *et al.*, 2017).

The installed capital cost of the biorefinery (ISBL) was used to determine the cost of utilities and storage (11.1% of ISBL) and indirect costs of warehousing (4% of ISBL), site development (9% of ISBL), and additional piping (4.5% of ISBL) (Nieder-Heitmann *et al.*, 2018). The ISBL cost, CHP plant, storage, utilities and indirect costs summate to the total direct costs (TDC), which was used to determine the total indirect costs (60% of TDC) (Nieder-Heitmann *et al.*, 2018). Together, the total indirect and direct (TDC) costs provided the fixed capital investment (FCI). The working capital was taken as 5% of the FCI, and a land factor of 1 was applied, to determine the total capital investment (TCI) (Nieder-Heitmann *et al.*, 2018).

5.2.4.2 *Operational cost estimate*

The mass and energy balances were used to determine the total operating costs (TOC). The TOC include the variable costs, fixed costs and annual capital charge expenses. More information on the operating costs used for the succinic acid biorefinery are available in previous work (Chapter 4). The feedstock cost is 10.79 US$/t and it is assumed that excess electricity is sold back into the network at 0.08 US$/kWh (Nieder-Heitmann *et al*., 2018). Additional variable costs for the pretreatment scenarios were adjusted for time (E-2) and include the cost of sodium hydroxide (NaOH) alkaline solvent (0.10 US$/kg (2016) (Mussatto *et al*., 2013), ethanol (1.08 US$/kg) (Mussatto *et al*., 2013), and calcium hydroxide (0.09 US$/kg) (Tao *et al*., 2011).

5.2.4.3 *Profitability estimate*

The TCI and TOC values were used in a discounted cash flow (DCF) rate of return analysis to determine the internal rate of return (IRR) for a discount rate of 9.7%. A real term approach was used whereby the selling prices and TOC were not adjusted for inflation over the plant life span of 25 years, and therefore the project resulted in a net present value (NPV) of 0 US$ for a discount rate of 9.7%. To this end, an IRR of more than 9.7% is an indication of a profitable project, with a positive NPV, for a succinic acid selling price of 1500 US$/kg (Orjuela *et al*., 2011; Vaswani, 2014) and electricity selling price of 0.08 US$/kWh (Nieder-Heitmann *et al*., 2018). Details on the economic parameters used in the DCF have been provided previously and include straight line depreciation over 5 years (20%) and an income tax rate of 28% (Nieder-Heitmann *et al*., 2018).

5.2.4.4 *Simulation delimitations*

Although experimental or pilot plant work provides qualitative results, it can be time consuming and expensive. Therefore simulations can be used as a screening tool to identify the most favourable process options, which can then be verified through experimental or pilot plant work, or to direct the focus of experimental work (Eggeman and Elander, 2005), to ensure the pretreatment methods have industrial application within the biorefinery context. To this end, predictive models or simulations enable the selection, design, and optimisation of pretreatment methods that are suitable for a specific type of biomass and downstream process configuration (Maurya et al., 2015; Mosier et al., 2005).

The assumption that the conversion efficiencies for succinic acid production from fermentable sugars remained constant was made to address the missing link between the experimental data

used for pretreatment methods and the subsequent succinic acid fermentation, since all the necessary experimental data is not available. Although the fermentation results did not reflect any unforeseen fermentation inhibition, such as the impact of the 1 ppm dissolved oxygen in the fermentation feed stream on the anaerobic fermentation performance with the WO pretreatment method, the overall succinic acid produced per tonne feedstock varied, depending on the total and type of fermentable sugars available after pretreatment and enzymatic hydrolysis. Moreover, the uncertainty was dealt with by adhering to the known fermentation requirements and conditions for succinic acid production, thereby assuming that the fermentation performance was not over- or under estimated.

5.3 Results and Discussion

Although experimental or pilot plant work provides qualitative results it can be time consuming and expensive. Therefore simulations can be used as a screening tool to identify the most favourable process options based on bench-scale optimisation, which can then be verified through experimental or pilot plant work. Simulations can also be used to direct the focus of experimental work (Eggeman and Elander, 2005), to ensure the pretreatment methods have industrial application within the biorefinery context. To this end, predictive models or simulations enable the selection, design, and optimisation of pretreatment methods that are suitable for a specific type of biomass and downstream process configuration (Maurya *et al*., 2015; Mosier *et al*., 2005).

5.3.1 Mass and Energy balance

The monomeric, fermentable sugar yields obtained by the combinations of alternative pretreatment methods with subsequent hydrolysis are provided in Table 4, together with the reported sugar yields. The minor differences between the reported and simulated values were attributed to variation in feedstock composition and additional processing steps in the simulation, such as cellulignin washing and detoxification. The largest yield of succinic acid per dry tonne of feedstock fed to the biorefinery was obtained for the STEX and STEX with SO_2 pretreatment methods, resulting in 45.7 and 43.5 kg succinic acid, respectively (Table 3).

The biorefinery utilised low pressure steam (LPU 293°C at 650 kPa), high pressure steam (HPU 266°C at 1300 kPa) and electricity as energy sources (Table 3). The biorefinery's energy needs were different for each pretreatment method and are provided in Table 4. An iterative approach was used to determine the exact amount of feedstock that had to be bypassed from each biorefinery to its CHP plant, in order to generate sufficient steam and electricity to meet the biorefinery's energy needs, as well as the 120 t/h steam requirement for the existing sugar mill.

The bypass ratio was impacted significantly by the solubilisation of the lignin fraction. Even though the lignin was recovered through a precipitation step, the fractional conversion was only 0.51 – 0.63 (Table 4), resulting in some lignin losses. High bypass ratios were required for the Organosolv pretreatment method at 64%, the sodium hydroxide alkaline pretreatment method (NaOH) at 62%, the combined steam explosion and alkaline delignification method (STEX and NaOH) at 51%, and the wet oxidation (WO) method at a bypass ratio of 48% (Figure 4). In comparison, the AFEX™ and DAT without EH pretreatment methods had low bypass ratios of 25% and 10%, respectively, due to no or low solubilisation of the lignocellulosic feedstock.

The biorefinery energy requirement also had an impact on the bypass ratio, but to a lower extent than lignin solubilisation. Although the lignin solubilisation of the Organosolv pretreatment were low, at 0.092 fractional conversion compared to 0.9 and 0.92 for NaOH and STEX with NaOH, respectively (Table 4), the high energy requirement of 0.86 kW and 47.1 tonne steam per dry tonne feedstock resulted in a high bypass ratio of 64% for the Organosolv pretreatment method. The high energy requirement was due to the ethanol distillation column used for solvent recovery, which used 94.6 t/h HPU steam and 51.2% steam overall.

On the other hand, a high monomeric sugar concentration resulted in a lower bypass ratio compared to the other scenarios with low monomeric sugar concentrations (Table 3), since less HPU steam energy was required for the triple effect evaporator prior to fermentation. The WO pretreatment method resulted in a total sugar concentration of 132 g/L (Table 3), causing a low bypass ratio of 48%, even though it has a high fractional conversion of 0.661 for lignin solubilisation (Table 4).

The plant capacity of a bioenergy self-sufficient biorefinery is determined by the required bypass ratio of feedstock from the plant to the CHP plant. As a result, the way in which a pretreatment method impacts the biorefinery's energy *provision* (such as the available cellulignin stream after lignin solubilisation and EH) as well as the energy *required* (such as the amount of HPU steam energy required to concentrate the pretreatment and EH product stream prior to fermentation) have a major impact on the bypass ratio. From the pretreatment methods investigated, low lignin solubilisation, high monomeric sugar yields and low overall energy requirements contribute to a low bypass ratio. A low bypass ratio (i.e. large plant capacity) is one of the contributing factors to a profitable biorefinery scenario, together with

the capital costs, operational costs, and succinic acid yield on feedstock, as discussed in section 3.2.

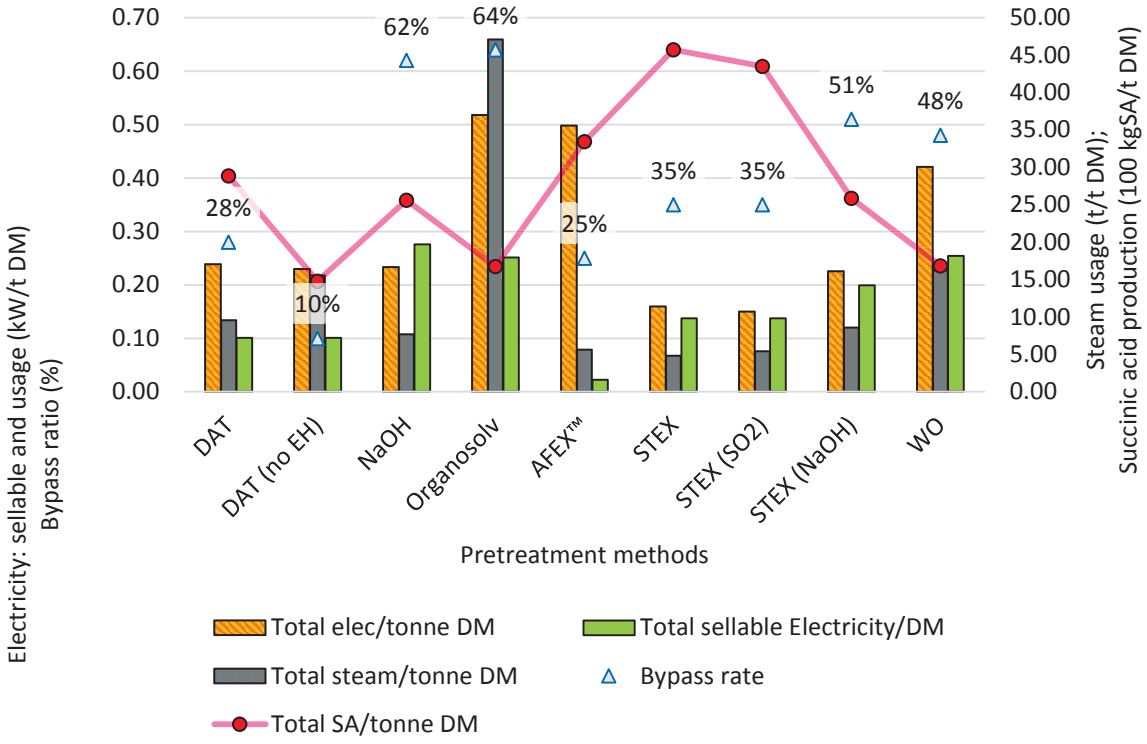

Figure 5-4: Mass and Energy balance results per tonne of lignocellulosic feedstock

Table 5-3: Mass and Energy balance results

Pretreatment methods	DAT	DAT (no EH)	NaOH	Organosolv	AFEX™	STEX	STEX with SO2	STEX with NaOH	WO
Feedstock fed to the biorefinery (t DM/h)	46800	58500	24700	23400	48750	42250	42250	31850	33800
Succinic acid per feedstock (kg SA/100 t DM)	28.9	14.8	25.6	16.8	33.4	45.7	43.5	25.9	16.9
Succinic acid produced (t/h)	13.5	8.6	6.3	3.9	16.3	19.3	18.4	8.2	5.7
Fermentable sugar stream concentration (g/L)	85.0	87.3	105.5	Footnote a	113.7	204.7	83.7	113.1	132.0
Saturated steam (t/h)	62.2	73.6	0.0	38.0	0.0	14.3	32.9	9.8	0.0
LPU (t/h)	6.59	6.27	8.59	6.55	13.28	13.96	11.93	5.90	23.64
HPU (t/h)	60.6	54.6	40.1	140.2	78.3	64.8	54.3	55.0	68.7
Electricity Produced (kWh)	7943.6	7903.3	8290.7	7907.6	9209.6	8880.5	8554.8	8208.7	10979.6
Electricity Required (kWh)	3220.3	1987.2	1476.7	2031.7	8112.1	3082.6	2756.6	1859.1	2395.6
Sellable electricity (kWh)	4723.3	5916.1	6814.0	5875.9	1097.4	5797.9	5798.3	6349.7	8584.0
COD (mg/L) of waste water stream	37.8	51.7	42.5	37.7	56.1	43.1	48.0	28.7	45.3

a) The sugar is sent to fermentation in two separate streams, where the ethanol rich stream is sent directly to the triple effect evaporator to ensure no ethanol in the fermentation feed stream, which could inhibit succinic acid production.

Table 5-4: Reported and simulated combined sugar yields with fractional conversions for the pretreatment, enzymatic hydrolysis and lignin precipitation as simulated for the different scenarios

Pretreatment	DAT	DAT (no EH)	NaOH	Organosolv	AFEX™	STEX	STEX with SO_2	STEX with NaOH	WO
Monomeric sugar conversions									
Glucan (s)[a] → Glucose	0.043	0.053	-	0.0062	-	-	0.050	-	-
Xylan (s)[b] → Xylose	0.731	0.769	-	0.343	-	-	0.688	-	-
Arabinan (s)[b] → Arabinose	0.812	1	-	1	-	-	1	-	-
Glucan (s) → Cellobiose[c]	0.003	0.003	-	-	-	-	-	-	-
By-products									
Xylan (s) → Acetic acid	*Foot note h*	0.088	-	-	-	-	0.107	-	-
Xylan (s) → Furfural	0.08	0.118	-	-	-	-	0.023	-	-
Glucan (s) → HMF	0.003	0.001	-	-	-	-	-	-	-
Solubilisation conversions									
Glucan (s) → Glucan-L	-	-	0.021[e]	0.006218	-	0.08[e]	0.0023	0.098[f] (0.153[e])[i]	0.503
Xylan (s) → Xylan-L	-	-	0.127[e]	-	-	0.89[e]	0.202[e]	0.826[f] (0.725[e])	0.868[e]
Arabinan (s) → Arabinan-L	-	-	-	1	-	1	-	1	0.83
Lignin (s) → Lignin (acid soluble)	-	-	0.9	0.092	-	-	-	0.13 (0.92)	0.661
Enzymatic Hydrolysis									
Glucan (s) → Glucose	0.684	0.684	0.7541	0.428438	0.705	1	1	1	1
Xylan (s) → Xylose	-	-	0.3875	-	0.765	0.35	0.35	0.350	0.353
Glucan (s) → Cellobiose	$5.68*10^{-3}$	$5.68*10^{-3}$	-	-	-	-	-	-	-
Arabinan (s) → Arabinose	-	-	-	-	0.97	-	-	-	-
Lignin precipitation									
Lignin (acid soluble) → Lignin (s)	-	-	0.509	0.60844	-	-	-	0.6255	-
Reported and Simulated Sugar Yield									
Combined sugar yield in simulation (g/100g DM)	40.14	23.87	32.47	22.15	57.13	59.90	58.61	31.29	20.16
Combined sugar yield reported (g/100g DM)	40.50	26.78	31.18	20.87	53.65	60.40	60.848[j]	35.19	20.40

a) $(C_5H_8O_4)_n$ with Mw = 162.14 g/mole b) $(C_5H_8O_4)_n$ with Mw = 166.13 g/mole c) $(C_{12}H_{22}O_{11})_n$ with Mw = 342.3 g/mole d) assumed same as xylan e) The solubilised polysaccharide is converted to the associated monomeric sugar during enzymatic hydrolysis with a fractional conversion of 1 f) The solubilised polysaccharide is converted to the associated monomeric sugar during post hydrolysis with a fractional conversion of 1 h) simulated from glucan(Ali Mandegari *et al*., 2017) with a fractional conversion of 0.219 i) The values provided in parenthesis are used during alkaline hydrolysis, the values without parenthesis is used to simulate steam explosion j) The simulated sugar yield for the STEX with SO_2 method was compared to the calculated yield of 60.85 g/100g DM, based on the stated 87% total yield for the total polysaccharides (glucan, xylan and arabinan) of 69.7 g/100 g DM sugarcane bagasse (Rocha *et al*., 2012).

5.3.2 Economic evaluation

5.3.2.1 Capital and operational cost estimate

On average, the pretreatment plant area A contributed 38% of the ISBL installed equipment cost and 12.1% of the FCI. The installed equipment cost per plant area, together with the bypass ratio, are shown in Figure 5. Due to no enzymatic hydrolysis, the least expensive pretreatment method was DAT without EH at an installed equipment cost of 14.3 million US$ for plant area A. For DAT without EH, the separation equipment (centrifuges and filters) contributed most to the total plant area A cost (52.3%), followed by the cost of the GAC columns for detoxification (16.3%).

The most expensive pretreatment method was AFEX™ with an installed equipment cost of 54.0 million US$ for plant area A, due to the high cost of enzymatic hydrolysis (46.23 million US$). The enzymatic hydrolysis feed stream is large at 197 m^3/h compared to the other methods such as NaOH with a feed stream of 66.3 m^3/h, since no solubilisation of lignin or hemicellulose occur during AFEX™ pretreatment. Low solubilisation of the biomass during pretreatment results in a larger solid content (cellulignin stream) after pretreatment than when solubilisation of the biomass does occur. In turn, more process water must be added to dilute the stream prior to enzymatic hydrolysis. The large feed stream increases the required capacity and thus the number of enzymatic hydrolysis reactors needed, which then increases the capital cost. Consequently, the capital cost of enzymatic hydrolysis contributed the most at 37.9 – 85.7% of the total capital cost for PA-100 (Table 5) for all the pretreatment methods except DAT (no EH) and Organosolv. The installed equipment cost break down for plant area A (Figure 1) is provided in Table 5.

The enzymatic hydrolysis residence time also has an impact on the capital costs. The capital cost of enzymatic hydrolysis was high for STEX (35.1 million US$) and WO (16.8 million US$) due to the long residence time of 96 h, compared to 72 h, 30 h and 24 h for the other methods (section 5.3.3). Reducing the residence time to below 72 h (Alvira *et al*., 2010; Dias *et al*., 2011) is advisable for the development of commercially viable processes, since it has been shown that the small increase in sugar yield does not justify the high capital cost of enzymatic hydrolysis tanks required (Dias *et al*., 2011).

The cost of milling and feed handling was excluded from the capital cost estimate since it was assumed that milling was only done on an experimental level to overcome potential feed size

restrictions of the bench scale equipment. This means that the industrial size equipment would be able to handle the bagasse and trash particle size distribution (PSD) as received by the biorefinery from the sugar mill. It should be noted that the feedstock PSD, and the variability thereof, will have an impact on the mixing and mass transfer effects which may impact the pretreatment and EH performance, but the significance or extent thereof is unknown. Therefore, the impact on process performance caused by the different PSD used was not taken into consideration. To this end, the impact on process performance due to no milling was not taken into consideration for the pretreatment methods (NaOH, AFEX™, STEX with NaOH and WO) that specified milling in the experimental setup.

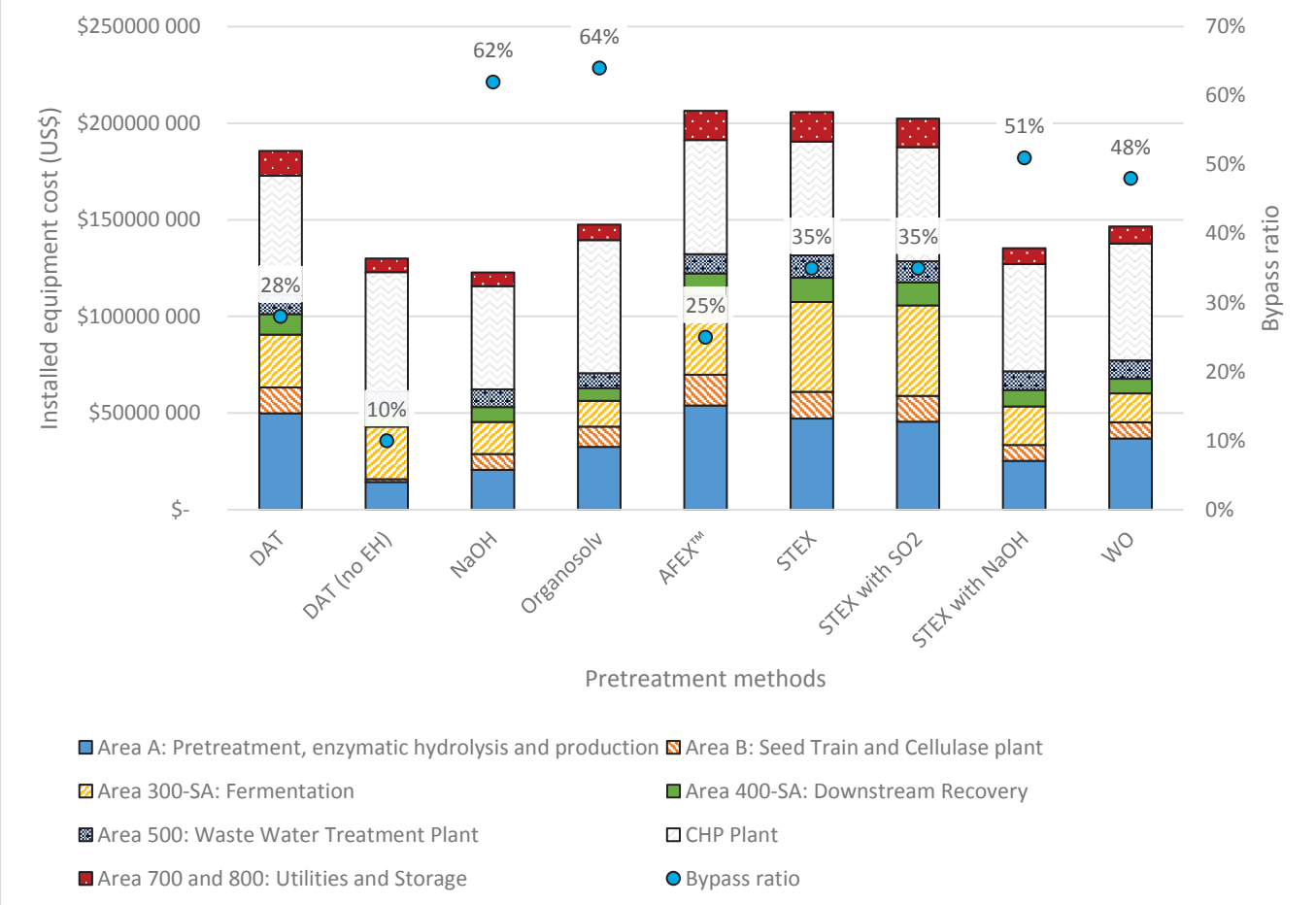

Figure 5-5: Bypass ratio and installed equipment cost per plant area for each pretreatment method

The Organosolv pretreatment method resulted in the largest total operating cost due to the high cost of ethanol required for the solvent make-up stream (95% ethanol recovery), at 46.53 million US$ per annum and 1988 US$/t DM fed to the biorefinery, contributing 64% of the total variable operating costs (37.41 million US$). The total operating costs are shown in Figure 6 for each pretreatment method. In comparison, the WO method has the lowest total operating

cost at 24.35 million US$ per annum. Although this method requires oxygen as a consumable raw material, an on-site cryogenic oxygen production plant was included in the capital cost, and therefore oxygen was not accounted for as a variable operating cost. The CHP plant provides the oxygen plant with electricity (200 kWh).

Table 5-5: Major pretreatment equipment costs per pretreatment method for plant area A (results are provided as million US$ with the cost contribution (%) of the total plant area A capital cost given in parenthesis)

Major Pretreatment equipment costs	DAT	DAT (no EH)	NaOH	Organosolv	AFEX™	STEX	STEX with SO_2	STEX with NaOH	WO
Tanks and Hoppers	7.41 (14.9%)	2.09 (14.6%)	2.17 (10.5%)	2.72 (8.3%)	2.22 (4.1%)	2.45 (5.2%)	3.30 (7.3%)	2.69 (10.7%)	3.00 (8.1%)
GAC Detoxification	2.01 (4.0%)	2.33 (16.3%)	-	-	-	-	2.44 (5.4%)	-	-
Solvent Recovery	-	-	-	4.31 (13.2%)	-	-	-	-	-
Oxygen Plant	-	-	-	-	-	-	-	-	2.18 (5.9%)
Separation equipment (Centrifuges, screens, filters)	6.81 (13.7%)	7.45 (52.3%)	7.29 (35.5%)	12.73 (39.1%)	0.45 (0.8%)	8.13 (17.2%)	8.31 (18.3%)	6.90 (27.3%)	12.97 (35.1%)
Heat exchangers	0.61 (1.2%)	0.13 (0.9%)	0.35 (1.7%)	0.51 (1.6%)	0.25 (0.5%)	0.09 (0.2%)	0.53 (1.2%)	0.38 (1.5%)	0.16 (0.4%)
Pumps and Conveyors	0.81 (1.6%)	0.59 (4.1%)	0.57 (2.8%)	0.64 (2.0%)	0.80 (1.5%)	0.59 (1.3%)	0.70 (1.5%)	0.58 (2.3%)	0.58 (1.6%)
Pretreatment reactor	0.36 (0.7%)	0.39 (2.8%)	1.39 (6.8%)	1.86 (5.7%)	2.34 (4.3%)	0.28 (0.6%)	0.44 (1.0%)	0.93 (3.7%)	0.45 (1.2%)
Enzymatic Hydrolysis reactors	30.74 (61.6%)	-	7.79 (37.9%)	8.63 (26.5%)	46.23 (85.7%)	35.09 (74.3%)	29.06 (64.1%)	12.39 (49.1%)	16.75 (45.4%)
Dust suppression	0.54 (1.1%)	0.54 (3.8%)	0.54 (2.6%)	0.54 (1.6%)	0.54 (1.0%)	0.54 (1.1%)	0.54 (1.2%)	0.54 (2.1%)	0.54 (1.4%)
Other	0.61 (1.2%)	0.75 (5.2%)	0.45 (2.2%)	0.64 (2.0%)	1.13 (2.1%)	0.03 (0.1%)	0.03 (0.1%)	0.85 (3.4%)	0.29 (0.8%)
Total	49.88 (100%)	14.27 (100%)	20.55 (100%)	32.56 (100%)	53.95 (100%)	47.20 (100%)	45.34 (100%)	25.26 (100%)	36.91 (100%)

Table 5-6: Profitability indicators for each pretreatment method

Profitability indicators	DAT	DAT (no EH)	NaOH	Organosolv	AFEX™	STEX	STEX with SO_2	STEX with NaOH	WO
TCI (million US$)	344.5	236.3	224.6	268.7	385.7	384.2	377.8	248.4	268.9
TOC (million US$)	32.7	32.4	26.0	46.5	39.4	37.6	38.2	32.6	24.4
NPV (million US$)[a]	352.03	150.47	62.81	(314.96)	440.95	644.97	590.41	116.79	1.88
IRR[a]	21.57%	17.43%	13.24%	n/a	22.81%	28.04%	26.94%	15.51%	9.79%
MRSP[b] (US$/t)	870	1 084	1 265	3 116	844	685	717	1 162	1 492

a) Net present value (NPV) and Internal rate of return (IRR) for a succinic acid selling price of 1500 US$/t and electricity selling price of 0.08 US$/kWh. b) Minimum required selling price of succinic acid for a NPV of 0 US$ and electricity selling price of 0.08 US$/kWh.

5.3.2.2 *Profitability evaluation*

The STEX pretreatment method resulted in the most profitable succinic acid biorefinery configuration, with a MRSP of 685 US$/t succinic acid or an IRR of 28.04% for a selling price of 1500 US$/t succinic acid and 0.08 US$/kWh electricity (Table 6), mainly due to a high fermentable sugar yield and low residence time. The STEX method had the highest SA yield per tonne of biomass fed to the biorefinery (45.7 kg SA/t DM) due to sugar preservation (low inhibitor production) and digestibility of cellulignin (Table 4). The low residence time of 7.5 minutes contributes to lower energy use per unit feedstock treated and reduced capital costs due to the continuous nature of the processing step. In batch configurations energy and equipment capacity is required to 'hold' the material for the required residence time which increases the bypass ratio and capital costs, respectively.

Furthermore, no costs were required for detoxification and the capital cost of separation equipment (i.e. centrifuges) was low due to ease of separation caused by the evaporation of 26.2 wt% of the material fed to the pressure vessel after pressure release (Rocha *et al*., 2012). Moreover, a high fermentable sugar stream concentration of 204.7 g/L resulted in less HPU steam required for the triple effect evaporator in the fermentation plant area (B), which had a positive impact on the bypass ratio and thus plant capacity for economies of scale. Therefore, any pretreatment method combined with EH that results in a high overall fermentable sugar yield, while avoiding large residence times, detoxification and extensive liquid-solid separation, is suitable for succinic acid production.

The profitability results of this study are similar to a previous study on corn stover, where the STEX, AFEX™ and DAT methods were identified as cost competitive pretreatment methods (Baral and Shah, 2017; Hendriks and Zeeman, 2009). However, it is contradictory to the results found for the comparison of acid-alkaline-, STEX and STEX with NaOH pretreatment methods for oil palm fruit bunches (Medina *et al*., 2018), where the STEX pretreatment method resulted in the least profitable scenario. This is due to the product profile of the oil palm fruit branches biorefinery, where only ethanol was produced from the STEX pretreated biomass, and xylitol and lignin was co-produced as high-value bioproducts with ethanol from the acid-alkaline and STEX with NaOH pretreated biomass (Medina *et al*., 2018). This confirms the notion that the pretreatment method must be selected with both the type of biomass and bioproduct(s) in mind for a favourable economic outcome (Alvira et al., 2010; Menon and Rao, 2012).

The most profitable pretreatment methods, DAT (IRR of 21.6%), AFEX™ (21.3%), STEX (28.0%) and STEX with SO_2 (26.9%), are also those that adhere best to the key factors for effective pretreatment of lignocellulose (Alvira *et al*., 2010), which include items such as:

- no or low sugar degradation,
- minimum fermentation inhibitor production,
- no size reduction required (i.e. milling),
- effectiveness at low moisture content,
- obtaining high sugar concentrations,
- lignin recovery and
- minimum heat and power requirements (Alvira *et al*., 2010; Bensah and Mensah, 2013).

Consequently, the results from this study confirm these factors as key selection criteria for effective pretreatment methods.

The Organosolv and WO method resulted in unprofitable biorefinery configurations with MRSPs of 3116 US$/t and 1585 US$/t, and negative NPVs of 315.0 million US$ and 20.7 million US$, respectively. WO and solvent based pretreatment methods (i.e. Organosolv) were also previously found to be unprofitable since the solvent is too expensive when compared to the value of glucose (Hendriks and Zeeman, 2009). The Organosolv method also resulted in high steam and electricity requirements (47.1 t steam/t DM and 0.52 kWh/t DM), high capital cost of the distillation column required for ethanol solvent recovery (4.3 million US$) and cost of ethanol (23.83 million US$ per annum).

Although this pretreatment method is not suitable for succinic acid production, it may be more suitable for a cellulosic ethanol biorefinery where the bioethanol produced can be used as the solvent, and the energy requirement can be integrated with ethanol recovery in the downstream process (Archambault-Leger *et al*., 2014). Organosolv may also be more suitable for biorefineries where lignin is also valorized as valuable bioproduct, and not only burned for energy production, since the added revenue may justify the high energy, operating and capital costs of this pretreatment method.

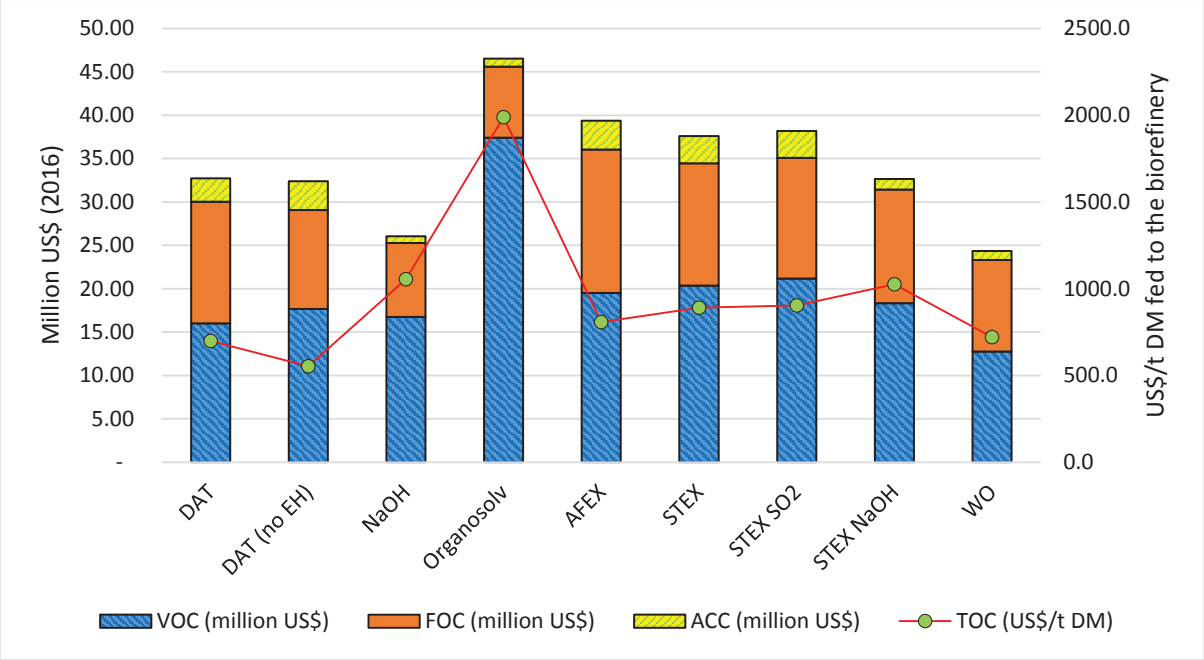

Figure 5-6: Operating cost summary for each pretreatment method (VOC: variable operating cost; FOC: fixed operating cost; ACC: Annual capital charge)

5.3.3 Case studies

The simulations have been developed based on the experimental operating conditions of the respective pretreatment methods reported in literature. However, two major process design changes were made for the industrial processes based on the NREL report on DAT and enzymatic hydrolysis of corn stover (Humbird, 2011). These two design changes are addressed in Case study 1 and 2 respectively.

Firstly, the solids concentration of the enzymatic hydrolysis feed was simulated as 20 %wt (Humbird, 2011), since it is well known that a biomass-water mixture of 15 - 25 %wt is required for profitable bioethanol production (Villadsen *et al*., 2011). Whether the same solids loading (15 – 25 %wt) is required for profitable succinic acid production, is yet to be determined. Therefore, the first case study was included to investigate the impact of the EH solids loading on the process economics by taking only the effect of dilution into consideration. The major assumption is that the process performance, such as sugar yield and residence time, remains constant. It should be noted that this is not a realistic assumption, since the solids loading will most certainly have an impact on the process performance.

A solids loading of 15 – 25 wt% results in a dry viscous mixture which makes effective stirring difficult. Poor stirring or mixing causes low mass and heat transfer, which in turn will result in low monomeric sugar release (Benjamin, 2014). This is because the enzymes cannot hydrolyse

the polysaccharides if they are not close enough to the polysaccharide fibers (Villadsen *et al*., 2011). Moreover, conventional mixing techniques may prove to be unfeasible due to high mechanical strength or power required (Villadsen *et al*., 2011). However, the assumption is necessitated by a lack of data to simulate the change in process performance caused by a change in EH solids loading.

The challenge of proper mixing in industrial pretreatment and EH applications leads to the need for new process technologies and equipment to be developed (Villadsen *et al*., 2011). These can include "kneading-tearing-and-hydrolysis" aggregates which are seen in the baking industry. Tearing of the biomass fibers is combined with folding or 'kneading' of the dry mixture for improved mixing (Villadsen *et al*., 2011). Another potential pretreatment technology where shearing and mixing are combined is the twin screw reactor.

Duque *et al.*, (2014) reported combined alkali and enzymatic extrusion to pretreat barley straw in a twin screw reactor. Subsequent enzymatic hydrolysis in a batch reactor could then be carried out at a high solids loading of 30% (w/v), producing 32 g glucose (96 g/L) and 18 g xylose (52 g/L) per 100 g extruded barley straw lignocelluloses, resulting in 50 g combined sugar yield per 100 g DM. This is comparable to AFEX™ and enzymatic hydrolysis (SHF) pretreatment of sugarcane lignocelluloses with 53.7 g combined sugars per 100 g DM. The extruded material has been subjected to physical disruption due to the shearing forces as well as mixing of the enzymes with the biomass during the extrusion and prior to subsequent incubation for enzymatic hydrolysis (Duque *et al*., 2014).

Similar to the first case study, the impact of dilution, caused by the solid to liquid ratio of the dilute sulfuric acid and sodium hydroxide alkaline streams, on the process economics was investigated in the second case study. The solids concentration and solid to liquid concentration of the experimental and simulated values are provided in Table 7 for the pretreatment methods included in the case studies (DAT, NaOH, STEX, STEX with SO_2, STEX with NaOH, and AFEX™). A sensitivity analysis was done in the third case study to determine the impact of the different economic parameters used in the discounted cash flow analysis on the economic outcome and profitability indicators.

Table 5-7: Test conditions summary for Case study 1 and 2

Pretreatment methods	DAT	AFEX™	STEX	STEX with SO_2	STEX with NaOH	NaOH
Case study 1: Enzymatic hydrolysis loading						
Simulated value	20%	20%	20%	20%	20%	20%
Experimental value	2%	1%	12%	2%	n/a	n/a
Test conditions	5%, 12.5%, 20%					
Case study 2: Solid to liquid ratio						
Simulated value	1: 2	n/a	n/a	n/a	1: 2	1: 2
Experimental value	1:20	n/a	n/a	n/a	1:10	1:15
Test conditions:						
Assumed 1:2	1:2				1:2	1:2
50% of Actual	1:11	n/a	n/a	n/a	1:6	1:8.5
Actual S:L ratio	1:20				1:10	1:15

5.3.3.1 Case study 1: Impact of enzymatic hydrolysis solids loading on profitability

A high hydrolysis solids loading has been identified as a vital parameter for cost-effective pretreatment and hydrolysis processes (Baral and Shah, 2017; Dias *et al*., 2011; Jorgensen *et al*., 2007). Therefore the dilution impact, caused by a variation in enzymatic hydrolysis (EH) feed stream solids loading of 5%, 12.5% and 20% by mass, on profitability was investigated. The four most profitable scenarios (section 5.4.2.3) were assessed, namely DAT, AFEX™, STEX and STEX with SO_2.

The STEX and STEX with SO_2 pretreatment methods remained profitable with IRR values above 9.7% at 11.6% and 10.2%, respectively, at low enzymatic hydrolysis solids loadings of 12.5% and 5%, as shown in Figure 7. There was a high fermentable sugar concentration in the fermentation feed stream of 121.7 g/L and 47.9 g/L for STEX and STEX with SO_2, respectively, at 12.5% enzymatic hydrolysis solids loading. The fermentable sugar concentrations were 37.8 and 68.8 g/L for 5% enzymatic hydrolysis solids loadings for STEX and STEX with SO_2, respectively. No additional evaporation was required to obtain a minimum sugar concentration of 55 g/L.

However, an additional triple effect evaporator was required to concentrate the fermentation feed stream for the 12.5% solids loading AFEX™ pretreatment scenario, and for all the 5% solids loading pretreatment method scenarios included in this case study. The triple effect

evaporator required HPU steam, which in turn caused the bypass ratio to increase and the plant capacity to decrease, resulting in a negative economic impact.

The profitability of the biorefinery scenarios with AFEX™ pretreatment were impacted most by a change in solid loading, as seen in Figure 7. Although it is one of the most profitable methods for a 20% solids loading (section 5.3.2.3), it became unprofitable with IRR values of 2.6% and 8.2% for solids loadings of 5% and 12.5%, respectively. This is due to the low solubilisation of the lignocellulose during pretreatment, causing a larger solids cellulignin feed stream to EH. Therefore, more process water was required to dilute the AFEX™ product stream to the required solids loading for EH, compared to the DAT or STEX pretreatment methods. In turn, the EH product stream was also more diluted with a combined sugar concentration of 37.2 g/L for 12.5% loading, and 23.8 g/L for 5% from 113.7 g/L for 20% solids loading.

To this end, the STEX or STEX with SO_2 pretreatment methods may be preferred over DAT and AFEX™ if there are low levels of confidence that attainable enzymatic hydrolysis solids loading can be achieved. Potential solutions include new mixing techniques, such as the twin screw reactor, or simultaneous saccharification and co-fermentation (SSCF) coupled with a fed-batch strategy. In that case, the starting conditions can be at a low solids loading (5 %wt) to promote efficient mixing for proper heat and mass transfer, followed by feeding intervals with a high solids loading stream. Co-fermentation may then also reduce any potential sugar inhibition on the enzymes or microorganism used. Although this case study does not provide a clear solution, it highlights the challenge, sensitivity and resulting economic impact of the EH solids loading design parameter for succinic acid production from sugarcane lignocelluloses.

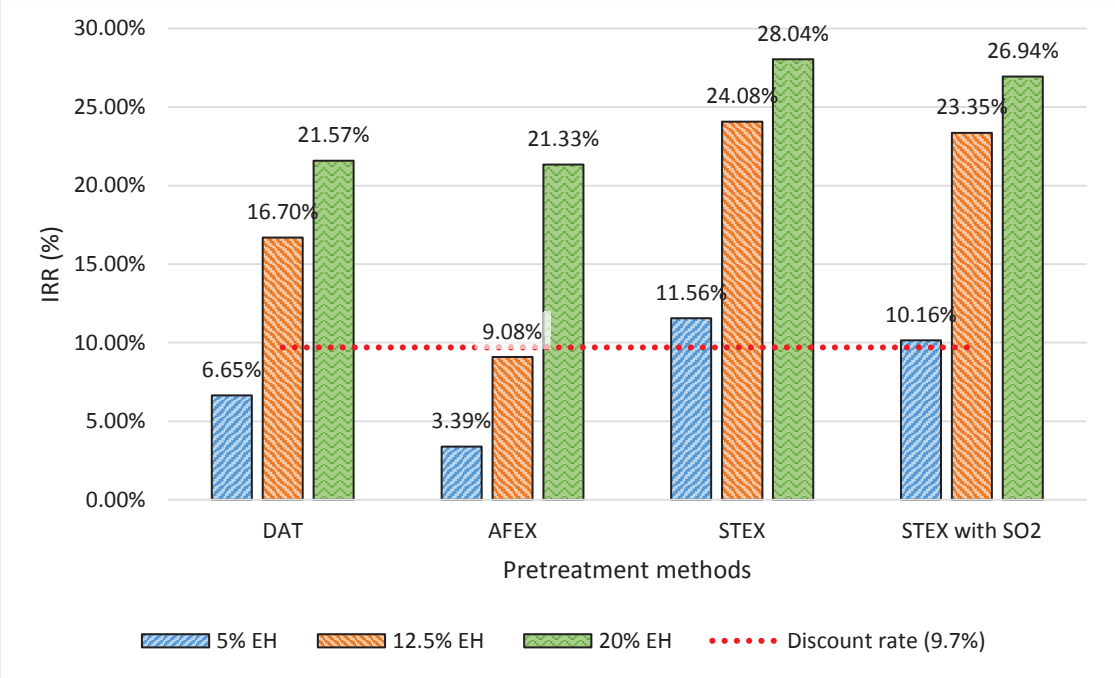

Figure 5-7: Change in profitability (IRR) for different EH solid loadings

5.3.3.2 Case study 2: Impact of the pretreatment solids to liquid ratio on profitability

The process assumption of a 1:2 solids to liquid ratio was applied to the DAT, NaOH and STEX with NaOH pretreatment methods and therefore they are included in this case study. A smaller solid to liquid ratio leads to a more diluted process stream, which in turn requires increased equipment capacity and more energy for heating and pumping. Since these pretreatment processes have high operating temperatures (100 – 180°C), the impact on process economics are mainly due to the steam energy required to heat the feed stream and maintain the pretreatment reactor temperature. For a higher steam demand more feedstock has to be bypassed from the biorefinery to the CHP plant to meet the new energy requirements, which will impact the economic outcome and profitability.

It was assumed that the fermentable sugar yields and residence times remained the same at different solid to liquid ratios and therefore the impact of feed dilution on pretreatment and the associated downstream processes was investigated. As discussed in section 5.3.3, the impact of the variation in solids to liquid ratio on the process performance, due to the mass and heat transfer limitations caused by inefficient mixing, is not available. Therefore, the change in process performance could not be simulated without additional process assumptions, which may further decrease the accuracy of the economic outcome.

For the experimental setup, a solids to liquid ratio of 1:20 was reported for the DAT pretreatment (Benjamin, 2014), a 1:15 ratio was reported for the NaOH pretreatment (Chen *et al*., 2016) and a 1:10 ratio was reported for the subsequent delignification step in the STEX and NaOH pretreatment methods (Rocha *et al*., 2012), as shown in Table 7 (section 5.3.3). The economic outcome was determined for each pretreatment method at three different intervals, namely the 1:2 assumed ratio, a midway ratio and the experimental value used. The midway solid to liquid ratios were 1:11, 1:8.5 and 1:6 for DAT, NaOH and STEX with NaOH pretreatment methods, respectively. The results are shown in Figure 8.

A changing solids to liquid ratio had the lowest impact on the STEX with NaOH pretreatment method scenario's profitability, compared to the change in profitability for the DAT and NaOH pretreatment methods (Figure 8). This is due to the small stream size of the delignification reactor feed stream (per 100 kg DM feedstock), compared to that of the other scenarios, since some lignin and hemicellulose solubilisation took place during the preceding STEX pretreatment step of the STEX with NaOH pretreatment method (Table 5).

Moreover, the step change was smaller for the STEX with NaOH method, since the experimental solids to liquid ratio of 1:10 was lower than those used for the DAT (1:20) and NaOH (1:15) pretreatment methods. As a result, the STEX with NaOH pretreatment was the only method that remained profitable at the midway ratio of 1:6 solids to liquid with an IRR of 12.74%. The use of the actual ratio (1:10) resulted in an IRR of 9.59%, which is close to the minimum of 9.7%, but still unprofitable with a negative NPV of 2 million US$.

In comparison, the DAT pretreatment method profitability was severely impacted by a decreasing solids to liquid ratio. The bypass ratio increased from 28% to 78% and the IRR decreased from 21.57% to 0.31% when the experimental solid to liquid ratio of 1:20 was used, in comparison to the assumed 1:2 ratio. The DAT and NaOH methods were unprofitable with IRR values of 7.90% and 6.46% for the midway ratio and 0.31% and 2.85% for the actual ratios, respectively, as shown in Figure 6.

The solids to liquid ratios for the dilute acid (DAT) and delignification (NaOH and STEX with NaOH) processing steps have a major impact on the biorefinery's energy requirement and thus profitability. A diluted stream results in a large energy requirement and change in the overall bypass ratio of feedstock from the biorefinery to the CHP plant. Therefore, optimised heat integration is recommended for detailed design phase (which includes plant and mechanical

layouts) to decrease the energy requirement of the dilute acid or delignification pretreatment steps. Until these high energy requirements are addressed to ensure profitable biorefinery scenarios, the DAT, NaOH and STEX with NaOH methods are unfavourable for commercial application compared to the AFEX™, STEX, or STEX with SO_2 pretreatment methods discussed in section 5.3.3.1.

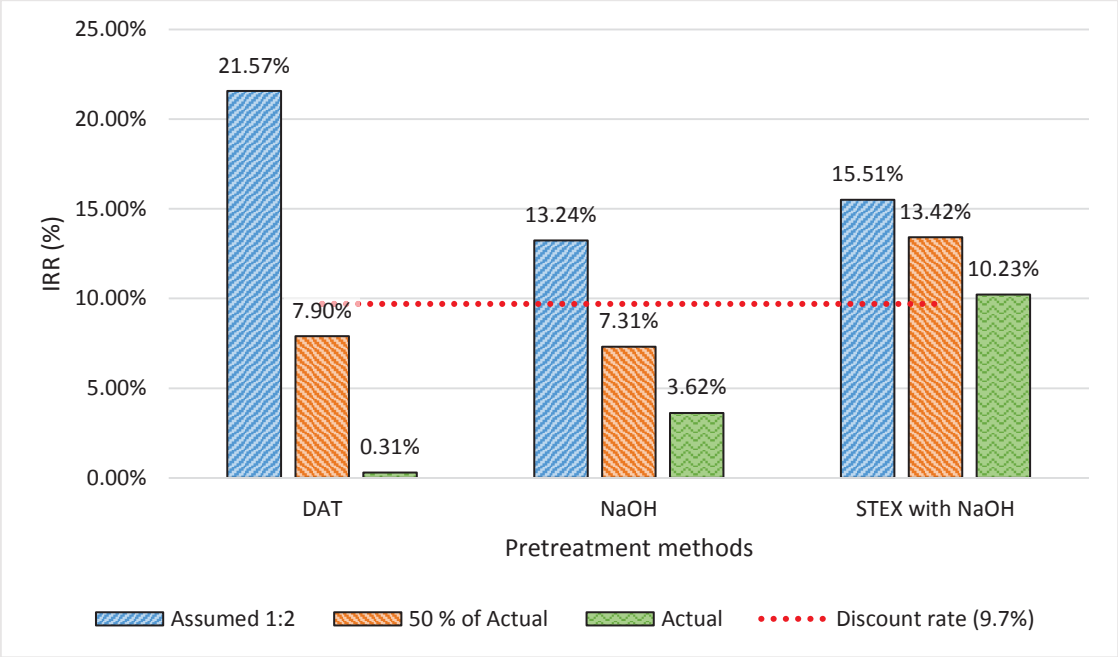

Figure 5-8: Case study for the change in solids to liquid ratio from the actual (chevron hatch), midway ratio (hatched right) and assumed ratios (hatched left) for DAT (1:20, 1:11, 1:2), NaOH (1:15, 1:8.5, 1:2) and STEX with NaOH (1:10, 1:6, 1:2)

5.3.3.3 Case study 3: Economic Sensitivity analysis

When the economic outcome is considered (section 5.3.2.3), together with the results from the first (section 5.3.3.1) and second case study (section 5.3.3.2), it is seen that the STEX and STEX with SO_2 are the most favourable pretreatment methods for commercial application based on their profitability (IRR%). Therefore, these methods were included in the economic sensitivity analysis, where the change in profitability was measured for a 30% variation of the economic parameters from their default values shown in Figure 9.

The IRRs determined through the DCF analysis were most sensitive to a change in the succinic acid selling price, resulting in an IRR of 18.9% for STEX and 17.9% for STEX with SO_2 for a 30% decrease in the succinic acid selling price from 1500 to 1050 US$/t succinic acid. Conversely, the IRR values were not sensitive to the selling price of electricity. A 30% increase (0.104 US$/kWh) and decrease (0.056 US$/kWh) in electricity selling price (0.08 US$/kWh) only increased and decreased the IRR with 0.14% for both pretreatment methods.

Moreover, the IRR values were not sensitive to a 30% change in feedstock cost, which is contrary to findings of previous studies (Baral and Shah, 2017; Chandel *et al*., 2012; Kazi *et al*., 2010; Saini *et al*., 2015). This is because the production cost of a high value chemical such as succinic acid is much higher than for ethanol or produced sugars. Consequently, the feedstock cost is only a small fraction of the total production cost. The feedstock cost (0.0108 US$/kg) contributes 3.6% of the STEX scenario production cost (0.30 US$/kg) and 3.4% of the STEX with SO_2 scenario production cost (0.32 US$/kg). This is low compared to that of other studies such as ethanol production, where the feedstock cost contributed 40% (Saini *et al*., 2015), or sugar production where the feedstock cost contributed 25.7% (Baral and Shah, 2017).

To this end, the IRR reaches 9.7% (i.e. NPV of 0 US$) for a feedstock cost of 150.13 US$/t, which is much higher than the cost used (10.8 US$/t) or the feedstock cost of 64.8 US$/t when the proposed selling price of bagasse (90 US$/t) (Petersen *et al*., 2017) is included. For a feedstock cost of 64.8 US$/t, the STEX and STEX with SO_2 pretreatment methods remain profitable with IRR values of 21.73% and 20.40%, respectively.

The IRR is second most sensitive to the FCI. The FCI was determined from the capital cost of equipment, which was based on the mass and energy balance, thereby indicating the significance of a rigorous process design. Overall, the STEX and STEX with SO_2 pretreatment methods could be implemented with a level of confidence, since these will still result in profitable succinic acid biorefineries even with a 30% increase in cost of capital equipment, resulting in an IRR of 22.34% and 21.41% for STEX and STEX with SO_2, respectively.

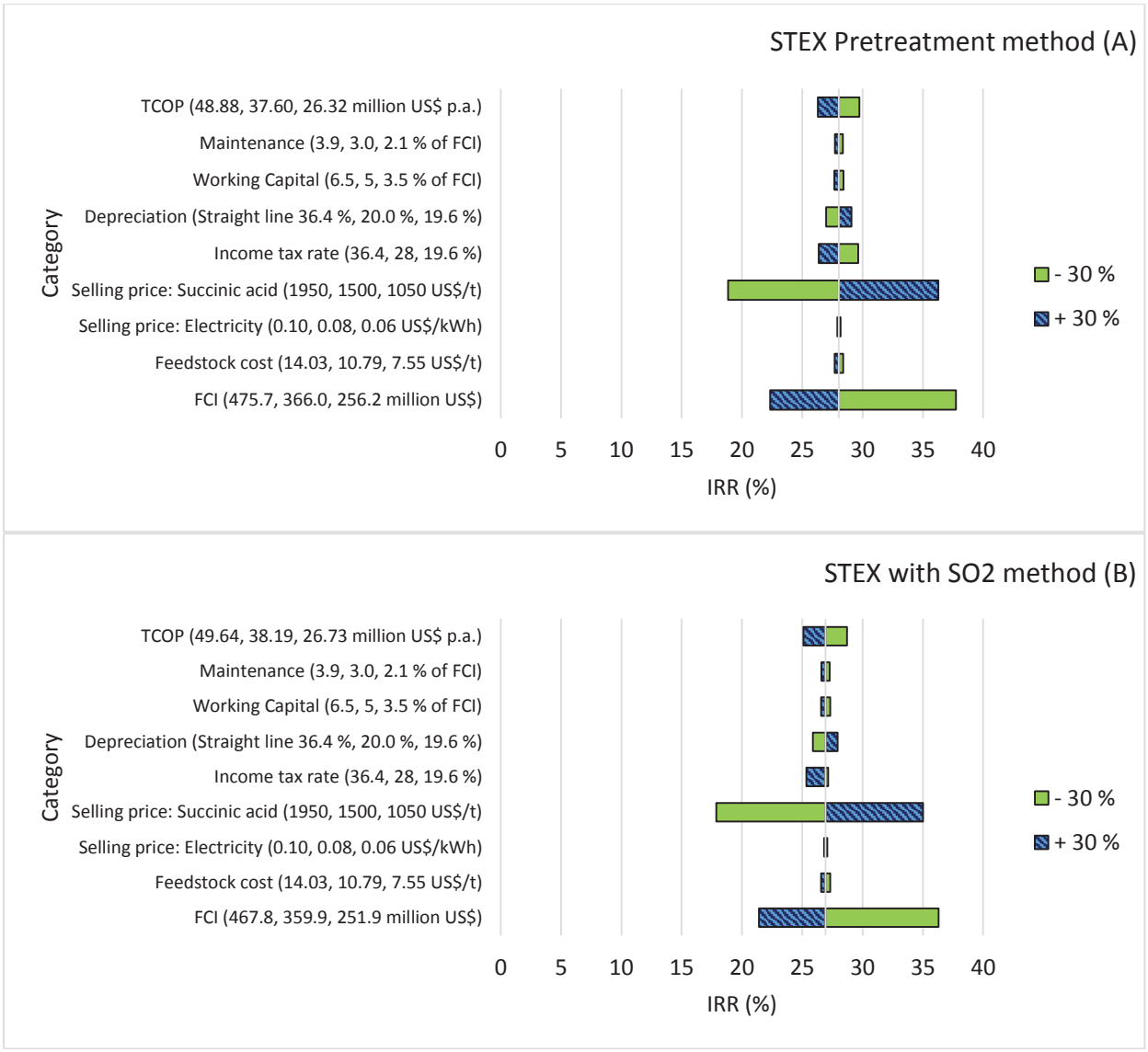

Figure 5-9: Economic sensitivity analysis for the STEX (a) and STEX with SO_2 (b) pretreatment methods

5.4 Conclusion

Of the nine pretreatment methods selected from a wide range of available chemical and physio-chemical pretreatment methods, for the co-production of succinic acid and electricity from sugarcane bagasse and trash lignocelluloses, the steam explosion (STEX) pretreatment method was the most profitable with an IRR of 28.04%, followed by STEX with SO_2 catalyst (26.94%), AFEX™ (22.81%) and DAT (21.57%), all with enzymatic hydrolysis (SHF).

Proper mixing for efficient heat and mass transfer is a design challenge in pretreatment and enzymatic hydrolysis scale-up to commercial applications. This challenge could be overcome by developing new process technologies and process design flowsheets for increased energy efficiency. Continued optimisation of pretreatment methods for commercial application will increase the profitability of biorefineries for the valorisation of lignocellulosic biomass.

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Supplementary information

The results of the economic evaluation and mass and energy results for Case study 1 and 2 are available in the supplementary information.

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Chapter 6

6. Life cycle assessment and multi-criteria analysis of sugarcane biorefinery scenarios: finding a sustainable solution for the South African sugar industry

In Chapters 3 – 5 various biorefinery scenarios were developed and profitable scenarios were identified through the respective techno-economic analyses. The economic performance is only one indicator of sustainability, together with the environmental and social indicators. By taking all three sustainability indicators into account, the biorefinery scenarios could be compared in order to achieve the overarching aim of this dissertation: to investigate whether a lignocellulose biorefinery is a sustainable and viable investment option to revive the South African sugar industry and associated farming communities.

A cradle-to-gate life cycle assessment (LCA) was conducted on six biorefinery scenarios selected from prior studies in Chapters 3 – 5. The LCA was used to determine the environmental impact of the selected biorefinery scenarios to identify the potential 'hot spots' for process improvement. The three sustainability indicators were then combined in a multi-criteria decision analysis (MCDA) tool to determine which biorefinery scenario is the most sustainable solution for the South African sugar industry. The selected biorefinery scenarios are:

- Scenario 1: Coal-supplemented itaconic acid and electricity co-production (Chapter 3)
- Scenario 2: Itaconic acid and electricity co-production (Chapter 3)
- Scenario 3: PHB and electricity co-production (Chapter 4)
- Scenario 4: Multiproduct plant (succinic acid, PHB and electricity co-production) (Chapter 4)
- Scenario 5: Succinic acid and electricity co-production (Chapter 5)
- Scenario 6: CHP plant (electricity production) (Chapter 4)

As a result, the outcome of this study contributed to Objective 4 and Objective 5 as stated in section 1.2.

The key outcomes of this chapter are the respective SimaPro® LCA simulations and comparison to fossil based systems, followed by the development of the MCDA tool. In doing so, this section introduces a method and tool to compare the different biorefinery scenarios with regards to sustainability.

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Declaration by the candidate:

With regard to Chapter 6, pg. 171 - 207, the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Project and scope definition, SimaPro® simulation work, interpretation of results and writing of manuscript.	80

The following co-authors have contributed to Chapter 6, pg. 171 - 207:

Name	e-mail address	Nature of contribution	Extent of contribution (%)
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Signature of candidate: ……

Date: 28/01/2019

Declaration by co-authors:

The undersigned hereby confirm that

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 6, pg. 171 - 207,
2. no other authors contributed to Chapter 6, pg. 171 - 207, besides those specified above, and
3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 6, pg. 171 - 207, of this dissertation.

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Life cycle assessment and multi-criteria analysis of sugarcane biorefinery scenarios: finding a sustainable solution for the South African sugar industry

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Abstract

The valorisation of sugarcane lignocelluloses is a potential solution to ensure the sustainability of the South African sugar industry. Moreover, the use of biomass for the production of biofuels, -chemicals and –products to replace fossil resources is vital in reducing our fossil resource dependency and carbon footprint. A life cycle assessment (LCA) was used to measure the environmental impacts caused by the cradle-to-gate life cycle of itaconic acid, succinic acid, PHB (polyhydroxybutyrate). The carbon footprint and water scarcity impact of a CHP coal supplemented itaconic acid biorefinery (Scenario 1), bioenergy self-sufficient (ESS) itaconic acid biorefinery (Scenario 2), ESS PHB biorefinery (Scenario 3), ESS succinic acid and PHB biorefinery (Scenario 4), ESS succinic acid biorefinery (Scenario 5) and a CHP plant (Scenario 6) were included in a multi-criteria decision analysis tool, with the techno-economic and social sustainability indicators, to determine the most sustainable solution for implementation by the South African sugar industry. A trade-off exists between the environmental and techno-economic performance. Scenario 4 is the most sustainable scenario due to the environmental advantage obtained by the high succinic acid production volume, since the environmental burden is shared across the bioproduct units, and the economic advantage of a high PHB selling price, while being bioenergy self-sufficient.

Key words: Environmental impact assessment, itaconic acid, PHB, socio-economic impact, succinic acid, sugarcane biorefinery

6.1 Introduction

Sustainable design solutions through green engineering, where the environment, economy and society are considered key design factors, are required to develop a more environmentally friendly industry (Julio *et al*., 2017). This will also contribute towards addressing the impact of increasing worldwide populations on depleting natural resources (Mihelcic and Zimmerman, 2010). A biorefinery is a good example of green engineering, since all three design factors can be addressed: sugarcane lignocelluloses can be valorised for economic benefit in a new

production facility which would create jobs for social upliftment and contribute towards the continuation of the sugar industry, while the lignocellulosic feedstock is a renewable resource (Ali Mandegari *et al*., 2017a; Görgens *et al*., 2016; Nieder-Heitmann *et al*., 2018). This green engineering solution could further decrease our fossil fuel dependency (Suriyamongkol *et al*., 2007), while the utilisation of a second generation feedstock does not endanger food security (Moncada *et al*., 2013).

Approximately 8 million tonnes of bagasse are produced annually in South Africa (Leibbrandt, 2010; Mashoko *et al*., 2013), making it an abundant biomass resource. A sugar mill treats 300 t/h sugarcane on average and produces 83.4 t/h bagasse, 12.3 t/h molasses and 20.4 t/h filter cake as by-products (Mashoko *et al*., 2013). In addition, if green harvesting practises are introduced, the environmental impact of sugarcane burning can be reduced (Ali Mandegari *et al*., 2017b), while the brown leaves can be valorised together with bagasse for the production of biofuels and –chemicals such as ethanol, lactic acid, furfural (Farzad *et al*., 2017a), itaconic acid (Nieder-Heitmann *et al*., 2018), succinic acid, and polyhydroxybutyrates (PHB) in a biorefinery system (Chapter 4.3).

However, even if a biorefinery system meets climate change mitigation objectives through reduced greenhouse gas emissions (GHG) and energy requirements, it may cause additional environmental impacts compared to fossil based systems in other impact categories (Gnansounou *et al*., 2015), which should not be ignored by policy makers (Cherubini and Jungmeier, 2010). Consequently, the environmental impact caused by the production of these biofuels and –chemicals should be measured. This can be done through a life cycle analysis (LCA).

A LCA is used to determine the environmental impact of a product for its entire life cycle: from the raw materials used, the manufacturing thereof, transportation and distribution, to eventual use and disposal or recycling (Luo *et al*., 2009). Although a cradle-to-grave LCA is preferred, it is not always possible to achieve due to broad-ranging use of final products, and therefore a cradle-to-gate life cycle analysis (LCA) is commonly preferred (Julio *et al*., 2017). The environmental impact can be determined using the problem-oriented (midpoint) approach, where parameters such as global warming, fossil fuel depletion, human toxicity, acidification, particle matter formation and eutrophication are considered (Petersen, 2012; Silalertruksa *et al*., 2017). Alternatively, the damage-orientated (endpoint) approach can be used, where

categories such as human health, ecosystem quality and availability of resources are considered (Renó *et al*., 2011).

The first attributional cradle-to-gate LCA was conducted on biorefinery scenarios for the co-production of itaconic acid, succinic acid, and PHB with electricity, to compare to the previously reported techno-economics. Once the environmental impacts are measured, the results can be used to i) identify opportunities to reduce detrimental environmental impacts, ii) marketing or iii) decision-making in industry. In the present study the LCA results were used in a multi-criteria decision analysis (MCDA) for decision-making in industry, largely based on the techno-economical and environmental indicators. The MCDA tool could be validated or further expanded by aligning the criteria with the stakeholder's targets (Julio *et al*., 2017). Stakeholders include the sugarcane farmers, biorefinery project developers, end-users, financial community, equipment suppliers, policy makers and planners, and the impacted members of the community (Elghali *et al*., 2007), or a group of local experts in economic, environmental or social sustainability (Myllyviita *et al*., 2013).

Due to the high level of variability that exists within the MCDA tool with regards to sustainability indicators and associated weighting (Myllyviita *et al*., 2013), no one solution exists (Julio *et al*., 2017). Therefore, different options were considered by changing the representative weighting of the techno-economical, environmental and social sustainability indicators to identify the most sustainable biorefinery scenario for implementation by the South African sugar industry.

6.2 Methodology

6.2.1 Biorefinery scenarios selection and description

An overview of itaconic acid, succinic acid and PHB, their uses and market values have been previously reported (Nieder-Heitmann *et al*., 2018; Chapter 4). The biorefinery for each bioproduct has four major plant areas, namely i) pretreatment, ii) bioconversion through fermentation iii) downstream processing and iv) waste water treatment. The biorefinery is annexed to a combined heat and power (CHP) plant, which provides the existing sugar mill with steam, and the new production facility with steam and electricity. Excess electricity is produced in the CHP plant which is sold back into the network as a co-product together with the biobased product produced in the biorefinery. The sugarcane lignocellulosic feedstock is split between the biorefinery and CHP plant to ensure energy self-sufficiency of both the sugar

mill and biorefinery. The general biorefinery BFD (block flow diagram) is shown in Figure 1 and the process description of the scenarios are provided in sections 6.2.2 – 6.2.4.

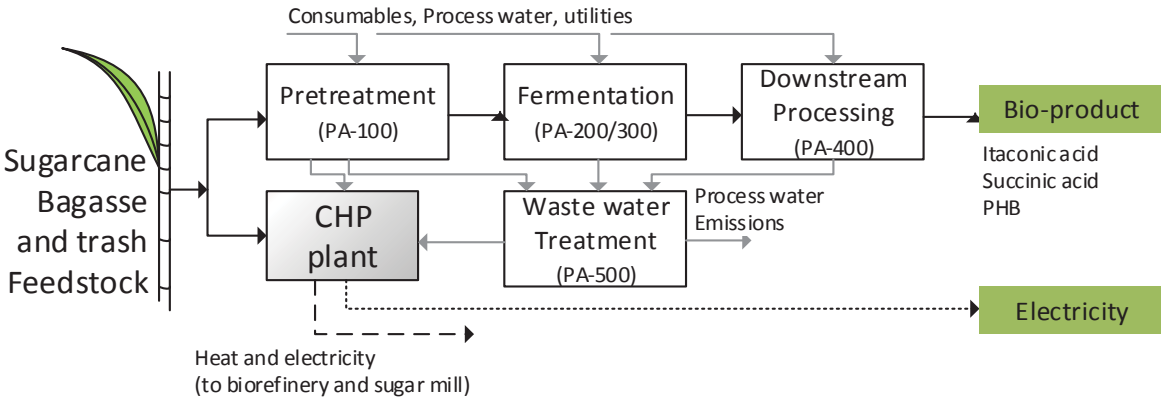

Figure 6-1: Biorefinery block flow diagram of biorefinery and CHP plant

Discussed in more detail below, the biorefinery scenarios included for comparison are:

- Scenario 1: Coal-supplemented itaconic acid and electricity co-production
- Scenario 2: Itaconic acid and electricity co-production that is bioenergy self-sufficient.
- Scenario 3: PHB and electricity co-production that is bioenergy self-sufficient.
- Scenario 4: Multiproduct plant (succinic acid, PHB and electricity co-production) that is bioenergy self-sufficient.
- Scenario 5: Succinic acid and electricity co-production that is bioenergy self-sufficient.
- Scenario 6: CHP plant (electricity production)

6.2.2 Itaconic acid production scenarios 1 and 2

Two itaconic acid biorefinery scenarios were included in the LCA and MCDA. Itaconic acid production from sugarcane lignocelluloses is only profitable when all the available bagasse and trash is utilised in the biorefinery and the energy deficit is met by supplementing the CHP plant with coal (Nieder-Heitmann *et al.*, 2018). Therefore, this configuration was included as the first scenario, though the use of coal is evidently not environmentally friendly. The unprofitable, bioenergy self-sufficient itaconic acid scenario without coal supplementation was included as the second scenario, which has the same energy configuration as the other scenarios.

For itaconic acid production the lignocellulosic feedstock is pretreated with dilute H_2SO_4 (sulphuric acid), followed by detoxification (washing of the cellulignin and granular activated

carbon absorption of the hemicellulose hydrolysate) and enzymatic hydrolysis. The fermentation sugar feed stream is then concentrated using an evaporator and pumped to the fermentation area where the microorganism *Aspergillus terreus* utilises the fermentable sugars for itaconic acid production during batch fermentation. After fermentation the itaconic acid is recovered and purified in the evaporation and crystallisation downstream process recovery area. All the waste streams are collected and sent to the biodigester in the WWT plant. The bypassed feedstock, solid cellulignin stream after enzymatic hydrolysis, biodigester sludge and evaporator dried solids are sent to the CHP plant for steam and electricity generation.

6.2.3 PHB and succinic acid production scenarios 3, 4 and 5

6.2.3.1 PHB production scenario 3

Although the PHB biorefinery scenario is not profitable, as seen from the techno-economic discussion in Chapter 4, it was also included in the LCA and MCDA to determine the environmental benefit of using a biobased and biodegradable plastic compared to conventional fossil-based plastics. Similarly to the itaconic acid pretreatment area, the bagasse and trash feedstock is pretreated with dilute H_2SO_4, followed by detoxification (washing of the cellulignin) and enzymatic hydrolysis for the co-production of PHB and electricity.

Specific to the PHB stand-alone plant, the hemicellulose hydrolysate is not detoxified, but instead sent to the WWT plant for biogas production, since the PHB producing microorganism, recombinant *Escherichia coli*, cannot utilise pentose sugars. PHB is collected intracellularly by the microorganism during PHB synthesis, whereafter the microbial cells are filtered out and sent to the downstream process recovery where PHB is recovered from the cells through sodium hydroxide alkaline digestion.

6.2.3.2 Combined PHB and succinic acid production in a multiproduct plant scenario 4

A profitable multiproduct plant configuration was included in the list of scenarios for LCAs, where PHB is produced from 25% of the fermentable glucose rich stream after H_2SO_4 dilute acid pretreatment and enzymatic hydrolysis of the sugarcane lignocelluloses. The remaining 75% of the glucose rich stream is combined with the detoxified hemicellulose hydrolysate and sent to succinic acid production (Section 4.3). The hemicellulose hydrolysate is detoxified using granular activated carbon (GAC) absorption. The techno-economics of this scenario was discussed in Chapter 4.

Similar to the PHB stand-alone plant, the PHB is collected intracellularly by recombinant *E. coli* during the PHB synthesis fermentation phase and recovered through sodium hydroxide alkaline digestion. In the succinic acid fermentation area, the microorganism *Actinobacillus succinogenes* produces succinic acid from glucose, cellobiose, xylose and arabinose sugars. A fed-batch fermentation strategy is used for both PHB and succinic acid bioconversion.

SA is recovered from the fermentation broth. The biomass cells are removed using ultrafiltration and sent to the WWT plant. There are three extraction columns in series, using an organic solvent (87 %wt 1-octanol and 13 %wt trioctylamine) counter current flow. After the three extraction columns, the SA rich solvent was pumped to the back-extraction column, where the SA migrated to an aqueous phase (25 %wt trimethylamine (TMA) and 75 %wt water). From the back-extraction column, the SA rich aqueous phase was pumped to a crystalliser. The crystals were centrifuged and separated from the liquid, washed, dried and packaged for redistribution.

All the waste streams are collected and sent to the biodigester in the WWT plant. The bypassed feedstock, solid cellulignin stream after enzymatic hydrolysis, biodigester sludge and evaporator dried solids are sent to the CHP plant for steam and electricity generation.

6.2.3.3 Succinic acid production scenario 5

In the stand-alone succinic acid biorefinery, the feedstock was pretreated using steam explosion followed by enzymatic hydrolysis. Since there is no separate hemicellulose hydrolysate stream, no GAC detoxification was required and all the available fermentable sugars are pumped to the succinic acid fermentation area. After fermentation by *A. succinogenes*, the succinic acid is recovered using ultra-filtration and reactive extraction followed by back-extraction, crystallisation, evaporation and drying.

6.2.4 CHP plant

The total available feedstock stream (65 t/h) is fed to the CHP plant where it is used as fuel in a high pressure, high efficiency boiler for steam and electricity production. Electricity is produced from the high pressure steam using condensing extraction turbines (CEST).

6.3 Life cycle assessment (LCA) methodology

There are four stages of a LCA as standardised by the international standards ISO 14040 and 14044 (Amores *et al*., 2013; Julio *et al*., 2017). First, the goal, scope and functionality are defined. In the second stage a life cycle inventory (LCI) is compiled and used to assess the potential environmental impact of each item listed in the inventory. In the final stage the environmental impacts are interpreted (Renó *et al*., 2011). The interpretation of results were done by comparing the life cycle impact assessment (LCIA) results between biorefinery scenarios, as well as to a fossil reference system (Cherubini and Jungmeier, 2010; Rahimi *et al*., 2017). PHB was also compared to starch/polyolefin blend polymer and polylactic acid (PLA) available in the SimaPro® v8.0 database.

6.3.1 Goal, boundary scope and functional unit

The goals of the LCAs are to determine the environmental impact of each biorefinery scenario in order:

i) to identify process 'hot spot' areas where the environmental impact could be reduced through process improvements for a more environmentally friendly bioproduct,

ii) to compare biorefinery scenarios to each other and to fossil based systems to better understand the relative contributions of the environmental impacts, and

iii) to include the carbon footprint (measured in CO_2 equivalents) and water scarcity impact results in a multi-criteria decision analysis (MCDA) tool for internal decision-making on selecting the most sustainable green engineering solution for the South African sugar industry.

The target audience is environmental policy makers, potential investors (Rahimi *et al*., 2017), and sugar industry stakeholders. The boundary scope includes the sugar cane plantation, sugar mill, combined heat and power (CHP) plant and biorefinery for the production of the bioproducts shown in Figure 2. While the biorefineries in the different scenarios co-produce more than one product, the total available lignocelluloses remain constant at 65 dry t/h feedstock for either bioproduct(s) or/and electricity production. Therefore the environmental indicator included for the MCDA tool in goal iii) were based on 65 t/h available lignocelluloses.

However, the comparison of all the scenarios was based on the functional unit of "1 kWh electricity produced" in the CHP plant, since all the scenarios had electricity production in common. In addition, scenarios were also compared to each other and their associated fossil-based systems based on the functional unit of "1 kg bioproduct produced". In the case of the multi-product plant scenario (Scenario 4), the succinic acid and PHB production volumes were jointly seen as 'bioproduct produced'.

6.3.2 Life cycle inventory (LCI)

The second stage is to compile a life cycle inventory (LCI) which is used to evaluate the potential environmental impact of each item listed in the inventory. The LCI inventory values used to define the harvested sugarcane and trash are provided in the Supplementary data. The inventory values for the bioproduct and electricity production in the biorefinery and CHP plant for each scenario are provided in the Supplementary information.

The LCI values were used to build the biorefinery scenarios in SimaPro® v8.5.0. The biorefinery scenarios were compared using the CML-IA baseline V3.05 methodology. The allocation of environmental burdens amongst the process products is based on mass contribution (Chrysikou *et al*., 2018; Julio *et al*., 2017) in the biorefinery, and 100% to generated electricity in the CHP plant. Allocation is widely discussed (Julio *et al*., 2017; Sandin *et al*., 2015), since no one solution exists and therefore a sensitivity analysis was performed for the comparison of biorefinery scenarios based on 1 kWh generated using the IMPACT 2002+ V2.14 methodology available in the Supplementary information. The relative environmental contributions of the scenarios were found to be similar between the two allocation methods and either could be used.

The final stage is to interpret the environmental impacts (Renó *et al*., 2011) and compare it to the other bioproduct biorefinery scenarios. The impact parameters considered were abiotic depletion, 100 year global warming potential (GWP_{100}), ozone depletion (ODP), human toxicity, fresh water-, marine aquatic- and terrestrial eco-toxicity, photochemical oxidation (POCP), acidification and eutrophication.

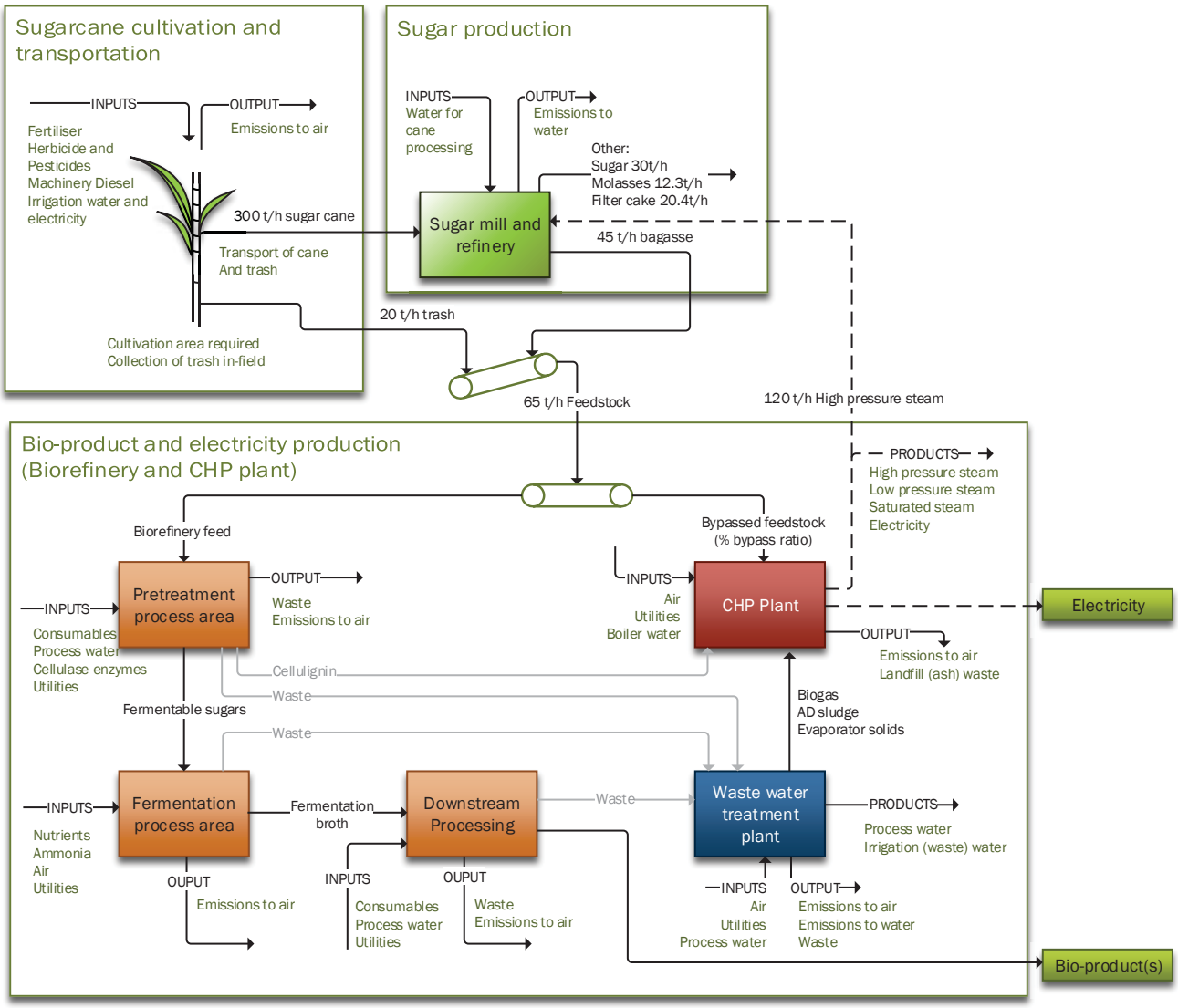

Figure 6-2: System boundary of the sugarcane cultivation, sugar production and biorefinery with CHP plant areas

6.3.3 LCA delimitations

The following assumptions and design decisions were made:

i) The impact of cane burning was omitted from sugar cultivation and transportation since the available feedstock feed rate of 65 t/h is based on green harvesting methods, where the sugarcane burning is omitted.

ii) Collection of the tops and trash (25 t/h) was included for 100% by road, 25 km in-field and 25 km average distance from the plantation to the NPP, based on the energy requirement of a truck at 1.08 MJ/tkm (Farzad *et al.*, 2017a).

iii) The steam and electricity required by the sugar mill are intermediate streams, since the high pressure steam required for steam and electricity production is supplied by the CHP plant.

iv) No coal supplementation is required for the CHP plant in Scenarios 2 – 6, since the available feedstock (65 t/h) is based on the installation of a high-efficiency boiler.

v) No data were provided for the production of capital goods. Contributions of capital goods to the environmental impact categories are based on the data inherent to the SimaPro Ecoinvent v3.0 database such as tractors, farm implements and rail tracks.

vi) The CO_2 uptake during cultivation has not been included (Renouf *et al*., 2010), and all the CO_2 emissions to air were included and indicated as biogenic, since the product use and thus lifespan is unknown.

6.4 Multi-criteria analysis

A multi-criteria decision analysis (MCDA) tool was used to determine the most sustainable investment option by taking the techno-economical, environmental and social indicators into account. Each scenario was measured according to the different criteria and normalised to a percentage, where 100% is the most desirable and zero is the least desirable. The respective categories are then added, according to the representative weighting (RW), for a total score out of 100.

The RW defines the relative importance of each factor and is assigned according to the stakeholders' interests. Therefore, the RW can vary, e.g. if more consideration should be given to environmentally friendly projects rather than economically competitive projects, the environmental indicator would receive a higher RW. However, no direct stakeholder input was sought after to date and therefore two case studies were considered to evaluate the sustainability performance of the respective biorefinery scenarios.

The RW's are adjusted for the focus on the techno-economic design factor in the first case study and then varied for the second case study. For the first case study the techno-economic indicator had a RW of 45%, with 35% for the environmental and 20% for the social indicators. For the second case study the social indicator remained constant at 20%, while the RW of the

techno-economic indicator varied from 20 to 80% and the RW of the environmental indicator varied from 80 to 20% in increments of 5%. The MCDA table is provided in Table 1.

Table 6-1: Multi-criteria decision analysis tool with RW (%) for Case study 1

Indicator	Description and score determination method	RW (%)
Techno-economic sustainability indicator		**45**
Profitability	Based on the IRR% normalised to the highest IRR. A profitable scenario will receive a high score.	20
TCI	Total capital investment – a high capital expenditure may not be favourable, as it increases financial risk. A low TCI will receive a high score.	5
TCOP	Total cost of production – a plant with high operating and maintenance cost is not favourable. A low TCOP will receive a high score.	5
Technical Maturity	The technical maturity of the scenarios is determined through the technical readiness level (TRL). A scenario with low risk will receive a high score.	10
Energy efficiency	The energy efficiency is determined by the steam and electricity required per tonne of feedstock fed to the NPP. A scenario that is energy efficient will receive a high score.	5
Environmental sustainability indicator		**35**
Carbon Footprint	The climate change impact calculated through the LCIA	20
Water Scarcity Impact	A plant with low water scarcity impact will receive a high score.	15
Social sustainability indicator		**20**
Job Creation	This criterion evaluates the number of employees required per scenario. A high score is awarded for a high number of employees.	20

6.4.1 Techno-economic design factor

The technical maturity or technology readiness of each scenario was taken into consideration with their respective economic performances, since the production of these bioproducts from sugarcane lignocelluloses has not been proven on a commercial scale. The techno-economic design factor includes the profitability (measured using the net present value), total capital investment (TCI), total cost of production (TCOP), technology readiness level (expressed as a %) and energy efficiency (%).

The Technology Readiness Level (TRL) method, first introduced by NASA, was used to indicate how successful technology can be implemented on a commercial scale (Booysen *et al*., 2016; E4tech *et al*., 2015). The levels range from a TRL 1 – 9. The first five levels (TRL 1 – 5) form part of the innovation phase and include basic research, technology application, feasibility demonstration, and pilot scale testing in TLR 5. The latter levels (TRL 6 – 9) form

part of the industry phase and include demonstration of the technology in TRL 6 – 8, with commercial implementation and application in TRL 8 – 9.

The TRLs for biochemicals produced via fermentation are shown in Figure 3. The TRL can also be applied on individual process stages, such as fermentation and separation. Therefore, it was used to evaluate each plant area, namely pretreatment, fermentation, downstream processing, the waste water plant and the CHP plant of the scenarios included (section 6.2.1).

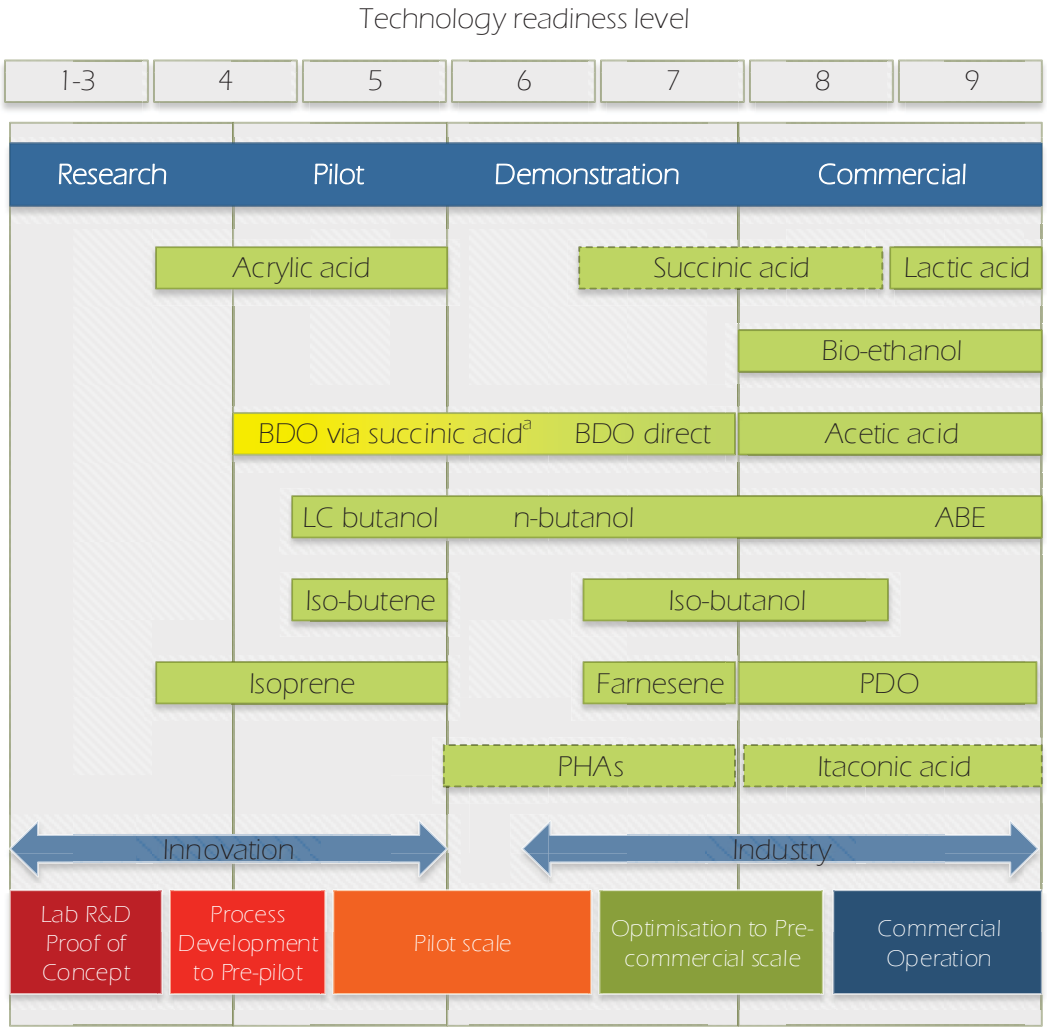

Figure 6-3: Technology readiness level for biochemicals produced through a biological conversion route, where superscript *a* denotes a chemical conversion route (redrawn (E4tech *et al.*, 2015))

6.4.2 Environmental design factor

Although a single score is desired to include in the MCDA tool, single issue methods are not aligned with ISO 14044, since the standard requires the assessment of environmental burdens across all the impact categories to ensure no impact category with a significant contribution is excluded (SimaPro Pré, 2018). However, ISO (the international organisation for

standardisation) has started to develop single issue standards such as the carbon and water footprint.

Therefore, the water and carbon footprints were used as the criteria under the environmental indicator in the MCDA tool. The carbon footprint (CO_2-eq) was calculated by using the IPCC GWP_{100} method and the water footprint was measured as the water scarcity impact (WSI) using the ISO 14046 compliant Hoekstra *et al.* (2012) method.

6.4.3 Social design factor

Employment or job creation was the only criterion considered under the social indicator, while health and safety, quality of working conditions, education and training, knowledge management, innovation potential, product acceptance, social benefit and dialogue as well as social sustainability criteria can also be included (Heinzle *et al*., 2006). However, these criteria cannot be quantified without communication between all relevant stakeholders, such as potential employees, consumers and local communities.

The representative weighting of the social factor remains low, since the job creation associated with collection and transport of the harvest residues (trash, tops and leaves) with green harvesting practises will be constant across the biorefinery scenarios at 89000 man-days per year for a biorefinery and CHP plant combined feed of 65 DM t/h bagasse and trash lignocelluloses (Farzad *et al*., 2017a).

Moreover, the number of jobs created within the biorefinery and CHP plant for each biorefinery scenario is dependent on the plant capacity and is scaled according to the bypass ratio of feedstock from the biorefinery to the CHP, based on the ethanol biorefinery scenario with a 35% bypass ratio (Ali Mandegari *et al*., 2017a). Although the number of jobs created within the biorefinery and CHP plant varies slightly for each scenario, the impact is limited in comparison with the jobs created through green cane harvesting, due to the high level of skill required (e.g. for plant managers, engineers and supervisors) as well as the high level of automation used (Farzad *et al*., 2017a). Therefore, there is no significant difference in the social score obtained between scenarios.

6.4.4 Normalisation of results for sustainability indicators

The results for each category, shown in Table 1 under the respective sustainability indicators, were normalised to a 100% in order to be included in the MCDA tool. For the categories within each design factor where a high value is favourable, namely the profitability, energy efficiency, TRL and job creation, the results were normalised against the highest value. For scenarios where a low value is desired, such as TCI, TCOP, LCIA and water demand, the results were normalised against the lowest value. It is worth mentioning that the scenarios included for comparison will ultimately impact the assigned scores due to normalisation. It should be noted that due to the normalisation of the sustainability indicator scores of each scenario, the MCDA tool can only be used to compare studies against each other if the information required for these indicators are available. Therefore, if other scenarios from literature or additional studies are added afterwards, the normalisation of indicator categories should be redone to ensure the values used are relative to all the scenarios included for comparison in the MCDA tool.

6.5 Life cycle assessment results and discussion

6.5.1 Sugar cultivation and processing LCIA

The results of the present study align with those previously reported (Farzad *et al*., 2017b; Mashoko *et al*., 2013; Pryor *et al*., 2017; Reno *et al*., 2011; Renouf *et al*., 2010), in that sugarcane cultivation and transportation contribute to the acidification and eutrophication impact categories, due to fertiliser use during cultivation and fossil fuels for agricultural machinery and transportation (Cherubini and Jungmeier, 2010; Farzad *et al*., 2017a; Reno *et al*., 2011; Renouf *et al*., 2010). Acidification could be reduced by using more efficient transportation (Reno *et al*., 2011), such as increasing the use of rail over road transport, which consumes less fossil fuel per kilometre travelled at 0.68 MJ/kmt compared to 1.08 MJ/kmt (Table S-1).

Eutrophication could also be reduced by minimising the use of nitrogen and phosphorous fertilisers or avoiding contact thereof with groundwater, rivers or lakes as suggested by Reno *et al.* (2011). Although this makes sense in theory it may not be practically possible, since high nitrogen application rates are used to remain competitive and achieve high crop yields, which ultimately lead to high nitrogen losses (Renouf *et al*., 2010). Therefore, the efficient management of fertiliser application is vital to reduce this impact category (Renouf *et al*., 2010), rather than the limitation thereof.

The sugarcane lignocellulosic feedstock (through sugarcane cultivation, trash collection and transport, and bagasse production in the sugar mill) also contributes to abiotic depletion (Farzad *et al*., 2017a), global warming impact (2 940 kg CO_2 eq per 65 t/h feedstock), and fresh water and marine aquatic eco-toxicity, as shown in Figure 4. However, unlike previous studies, (Farzad *et al*., 2017b; Mashoko *et al*., 2013; Pryor *et al*., 2017; Reno *et al*., 2011; Renouf *et al*., 2010), the feedstock had a significant impact on marine aquatic eco-toxicity. Marine eco-toxicity refers to the impact a chemical substance has on marine life, which includes fish (vertebrates), crustaceans (invertebrates) and algae (plants). The contribution to marine aquatic eco-toxicity is due to the aluminium used for the production of farm implements and machinery as well as fertilizer (Urea) use, contributing 45% and 20%, respectively.

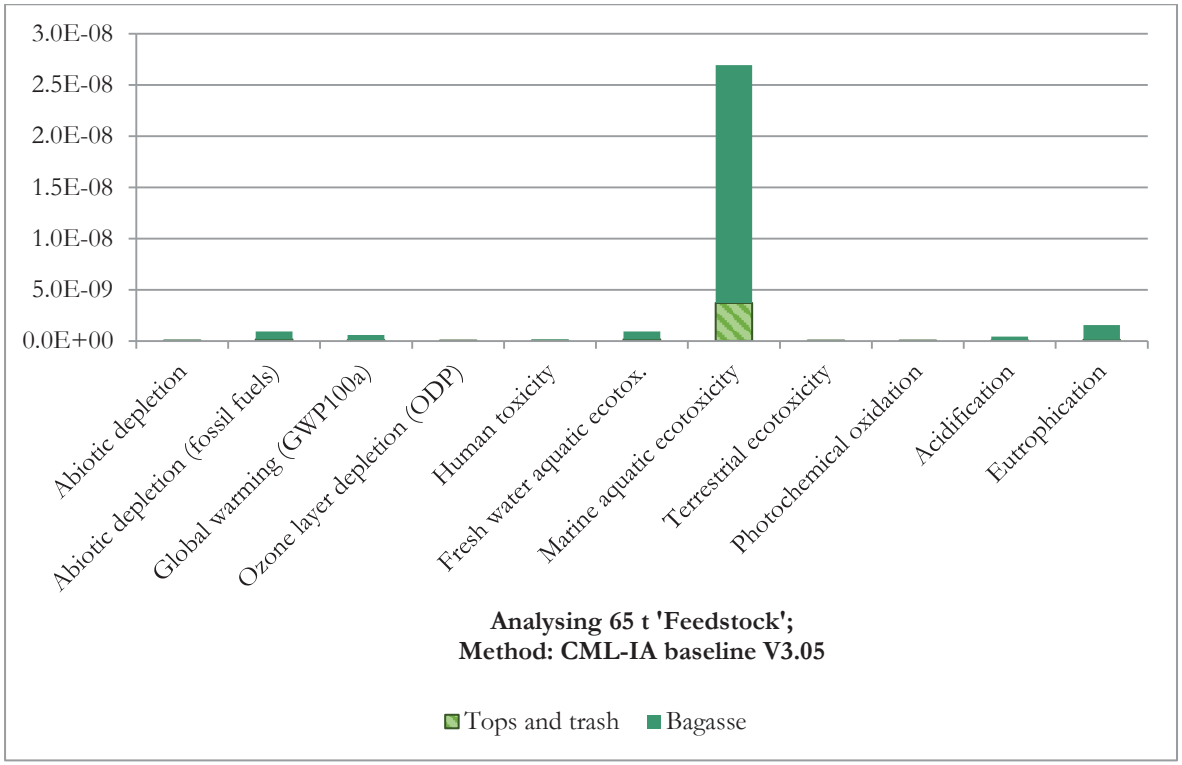

Figure 6-4: LCIA of the sugarcane lignocellulosic feedstock on the respective impact categories

Although sugarcane cultivation is a major contributor to environmental burdens, the use of sugarcane lignocellulosic feedstock has the advantage of CO_2 sequestration (although not included in the LCI) and thus contributes to a carbon neutral system (Reno *et al*., 2011). It is also worth mentioning that sugarcane agriculture uses less fertilisers than corn agriculture and therefore contributes less to the impact categories affected by fertiliser use (Luo *et al*., 2009), namely acidification, eutrophication, aquatic eco-toxicity and global warming.

Moreover, sugarcane cultivation has no detrimental environmental impact related to the land use change (LUC) emissions caused by the change in land use for the production of first generation feedstocks such as corn and wheat (Piemonte, 2012). As a result, sugarcane lignocelluloses remain a desirable bioresource despite the associated environmental burdens.

6.5.2 Comparison of biorefinery scenarios

The results for the mid-point, problem orientated CML-IA baseline V3.05 methodology are shown in Figures 6 to 9 for the comparison of biorefinery scenarios based on 1 kg bioproduct (Figures 5 and 6) and 1 kWh generated electricity (Figures 7). The characterisation results are shown as a relative percentage between scenarios (Figure 6 and 7) and as normalised units, which are dimensionless values provided for the impact categories' results divided by the annual environmental impact of an average person living in Europe (EU 25). Although this is not specific to South Africa, it provides a relative indication of how serious the contribution to environmental damage is for each impact category.

When the biorefinery scenarios are compared based on **1 kg bioproduct** produced, it is foreseen that the biorefinery with the lowest production volume will have the largest environmental contribution per production unit, as is the case for Scenario 3 (PHB) shown in Figure 6 and 7. This is true for all the impact categories, except fresh water eco-toxicity, ozone layer depletion and human toxicity. The fresh water eco-toxicity impact caused by Scenario 1 (coal supplemented IA biorefinery) was due to the use of coal in the CHP plant.

The ozone layer depletion caused by succinic acid production in Scenario 5 (SA) was due to the use of an organic solvent, specifically 1-Octanol, in the reactive extraction downstream process recovery. The CO_2 used during succinic acid fermentation was the main contributor to the human toxicity impact category due to the toxic compound monoethanolamine (ETA) used during CO_2 production.

The CO_2 used as input for succinic acid fermentation is described as having no environmental burdens, since it is a waste product from other processes such as ammonia and hydrogen production (SimaPro® v8.5.0). However, some inputs are still required to obtain, purify and liquefy the CO_2 for use and transport, which include the use of fossil based electricity to do so.

When the results are normalised, both the ozone layer depletion and human toxicity contribution of succinic acid is negligible, as seen in Figure 6. The impact on marine eco-

toxicity is due to sugarcane cultivation of the biomass feedstock (as discussed in section 6.3.1), as well as enzyme use, which contributed 36% of the normalised marine eco-toxicity for Scenario 1 (IA coal supplemented), 29.5% for PHB in Scenario 3 and 11.4% for succinic acid in Scenario 5 per kg bioproduct. The environmental contribution of cellulase enzymes should not be ignored as shown by the in-depth comparative attributional LCA on cellulase enzyme production for cellulosic ethanol production done by Gilpin and Andrae, (2017).

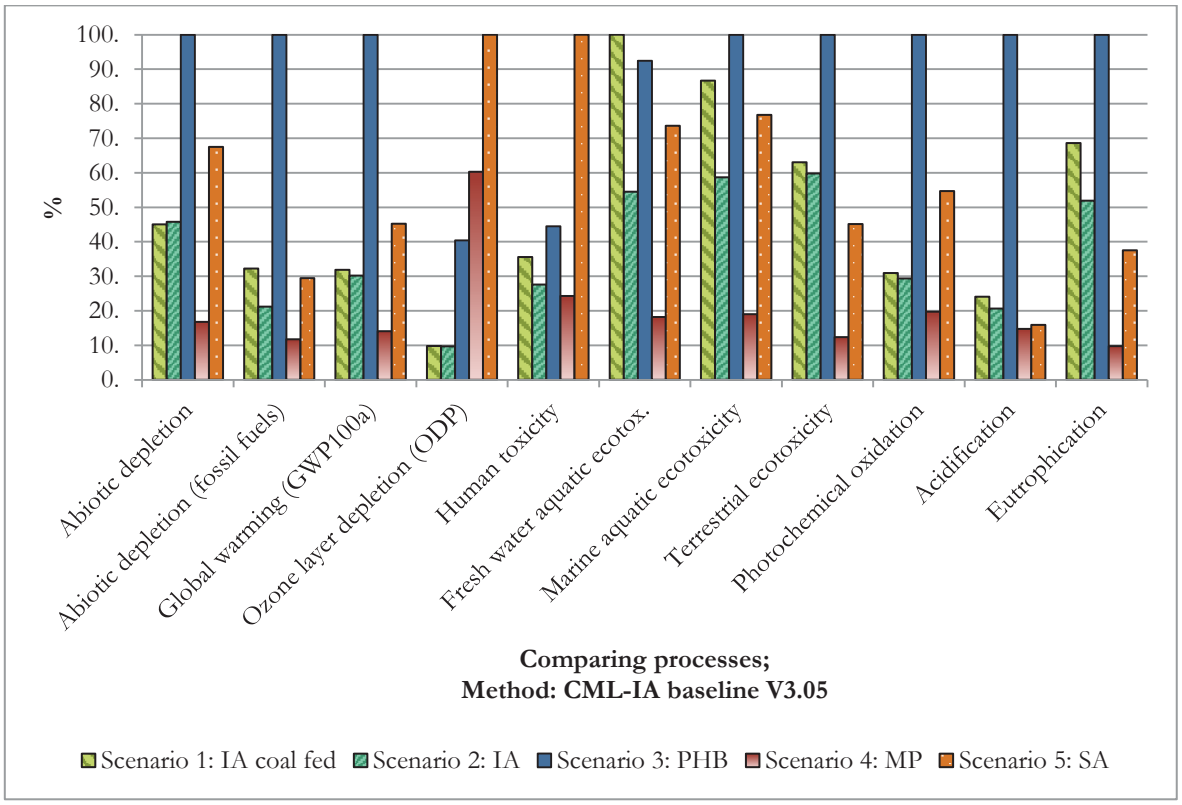

Figure 6-5: LCIA of biorefinery scenarios on respective impact categories (1 kg bio-product)

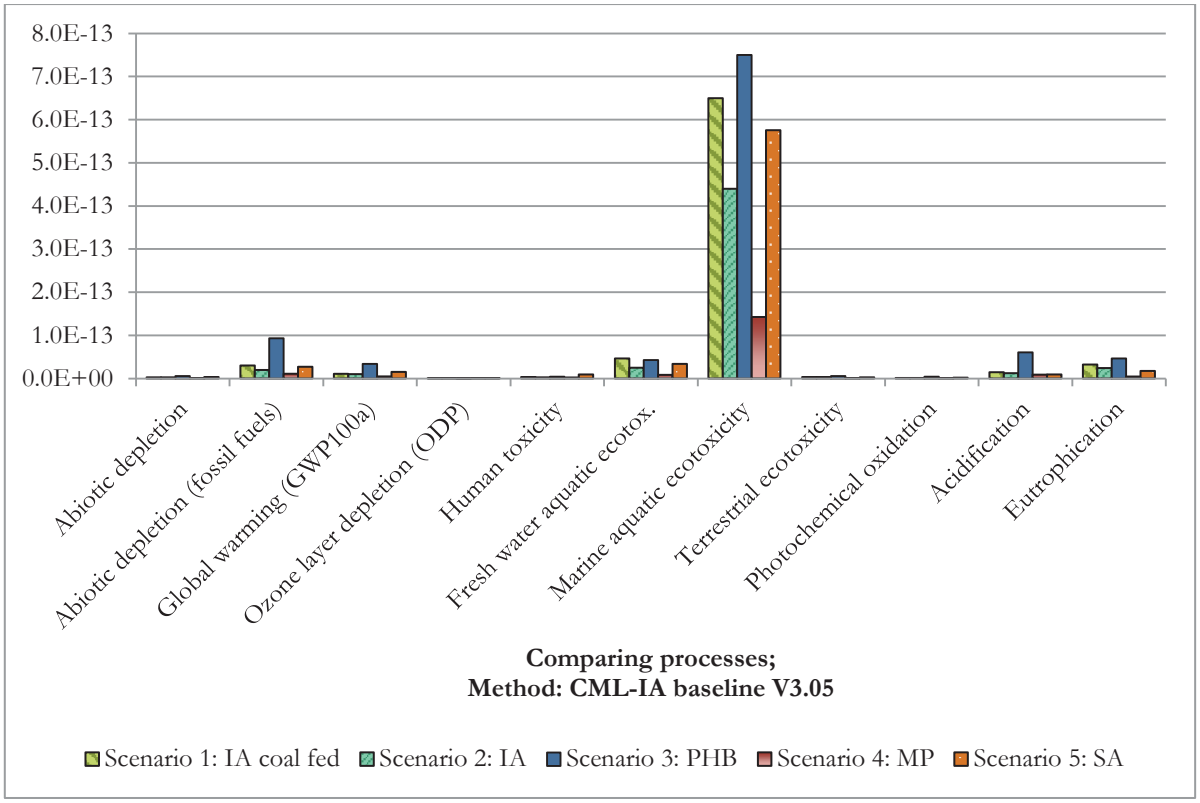

Figure 6-6: Normalised LCIA of the biorefinery scenarios on the respective impact categories (1 kg bio-product)

The environmental burden imposed by Scenario 1 (IA coal supplemented) on the impact categories becomes significant when the functional unit of **1 kWh generated electricity** is considered, especially on marine eco-toxicity, due to the use of fossil based coal in the CHP plant. The environmental impacts caused by Scenarios 2 – 6 have a negligible relative environmental impact, as seen in Figure 7.

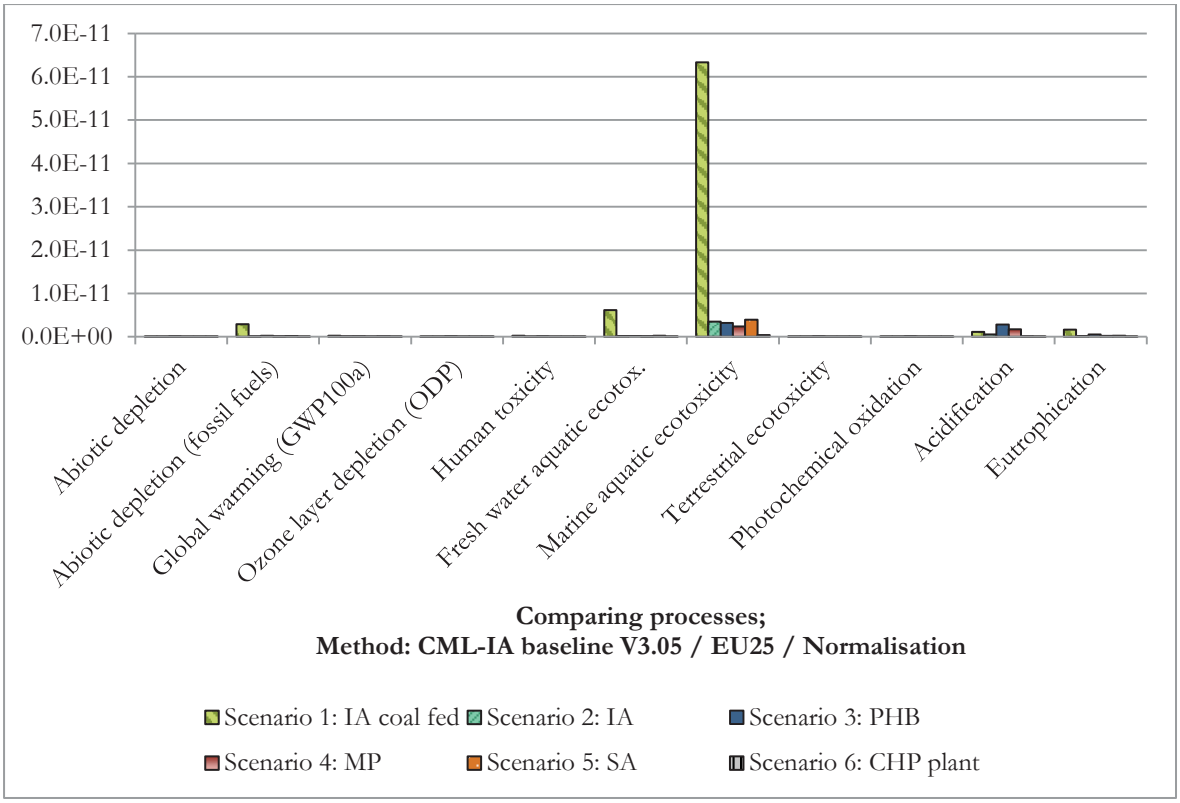

Figure 6-7: Normalised LCIA of the biorefinery scenarios on the respective impact categories (1 kWh generated electricity)

6.5.3 Comparison of biorefinery scenarios to fossil reference systems and literature

6.5.3.1 LCIA Itaconic acid production

Itaconic acid can replace fossil based chemicals such as acrylic acid for the superabsorbent polymer market, acetone cyanohydrin for the MMA (methyl methacrylate) market and maleic anhydride for the unsaturated polyester resin market (Weastra, 2011). As a result, the production of itaconic acid from sugarcane lignocelluloses (Scenario 1) was compared with these chemicals available in the SimaPro® V8.0 database, as shown in Figure 8.

The environmental contribution of 1 kg itaconic acid is small when compared to fossil based equivalent chemicals. The ozone layer depletion, human toxicity, fresh water, marine and terrestrial eco-toxicity and eutrophication impact categories are high for maleic anhydride.

The use of petroleum during maleic anhydride production from direct oxidation of n-butane contributes to ozone layer depletion and eutrophication, with the benzene used in maleic anhydride production contributing to human toxicity. The landfill caused by coal mining (in

benzene production) and lignite (in electricity production) contribute to marine eco-toxicity and eutrophication.

The abiotic fossil fuels (due to crude oil and natural gas use during production), global warming, photochemical oxidation and acidification impact categories were high for acetone cyanohydrin due to process emissions such as SO_2, NO_x, CH_4 and CO. Acrylic acid contributes to abiotic depletion due to the use of electricity and biogas during production.

The LCIA of polymerized itaconic acid (PIA), *Itaconix™ Dispersant DSP2K* produced from starch, was previously compared with PIA (DSP2K), produced from a lignocellulosic woody biomass though a cradle-to-gate attributional LCA (Nuss *et al*., 2013). However, the LCI data could not be compared to this study since it is not available due to the confidentiality of the Itaconix™'s process. The lignocellulosic based PIA has a GWP of 1.32 kg CO_2 eq compared to corn based PIA with a GWP of 2.19 kg CO_2 eq and fossil based PAA (polyacrylic acid) with 2.74 kg CO_2 eq (Nuss *et al*., 2013). This is higher than the 0.316 kg CO2 eq and 0.306 kg CO_2 eq per 1 kg bioproduct obtained for itaconic acid production in Scenario 1 and 2, respectively. This could be attributed to the difference in processing steps required for polymerized itaconic acid as well as the allocation method, geographic location and LCIA methodology that were used to interpret the results. Overall, the production of itaconic acid from sugarcane lignocelluloses does not contribute to additional environmental impacts compared to fossil based systems in other impact categories (Figure 8).

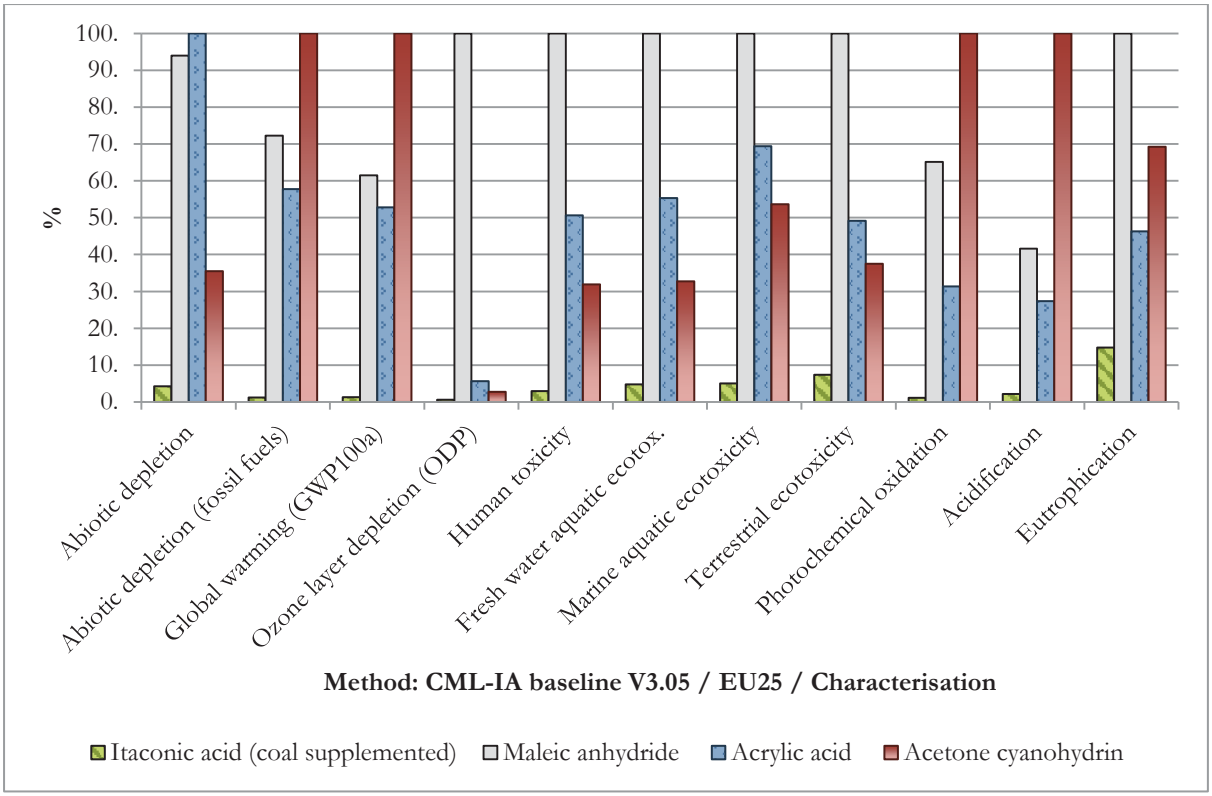

Figure 6-8: LCIA results of fossil reference system compared to 1kg itaconic acid produced

6.5.3.2 LCIA Succinic acid production

Succinic acid can potentially replace maleic anhydride in the production of 1,4-butanediol (BDO), γ-butyrolactone (GBL) and tetrahydrofuran (THF) (Delhomme *et al*., 2009), as well as adipic acid and phthalic anhydride for the production of plasticisers (Weastra, 2011). Succinic acid production from sugarcane lignocelluloses in Scenario 5 (SA) was compared to maleic anhydride and adipic acid LCIAs available in the SimaPro® V8.0 database, as shown in Figure 9. Maleic anhydride has the largest contribution to ozone layer depletion, caused by gas production from petroleum use during maleic anhydride production from direct oxidation of n-butane.

The other impact categories are affected most by adipic acid (Figure 9). Adipic acid is an organic carboxylic acid used predominantly for the production of nylon. Benzene is hydrogenated to cyclohexane, used to produce a mixture of cyclohexane and cyclohexanone (KA oil), which is then oxidized with nitric acid to produce adipic acid. Consequently, the use of benzene is the main cause of environmental burdens in the categories impacted by adipic acid.

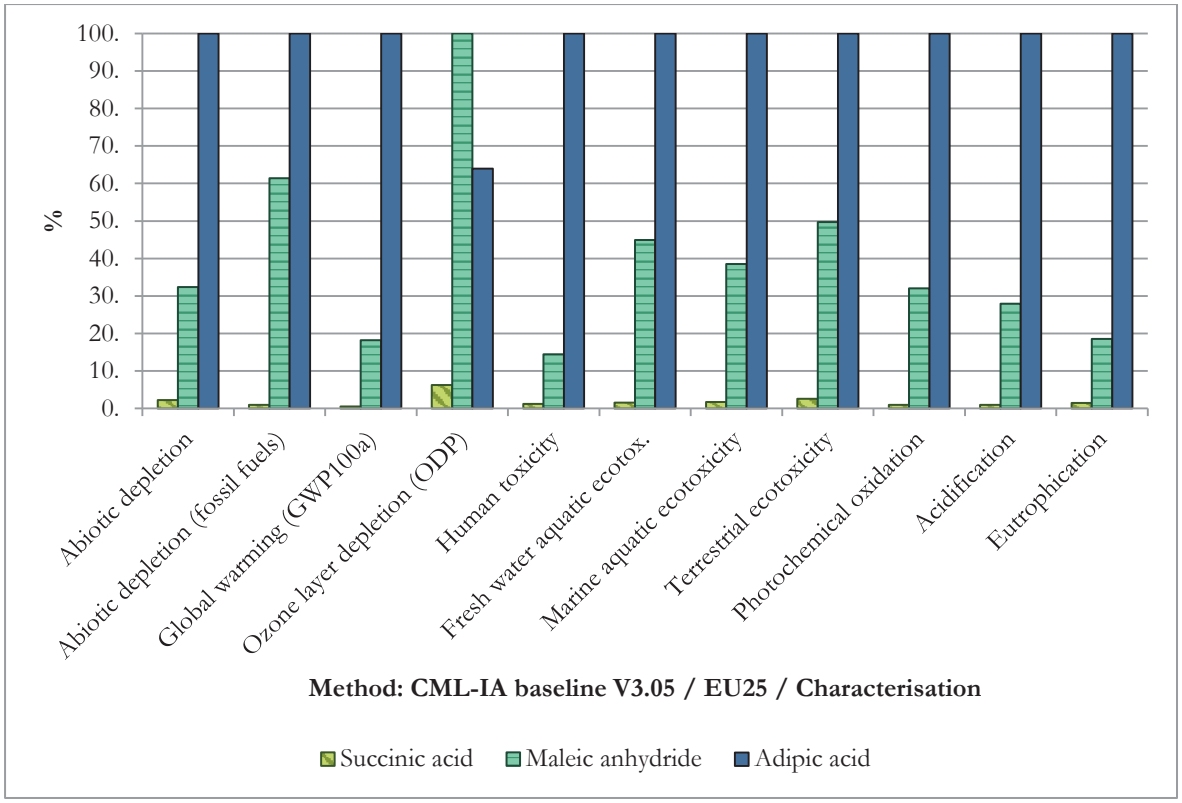

Figure 6-9: Comparison of biobased succinic acid to fossil based equivalent chemicals maleic anhydride and adipic acid

The LCA on biobased succinic acid production in Scenario 5 (SA) can be compared to the production of succinic acid from apple pomace through fermentation with *A. succinogenes* and reactive extraction downstream processing, as well as succinic acid and bioethanol co-generation from *Phalaric aquatic L.* (Harding grass) evaluated previously (Chrysikou *et al.*, 2018; González-García *et al.*, 2018).

A key hotspot identified in the apple pomace biorefinery was the use of fossil based heat and electrical energy. Likewise, the use of electricity in the Harding grass biorefinery was also identified as a major hotspot. Although the Harding grass biorefinery included combustion of biomass for electricity and steam production, the biorefinery was supplemented with electricity from the Greek electricity grid, which contributed significantly to greenhouse gas emissions. However, this is not the case for Scenario 5 (SA) since the energy needs of the biorefinery and existing sugar mill are met by the CHP plant and hence no additional fuel or energy source is required. Therefore, the damaging impact on climate change can be reduced beyond the use of bioresources, as opposed to fossil based resources, if it is operated as a bioenergy self-sufficient system using a biomass fed CHP plant.

The reported GWP's of succinic acid biorefineries vary significantly due to the source of energy used. For the biorefineries where fossil based energy were used, the GWP's are high: the apple pomace biorefinery reported a GWP of 5.30 kg CO_2 eq (González-García *et al*., 2018) and the Harding grass biorefinery reported a GWP of 193 kg CO_2 eq (Chrysikou *et al*., 2018).

The GWP of 0.544 kg CO_2 eq per kg succinic acid produced in Scenario 5 (SA) compares best with the bioenergy self-sufficient giant reed (*Arundo donax L.)* biorefinery in Southern Italy with a GWP of 1.95 kg CO_2 eq (Zucaro *et al*., 2017). The remaining differences between GWP's are due to fertilizer use. Of the 1.95 kg CO_2 eq, 51.3% was due to the organic N source, compared to 8.3% for the N source used for Scenario 5 (SA). It should also be noted that the different methodologies and allocation used between the various studies will impact the results.

Moreover, the CO_2 used for succinic acid production in Scenario 4 (MP) and 5 (SA) contributes to the global warming potential category due to electricity required during CO_2 production (i.e. capture, cleaning, liquefying and transport), whereas the CO_2 used during fermentation for the apple pomace and Harding grass biorefineries resulted in an environmental credit for the global warming potential impact category. This was due to the assumption that the CO_2 is supplied from a nearby bioethanol biorefinery (Chrysikou *et al*., 2018; González-García *et al*., 2018).

Therefore, a multiproduct plant producing both succinic acid and cellulosic ethanol could decrease the environmental impact of these bioproducts even more, since the CO_2 is produced on-site and the harmful impact of CO_2 production is avoided. Moreover, using an alternative DSP, where ion-exchange columns, nano-filtration and evaporation are used instead of reactive extraction with an organic solvent (1-octanol), has been shown to decrease the environmental impact between 82 and 97% across the impact categories. However, the impact on recovery and purity efficiency is unknown (González-García *et al*., 2018).

6.5.4 LCIA PHB production

The production of 1 kg PHB in Scenario 2 (PHB) from sugarcane lignocelluloses was compared with biobased plastic PLA and a polyester-complex starch plastic (starch/polyolefin blend), as well as to fossil based plastics PE (polyethylene) and PP (polypropylene), which have similar mechanical properties to PHB (Lopes *et al*., 2014; Verlinden *et al*., 2007). The PLA, polyester-complex plastic, PE and PP life cycle inventory data was available in the SimaPro® V8.0 database and the comparison of these scenarios are shown in Figure 10.

The production of PHB from sugarcane lignocelluloses in Scenario 3 (PHB) had the most significant environmental impact on the abiotic depletion, global warming potential, photochemical oxidation, acidification and eutrophication categories. The abiotic depletion is caused by the phosphoric acid used to produce the fermentation nutrient di-ammonium phosphate. Di-ammonium phosphate also contributes to photochemical oxidation, due to the sulphur required for the sulphuric acid production, used in phosphoric acid production for di-ammonium phosphate production. The impacts on global warming potential, acidification and eutrophication were due to fossil fuel and fertiliser use during sugarcane cultivation (section 6.3.1).

The polyester-complexed starch polymer had the most significant impact on ozone layer depletion due to the use of naphtha for olefin production used in the starch/polyolefin blend. Polylactic acid had the most significant impact on the human toxicity, fresh water-, marine and terrestrial eco-toxicity categories. The major contributor to human toxicity was the sulphide and lignite tailings produced from electricity and chemical consumables production. The electricity used for PLA production also contributed to the environmental impact on fresh water-, marine and terrestrial eco-toxicity categories due to the fossil sources used to generate the electricity.

It is interesting to note that the biobased polymers (PHB, PLA and polyester-complexed starch) are the major contributors across the impact categories, with PHB as the major contributor for 6 of the 10 impact categories. This aligns with previous studies where it was found that fermentation-derived PHB did not seem to be an appropriate replacement for conventional polymers if sustainable polymer production is desired (Chanprateep, 2010). PHB production is energy intensive compared to conventional plastics (Chanprateep, 2010), with low yields and efficiencies (Wolf *et al*., 2005). The high GWP of sugarcane lignocellulosic PHB can therefore be attributed to the low fermentation yield (0.27 w/w) compared to other substrates such as cane molasses (0.42 w/w) or sucrose (0.40 w/w) (Reddy *et al*., 2003).

Furthermore, using the IPCC GWP100a methodology, the PHB produced in Scenario 3 (PHB) had a GWP of 4.2 kg CO_2 eq, which is high when compared to 2.27 kg CO_2 eq for PE, 2.12 kg CO_2 eq for PP, 2.15 kg CO_2 eq for polyester-complexed starch polymer and 3.47 kg CO_2 eq for PLA, as summarised in Table 2. PHB production from waste water resulted in a GWP of 2.38 kg CO_2 eq compared to sugar-based PHA's 2.0 kg CO_2 eq and fossil based plastics' 2.15 kg CO_2 eq for PET (Dacosta *et al*., 2015). The carbon footprint of PHB can be reduced by

increasing the production volume of PHB per unit feedstock fed (i.e. increasing the fermentation yield and efficiency) or combining it with the co-production of another product, such as succinic acid in the multiproduct plant Scenario 4 (MP). In Scenario 4 the environmental burden can be shared across the range of biorefinery products (subject to the selected allocation method) to decrease the impact per functional unit of 1 kg bioproduct produced.

For the *cradle-to-gate* analysis, it does not seem evident that PHB production holds any environmental benefit over conventional plastics, due to the low yield, efficiency and high water use (section 6.3.4) associated with PHB production. This being said, PHB may still hold advantages over conventional plastics for the *gate-to-grave* analysis, depending on the eventual use. Therefore PHB may still be favoured due to its biodegradable properties for the marine eco-toxicity impact factor when the large amount of plastic waste within the ocean is considered.

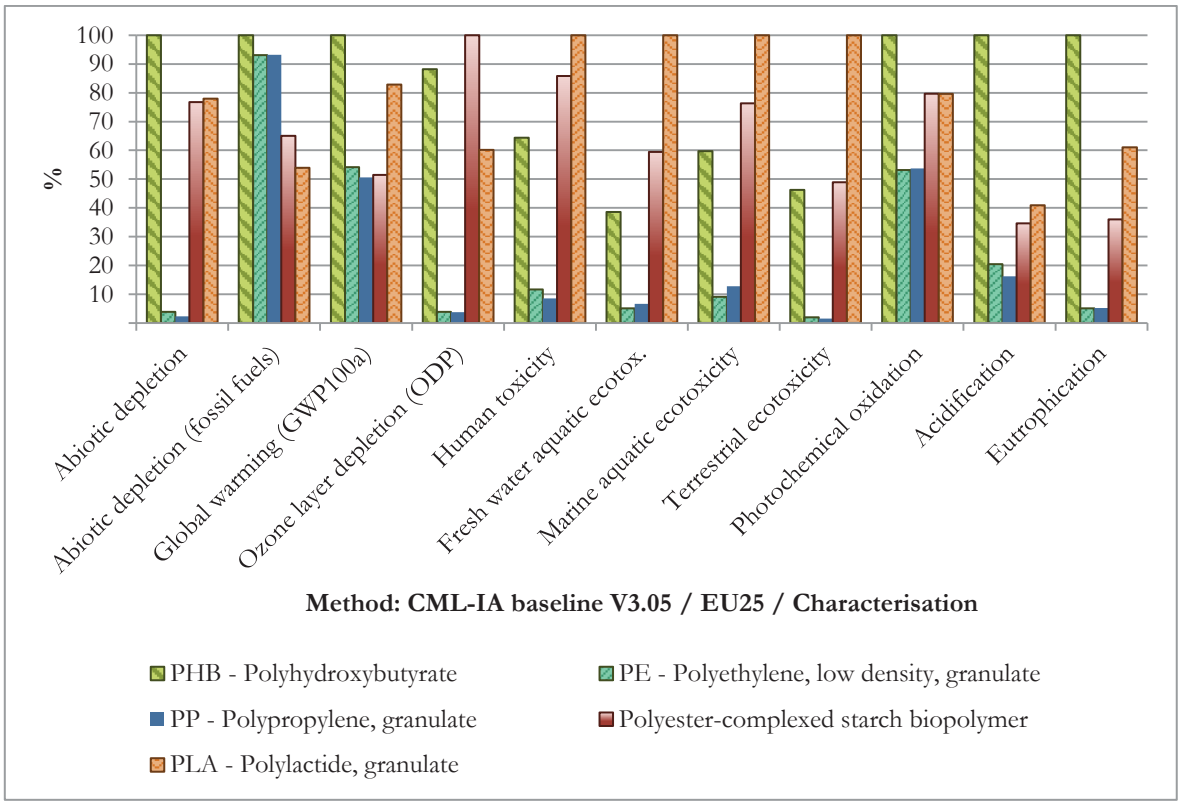

Figure 6-10: Comparison of bio- and fossil based polymers with PHB

Table 6-2: GWP (kg CO2 eq) of PHB, PLA, polyester-complexed starch polymer, PET and PP in literature

Description	Biobased		Fossil-based		Reference
	Polymer	(kg CO_2 eq)	Polymer	(kg CO_2 eq)	
Scenario 3 (PHB)	PHB	4.2			Present study
Glucose substrate	PHB	2.0			(Dacosta *et al.*, 2015)
Cradle-to-grave	PHB	3.7			(Wolf *et al.*, 2005)
Waste water and fossil reference	PHB	2.38	PET	2.15	(Dacosta *et al.*, 2015)
Cradle-to-grave	Starch polymer	2.8	Starch polymer	4.8	
Simapro® v8.0	Starch polyester PLA	2.15 3.47	PE PP	2.27 2.12	IPCC GWP_{100}
Cradle-to-grave	PLA	1.89	PLA	4.8	(Wolf *et al.*, 2005)

6.6 Carbon footprint and Water scarcity impact

The carbon footprint is calculated in SimaPro® using the IPCC GWP 100a method for the functional unit of 1 kWh generated, as shown in Figure 11. Scenario 1 (IA coal supplemented) had the largest carbon footprint at 100%. The CHP plant had the lowest carbon footprint of 3.8%, due to the biogenic CO_2 emissions from the burned biomass, no CH_4 (methane) emissions from a WWT facility and a high amount of electricity produced, making the environmental contribution per 1 kWh generated low. The carbon footprint score included for the MCDA tool is the characterised carbon footprint percentage subtracted from a 100%, since a low value indicates an unfavourable result in the MCDA table. Due to the arid nature of South Africa, the water scarcity is also included in the MCDA table.

The 100% WSI of Scenario 3 (PHB) is double that of Scenario 5 (SA) and far exceeds Scenarios 1, 2, 4 and 6 as seen in Figure 11. Once again, this is attributed to the small production volume of PHB in Scenario 3 at 2.7 t/h compared to another scenario such as 19.3 t/h succinic acid in Scenario 5 (Table 4). For Scenarios 1 – 5, steam was required during pretreatment and therefore a boiler water make-up stream was included. Scenario 6 (CHP) has the lowest WSI of 0.013% (Figure 11), since it does not require boiler make-up water for pretreatment and it is a closed steam system. Since a high WSI is not desirable, the score included for the MCDA tool is the WSI percentage subtracted from a 100%, since a low value indicates an unfavourable result in the MCDA table.

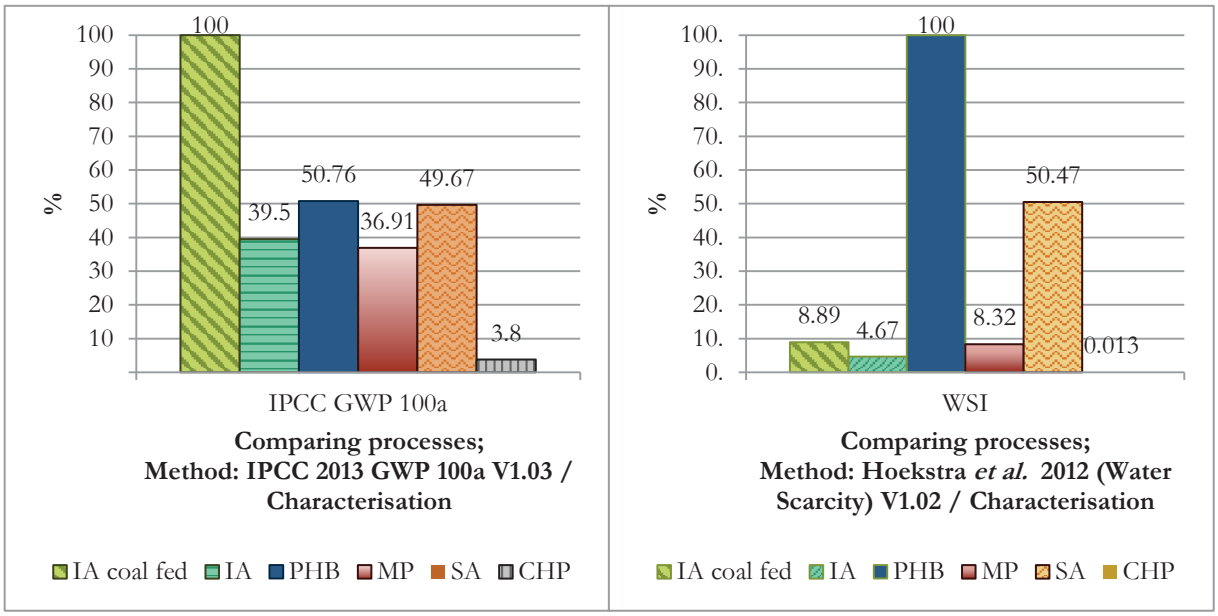

Figure 6-11: Scenarios compared on 1 kWh functional unit through single score method IPCC GWP 100a (left) for carbon footprint and Hoekstra *et al.*, 2012 v1.02 (right) for water scarcity impact (WSI)

6.7 Multi-criteria decision analysis results and discussion

6.7.1 Determination of key indicators

The profitability, TCI and TCOP economic indicators are provided in Table 3. The energy efficiency score is calculated from the results shown in Table 4 and the technical maturity is determined by evaluating the technology readiness level (TRL) of each plant area in Table 5. Additional information on selection of the TRL for each process area is available in the Supplementary information.

Table 6-3: Economic indicators (Extract from Chapter 3, 4 and 5)

Economic indicators	Scenario 1: IA coal	Scenario 2: IA	Scenario 3: PHB	Scenario 4: MP	Scenario 5: SA	Scenario 6: CHP plant
Profitability (IRR%)	10.29%	8.04%	0%	24.08%	28.04%	10.31%
NPV (million US$)	31.03	(48.27)	(216.9)	447.25	644.97	6.08
Selling price ($/kg)	1800	1800	2600	1 500 (SA); 11 424 (PHB)	1500	0.08 $/kWh
Total capital investment (TCI)	662.85	380.22	320.15	352.20	384.25	130.11
Total cost of production (TCOP)	47.77	23.08	35.74	33.16	37.60	13.16
Number of employees for biorefinery and CHP plant	82	40	72	82	48	12

Table 6-4: Mass and Energy balance results

	Scenario 1: IA coal	Scenario 2: IA	Scenario 3: PHB	Scenario 4: MP	Scenario 5: SA	Scenario 6: CHP plant	Calculation
Feed and Product							
Bypass ratio	0%	54%	13%	31%	35%	100%	-
Feedstock rate (t/h)	65.00	29.90	56.55	44.85	42.25	65	A
Bioproduct produced (kg/h)	12 179	5 601	2 680	11 774[a]	19 309	54 378 kWh	B
Steam							
Steam required (t/h)	288.89	133.52	146.82	134.71	93.02	15.02	C
Steam required per tonne of feedstock	4.44	4.47	2.60	3.00	2.20	0.23	D = C/A
Normalised to Scenario 2 (%)	*0.5%*	*0.0%*	*41.9%*	*32.7%*	*50.7%*	*94.8%*	E = 1-D/4.47
Electricity							
Electricity produced (kWh)	7291.507	7276.779	7437.32	7657.298	8880.467	54378.1854	F
Electricity required (kWh)	2224.23	1458.13	2501.15	2165.37	3082.57	1169.53	G
Sellable electricity (kWh)	5067.27	5818.65	4936.17	5491.93	5797.90	53208.66	H = F – G
Electricity required per tonne of feedstock	0.03	0.05	0.04	0.05	0.07	0.02	J = G/A
Normalised to Scenario 5 (%)	*53.1%*	*33.2%*	*39.4%*	*33.8%*	*0.0%*	*75.3%*	K = 1-J/0.07
Energy efficiency score	26.8%	16.6%	40.6%	33.3%	25.3%	85.1%	L = (E+K)/2

a) 514 kg/h PHB and 11 260 kg/h SA

Table 6-5: Technical readiness level for the respective biorefinery plant areas and CHP plant

Plant area	Description	Scenario 1: IA coal	Scenario 2: IA	Scenario 3: PHB	Scenario 4: MP	Scenario 5: SA	Scenario 6: CHP plant	References
PA-100	Dilute acid pretreatment	4	4	4	4	-	-	(Humbird, 2011)
	Steam explosion pretreatment	-	-	-	-	5	-	(Neves *et al*., 2016)
	Enzymatic Hydrolysis	3	3	3	3	3	-	(Benjamin, 2014; Humbird, 2011)
	GAC detoxification	2	2		2	-	-	(Hodge *et al*., 2009)
	Cellulase enzyme production	9	9	9	9	9	-	Novozymes®
PA-200 and/or PA-300	Itaconic acid fermentation from pretreated lignocelluloses	2	2	-	-	-	-	(Saha *et al*., 2018)
	Succinic acid fermentation from pretreated lignocelluloses	-	-	-	4	4	-	(Beauprez *et al*., 2010; Brink and Nicol, 2014)
	PHB fermentation from pretreated lignocelluloses	-	-	4	4	-	-	(Choi and Lee, 1999; Wang and Lee, 1997)
PA-400	Crystallisation and Evaporation	8	8	-	8	8	-	(Chenyu Du, 2014; Okabe *et al*., 2009; Pfeifer *et al*., 1952)
	Reactive extraction DSP	-	-	-	7	7	-	(Kurzrock and Weuster-Botz, 2011; Morales *et al*., 2016)
	Alkaline DSP	-	-	7	7	-	-	(Choi and Lee, 1999; Wang and Lee, 1997).
PA-500	WWT plant	8	8	8	8	8	-	(Humbird, 2011; Wooley *et al*., 1999)
PA-600 and PA-700	CHP Plant	7	9	9	9	9	9	
	Technical Maturity[a]	59.72%	62.50%	69.84%	65.66%	73.61%	100.00%	

a) The Technical maturity is calculated as the sum of the TRL values, divided by the number of plant areas (n) times TRL_{max} (9). i.e. TM = $\left(\frac{\sum TRL}{n}\right) * TRL_{max}$

6.7.2 Multi-criteria decision analysis results

For the first evaluation, emphasis was placed on the overall techno-economic indicator with a RW of 45%, 35% for the environmental and 20% for the social indicator. Scenario 4 (MP) has the highest score at 72.3, followed by 61.0 for Scenario 5 (SA) and 59.2 for Scenario 6 (CHP), as shown in Table 6. Although Scenario 2 (IA) is unprofitable, the high TCI and TCOP of Scenario 1 (IA coal supplemented) provided it with a lower overall techno-economic score at 8.3. As a result, Scenario 1 (IA coal supplemented) received a low overall score of 41.9, together with the unprofitable Scenario 3 (PHB) with a score of 33.5.

Table 6-6: Multi-criteria decision analysis table for Case study 1: Techno-economic focus

BIOREFINERY SCENARIOS	Scenario 1: IA coal	Scenario 2: IA	Scenario 3: PHB	Scenario 4: MP	Scenario 5: SA	Scenario 6: CHP plant	RW
Techno-economic	8.3	10.3	6.1	26.0	31.8	22.1	45
Profitability	4.8	-7.5	-33.6	69.3	100.0	0.9	20
TCI	0.0	42.6	51.7	46.9	42.0	80.4	5
TCOP	0.0	51.7	25.2	30.6	21.3	72.5	5
Technical Maturity	59.7	62.5	69.8	65.7	73.6	100.0	10
Energy efficiency	26.8	16.6	40.6	33.3	25.3	85.1	5
Environmental	12.5	28.8	15.9	20.4	11.1	35.0	35
GHG emissions	0.0	60.5	49.2	63.1	50.3	96.2	20
Water demand	91.1	95.3	0.0	91.7	50.0	100.0	15
Social	20.0	9.8	17.6	20.0	11.7	2.9	20
Job creation	100.0	48.8	87.8	100.0	58.5	14.6	20
TOTAL	41.9	46.5	33.5	**72.3**	61.0	59.2	100

The detailed MDCA results are provided in Table 6 for the expected values of 45% techno-economical, 35% environmental and 20% social contribution. However, when the impact of stakeholders' expectations was considered, the values were varied. For this scenario, the social indicator remained constant at 20% while the environmental RW was decreased from 80% to 0% and the economic RW was increased from 0% to 80% in increments of 5%.

Scenario 1 (IA coal supplemented) and 3 (PHB) remained relatively constant across the variation in RW. This is due to the fact that these scenarios received low scores for both the techno-economical and environmental indicators, as shown in Table 6 for each criterion and scenario before weighting is applied for the techno-economical, environmental and social indicators. However, for the other scenarios investigated, a trade-off between the environmental and techno-economical sustainability indicators are seen (Figure 12).

Scenario 2 (IA) received a low score for the profitability criterion under the techno-economical indicator and is therefore promoted by a high environmental indicator RW, where it received a score of 70.1 for an environmental RW of 80%, compared to a score of 28.1 for a high techno-economical RW. Likewise, Scenario 5 had a low water scarcity impact score in the environmental indicator and is therefore promoted by a high techno-economical RW of 80% with a score of 68.2, as seen in Figure 12.

For an environmental RW of 80%, Scenario 6 (CHP) had the highest score at 81.2%, followed by Scenario 4 (MP) at 80.3% and Scenario 2 (IA) at 70.1%. On the other hand, Scenario 5 (SA) had the highest score of 68.2% for a techno-economical RW of 80%, followed by Scenario 4 (MP) at 66.2% and Scenario 6 (CHP) at 42.2%. Consequently, it is seen that Scenario 4 (MP) and Scenario 6 (CHP) have the best results across the range of RW's.

As a result, a clear trade-off exists between the environmental and techno-economical indicators for all the scenarios except the multiproduct plant for the co-production of electricity, succinic acid and PHB from sugarcane lignocelluloses in Scenario 4 (MP). The overall score of this scenario remains high across the varying indicators. Consequently, Scenario 4 (MP) is the most sustainable biorefinery, followed by the production of electricity only in a CHP stand-alone CHP plant in Scenario 6 (CHP). The MCDA tool could be improved by involving local experts within the sugar industry or relevant stakeholders into selecting additional criteria for the social sustainability indicator and refine the techno-economical and environmental indicators.

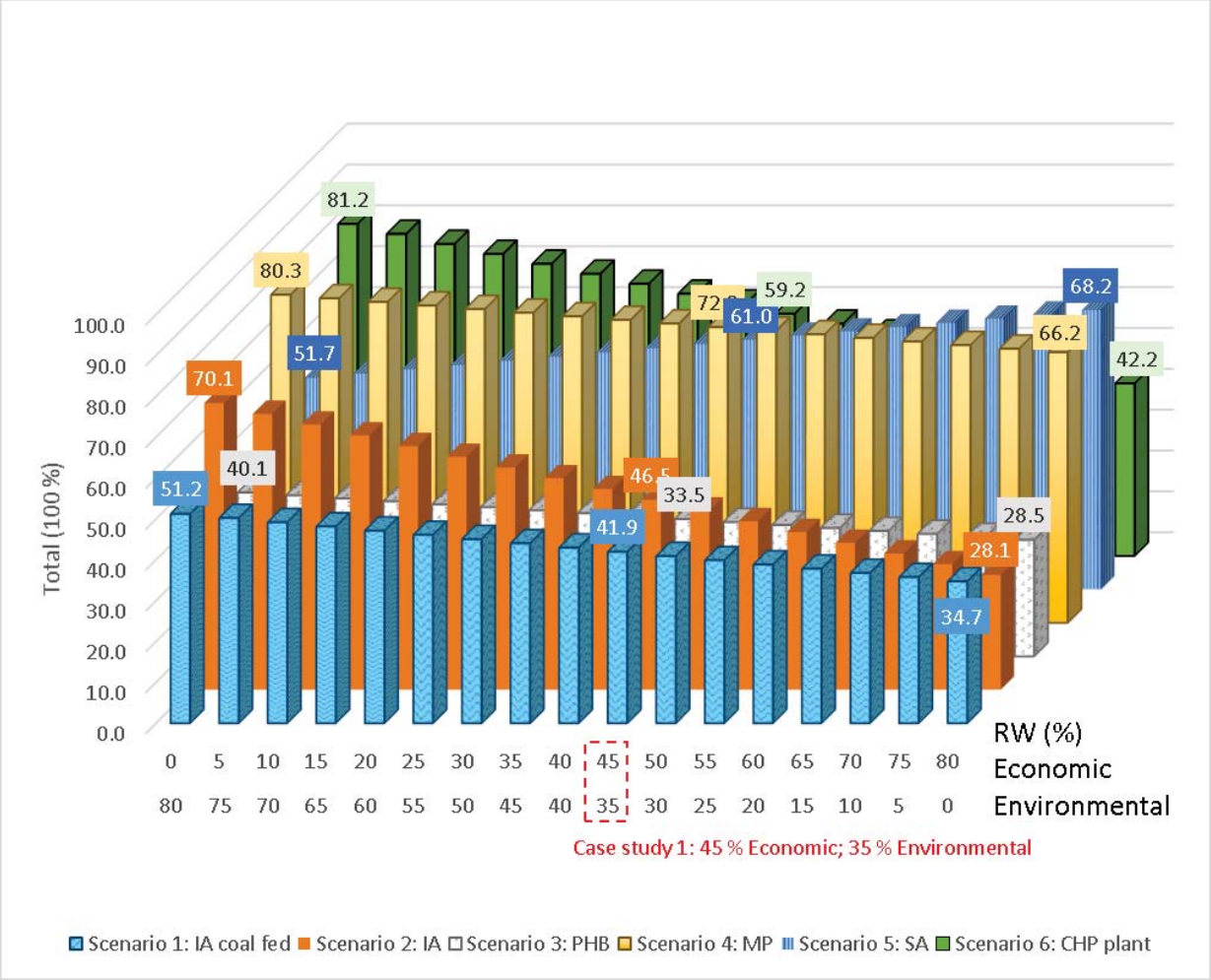

Figure 6-12: Multi-criteria analysis tool for an increasing economical RW and decreasing environmental RW (from left to right) at a constant social indicator of 20 %

6.8 Conclusions

Sugarcane cultivation contributed most to the abiotic depletion, aquatic eco-toxicity, eutrophication and acidification impact categories which could be mitigated by using more railway transport and applying more effective fertiliser use. The environmental advantage of sugarcane biorefineries is that there are no detrimental impacts related to LUC (land use change) emissions. In addition, the integration of a biorefinery with a CHP plant is key to obtaining a reduced carbon footprint when producing biobased products. No critical 'hot spot' areas were found for the production of itaconic acid, succinic acid or PHB. Succinic acid and itaconic acid production also have negligible environmental impacts when compared to their fossil reference products. The co-production of succinic acid, PHB and electricity in a multiproduct plant (Scenario 4) is the most sustainable scenario, followed by the co-production of electricity in a CHP stand-alone plant (Scenario 6).

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Supplementary information

The allocation sensitivity analysis, damage orientated LCIA comparison, TRL selection and LCI of Scenarios 1 – 6 are available in the supplementary information.

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Chapter 7

7. Conclusions and Recommendations

7.1 Chapter overview with novel contributions, key findings and future research

The biorefinery concept was investigated as a potential solution to relieve the economic strain on the South African sugar industry and farmers. The production of three bioproducts, itaconic acid, succinic acid and polyhydroxybutyrate (PHB), from sugarcane lignocelluloses was investigated in pursuit of providing such a potential solution. All the profitable biorefinery scenarios generated in this study is summarized in Table A-1, in Appendix A.

Following the introduction in Chapter 1, the literature review in Chapter 2 provided an overview of the feedstock considerations, biorefinery concept, and the respective bioproducts and their existing production processes. To date, no techno-economic studies have been previously done for the production of succinic acid, itaconic acid or PHB from sugarcane lignocellulosic bagasse and trash. From the literature review, the following objectives were developed:

- Objective 1: Design and develop conceptual biorefinery process designs for the production of biobased chemicals
- Objective 2: Determine which biorefineries are profitable in accordance with South African economic conditions
- Objective 3: Determine which pretreatment method will maximise the valorisation of sugarcane bagasse and trash lignocelluloses
- Objective 4: Determine the environmental impact of succinic acid, itaconic acid and PHB production from sugarcane lignocelluloses.
- Objective 5: Determine which biorefinery is the most sustainable solution for implementation by the South African sugar industry

In Chapter 3, *Process design and economic analysis of a biorefinery co-producing itaconic acid and electricity from sugarcane bagasse and trash lignocelluloses*, the bioenergy self-sufficient **itaconic acid** biorefinery scenario was found to be unprofitable. This scenario was compared to a glucose feedstock based biorefinery and a coal supplemented biorefinery,

followed by an assessment of the key bioconversion process parameters. Although the bioenergy self-sufficient scenario resulted in more favourable techno-economical results than the glucose based biorefinery, only the coal supplemented biorefinery was considered profitable.

Novel contributions of this chapter include the design and simulation of the respective itaconic acid biorefinery processes and the **key finding** that future bench scale research on the itaconic acid microorganism, *Aspergillus terreus,* should focus on improving the itaconic acid yield on pentose sugars through genetic engineering of the microorganism, rather than improving the product titre.

Moreover, from the life cycle assessment in Chapter 6, the coal supplemented biorefinery is environmentally favourable compared to fossil based chemicals such as acrylic acid, acetone cyanohydrin and maleic anhydride. Therefore, the investigation of coal supplemented biorefinery scenarios for the production of succinic acid and PHB for improved profitability is recommended, provided that the environmental impact remains favourable in comparison to their fossil based equivalent chemicals.

Additional **future research** could focus on the implementation of more energy efficient itaconic acid downstream process technologies, such as membrane separation or reactive extraction, to replace the current energy intensive crystallization and evaporation process. In doing so, the bypass of feedstock from the biorefinery to the CHP plant could be reduced, which will increase the plant capacity for economies of scale benefit. Consequently, a key design target for energy self-sufficient biorefineries could be to select process technologies that minimise energy use, specifically steam, in order to maximize the plant capacity for the economies of scale benefit.

However, as seen in Chapter 4 *Process design and economic evaluation of an integrated, multiproduct biorefinery for the production of bioenergy, succinic acid and polyhydroxybutyrate (PHB) from sugarcane bagasse and trash lignocelluloses,* the pursuit of economies of scale should be done by taking the impact of the production volume on selling price into consideration in the techno-economical analysis. For the current selling price of PHB (11 424 US$/t) the stand-alone PHB and electricity co-production biorefinery is profitable. However, the production volume will contribute 29% of the total PHA market volume (60 050 tpa) and therefore ‘flood’ the PHB market, which will cause a decrease in selling price due to

the effect of supply and demand. Consequently, the selling price could be adjusted to that of biodegradable bioplastic (2 600 US$/t), so that PHB can enter the larger bioplastics market with 0.2% contribution.

However, by decreasing the selling price, the techno-economic outcome changed from highly profitable to unprofitable. The NPV (net present value) decreased from 771.14 million US$ to negative 216.90 million US$ (Figure 4-8) for the PHB biorefinery scenario. To mitigate the detrimental impact of a low **PHB** selling price on the profitability, it was combined with **succinic acid** and electricity production in a multiproduct plant. In doing so, the production volume of PHB could be controlled through the amount of fermentable sugars sent to PHB production. As a result, the production volume could be reduced sufficiently to justify that the current, high PHB selling price could be used in the techno-economics. Moreover, the economies of scale benefit could be realized in shared process areas, such as pretreatment, CHP plant and WWT plant, which has significant capital cost contributions to the TCI (total capital investment).

Novel contributions of this chapter include the design, simulation and techno-economic analyses of the respective PHB, succinic acid and electricity co-production biorefinery scenarios, as well the first multiproduct plant for the co-production of succinic acid, PHB and electricity. The multiproduct plant was further optimised for maximum profitability by changing the glucose sugar split between the two bioproduct fermentation (bioconversion) areas.

The **key finding** from this chapter is that the techno-economic evaluation of high value, low volume bioproducts should take the impact of production volume on the potential selling price into account for realistic techno-economic results. However, this may imply that the biorefinery capacities should be reduced, or that only high volume, low value bioproducts could be pursued for lignocellulosic feedstocks in order to justify the capital costs incurred through pretreatment steps. To avoid the aforementioned, the combination of high value, low volume bioproducts into multiproduct biorefineries is recommended.

Therefore, **future research** could be done on the potential combinations of bioproducts to include in multiproduct biorefineries for favourable economic results. In doing so, it may also be possible to decrease the environmental impact. For example, when bioethanol and succinic

acid are combined in a multiproduct biorefinery, the CO_2 produced through ethanol fermentation can be used during succinic acid fermentation, as discussed in Chapter 6.

In addition, the production of these low volume, high value bioproducts from simple substrates could be investigated. PHB could be produced from sucrose which does not require pretreatment steps and therefore no additional capital costs. Moreover, if the PHB production facility is annexed to an existing sugar mill and integrated with a lignocellulosic CHP plant, sucrose and energy (steam and electricity) could be obtained at cost price which may further benefit the techno-economic outcome. Therefore, the investigation of the production of PHB, or any other potential biobased products from simple sugarcane substrates, such as sugar juice, sucrose or molasses, is recommended.

Pretreatment impacts the capital costs required and the bioproduct production volume. The techno-economic outcome is sensitive to both these parameters as shown in the techno-economic sensitivity analysis in Chapter 3. Therefore, the aim of Chapter 5 "*Economic evaluation and comparison of succinic acid and electricity co-production from sugarcane bagasse and trash lignocelluloses in a biorefinery, using different pretreatment methods: Dilute acid (H2SO4), Alkaline (NaOH), Organosolv, Ammonia Fibre Expansion (AFEX™), Steam explosion (STEX), and Wet oxidation*" was to select the most favourable pretreatment method for industrial application and maximise the valorisation of lignocelluloses in a biorefinery.

Nine pretreatment methods were selected from a wide range of available chemical and physio-chemical pretreatment methods for the co-production of succinic acid and electricity from sugarcane bagasse and trash lignocelluloses. The **novel contributions** of this chapter include the respective pretreatment Aspen Plus® simulations which can be adapted and used for different lignocellulosic feedstocks or bioproducts. Through this study the most favourable pretreatment method, steam explosion with autohydrolysis and enzymatic hydrolysis (STEX), was determined for a succinic acid biorefinery.

Moreover, the **key finding** is that the techno-economic outcome can be improved by selecting a pretreatment method while taking both the feedstock and bioproduct into consideration. Therefore, pretreatment methods could be optimised in **future research** with a specific bioproduct in mind, together with the feedstock, rather than maximum enzymatic digestibility. To this end, the pretreatment could be optimised on an experimental level with the aim to

ensure a high yield of bioproduct on fermentable sugars. This may reduce the extent of 'over design' whereby the small increase in enzymatic digestibility does not justify the high amount of processing steps, and thus equipment or consumables, required. For example, STEX followed by alkaline delignification results in high enzymatic digestibility, but the additional processing steps such as delignification, lignin precipitation, and additional waste treatment does not justify the small increase in enzymatic digestibility, when the STEX treatment is sufficient.

In addition, it is recommended that pretreatment methods should minimise the extent of feed dilution, i.e. decrease the liquid to solids ratio, to avoid energy use in the biorefinery required to concentrate the fermentable sugar stream prior to bio- or catalytic conversion. It is also recommended that the developed pretreatment simulations be used to determine the pretreatment method that will maximise the techno-economic outcome (i.e. profitability) for itaconic acid, PHB and the multiproduct plant (succinic acid, PHB and electricity) biorefinery scenarios.

Lastly, the pretreatment method should also be taken into consideration when determining potential combinations of biobased products to include in a multiproduct biorefinery. In the case of succinic acid and PHB, a pretreatment method where two distinct sugar streams are obtained (i.e. a glucose rich and pentose rich stream) are more favourable than a combined sugar stream.

To determine the most sustainable biorefinery scenario, the economic performance was taken into consideration together with the environmental and social sustainability indicators in a **multi-criteria decision analysis (MCDA)** tool. In Chapters 3 – 5 various biorefinery scenarios were developed and their profitability were evaluated through the respective techno-economic analyses. The environmental impact of each biorefinery scenario was determined through an attributional cradle-to-gate **life cycle assessment (LCA)** in Chapter 6 "*Life cycle assessment and multi-criteria analysis of sugarcane biorefinery scenarios: finding a sustainable solution for the South African sugar industry.*"

The environmentally competitive advantage of utilising sugarcane lignocelluloses is that it is not associated with any detrimental environmental impact related to LUC (land use change) emissions. Furthermore, the boiler technology, for utilising sugarcane lignocelluloses, already exists. Moreover, no process 'hot spot' areas were identified in the evaluated biorefinery

scenarios. The biorefinery scenarios are favourable in comparison with their fossil based equivalents, except for PHB production in a bioenergy self-sufficient PHB and electricity biorefinery. PHB production is less favourable in the production (cradle-to-gate) phase due to the low yield and poor energy efficiency compared to conventional plastic production. Therefore, extention of the LCA to include the environmental impact of the use and disposal phases (cradle-to-grave analysis) is recommended as well as comparison of this to that of conventional plastics.

The **novel contributions** of this chapter include the LCI (life cycle inventory) and LCA of the selected biorefinery scenarios. Most importantly, it includes the development of the MCDA tool that can be used to rank the available sugarcane biorefinery scenarios according to sustainability, with the flexibility to assign or change the sustainability indicators' representative weighting.

The **key finding** is that the next key design decision should be to integrate the biorefinery with a CHP plant in order to decrease a bioproduct's climate change footprint significantly. Although the use of biobased feedstock has a lower climate change impact compared to fossil based feedstocks, it is recommended that **future research** on biorefinery scenarios should focus on energy self-sufficiency to further decrease the climate change impact. This may include coal supplementation since the biorefinery waste streams are also used during energy generation. In the case of the coal supplemented itaconic acid biorefinery scenario, the residual cellulignin (solids stream after enzymatic hydrolysis), solid residue and biogas produced in the WWT plant were also used as fuel in the CHP plant. To this end, investigation is recommended on the sustainability of utilising the lignin fraction, in addition to the cellulose and hemicellulose biomass fractions, to produce high value bioproducts. If the environmental impact of coal supplementation is low enough, the valorization of lignin may further increase the profitability.

Although this may result in a number of potential trade-off studies, the indicators listed in the MCDA tool may also assist the researcher or process engineer to *design* sustainably, rather than measuring a scenario's sustainability afterwards. Moreover, the MCDA tool could be expanded by including more social sustainability criteria and refine the techno-economic and environmental indicators based on communication with relevant stakeholders such as sugarcane farmers, potential employees, local communities and sugar industry experts. Further development of the social sustainability indicator is especially important for developing

countries such as South Africa, where social upliftment and eradication of poverty might receive a higher representative weighting than for a developed country.

The techno-economic indicator could also be expanded to include IRR or NPV brackets for indicating when a scenario becomes viable by taking the project risk into account, since a profitable scenario may not necessarily indicate a viable option. For a high risk scenario, a larger IRR or NPV may be desired by investors before it can be considered viable. Therefore, this IRR or NPV 'bracket' can be developed by taking the technical maturity (or TRL) and process complexity into account. This may further increase the robustness of the MCDA tool for selecting sustainable biorefinery scenarios for future research and implementation.

7.2 Summary of recommended work for future research

This study identified various potential biorefinery scenarios and assessed them according to their sustainability, which can be used for internal decision making on which bioproducts to include for future work. To improve the techno-economics, add additional scenarios and move towards the commercial application of the respective scenarios, the following is recommended:

- Validate the simulation outcome and techno-economic results by experimental confirmation (preferably through a pilot scale study) on the STEX with enzymatic hydrolysis pretreatment method for succinic acid production from sugarcane bagasse and trash.
- Conduct a research study to find the most suitable pretreatment method for the multiproduct plant (co-producing succinic acid, PHB and electricity) and then validate the simulation performance (e.g. fermentation yield, productivity and titre) through experimental work.
- Extend the simulation work to investigate the techno-economic outcomes of coal supplemented biorefinery scenarios for succinic acid with electricity production and the multiproduct plant (succinic acid, PHB and electricity) scenario, where all the available biomass (65 t_{DM}/h or 0% bypass) is used as feedstock to produce bioproducts.
- Conduct research work to determine the techno-economic outcome of a PHB biorefinery using simple sugar substrates (1G or 1G/2G), so that no or very little feedstock pretreatment is required prior to fermentation. This biorefinery is annexed to an existing sugar mill and integrated with a CHP plant utilising sugarcane lignocelluloses for energy production.

- This can be extended to any other high value biobased products that can be produced from sucrose, sugar cane juice or molasses as feedstock.
- The production- or feedstock rate should be selected while taking the impact of supply and demand on the process economics into consideration. For example, a new selling price can be determined for a selected market, the production rate can be chosen as to not impact the selling price, or the production rate can be chosen to allow the product to enter into a new or existing market.
- Utilising the 1G or 1G/2G sugarcane feedstock can also be applied for a multiproduct biorefinery, based on favourable economic and/or environmental biobased product combinations.

- Investigate more multiproduct plant options: Conduct research work to investigate and determine potential combinations of biobased products that can be produced in a biorefinery from sugarcane bagasse and trash. This could be done to maximise favourable techno-economic or/and environmental results.
  - The resulting biorefinery scenarios can be simulated to obtain the mass and energy balances. This can be done for a coal supplemented and/or bioenergy self-sufficient scenario.
  - The techno-economic outcome and environmental impact of the resulting scenarios could be determined.
  - The input values of the pretreatment and conversion processing steps of the most sustainable scenario(s) could be validated through experimental work.
- Conduct an experimental investigation on the efficient integration of succinic acid and ethanol fermentation for CO_2 use. It is recommended that the mechanism should be developed in which the CO_2 produced during ethanol fermentation can be successfully used in succinic acid fermentation without severely impacting the succinic acid conversion performance, such as yield.
- Investigate the sustainability of a biorefinery where lignin is valorised, and not only burned for energy production.
  - In this case, the sodium hydroxide pretreatment method (NaOH) may be more favourable due to the separation of lignin from the resultant simple sugars.
  - Lignin valorisation can be included in a multiproduct plant with other bioproducts such as succinic acid and PHB.

- An LCA can be performed to determine and understand the environmental impact of coal supplementation, so that lignin may be valorised and not burned for providing the biorefinery and sugar mill with energy. Instead of coal supplementation, the valorisation of lignin might justify the capital expenditure of investing in solar energy to provide the biorefinery and sugar mill's energy needs.

- Conduct a research project on improving the MCDA tool through communication with the relevant stakeholders, with the focus on expanding the social sustainability indicator specific to developing countries such as South Africa. Moreover, existing sugarcane biorefineries could be added to the list of scenarios investigated in Chapter 6.

As more sugarcane biorefinery scenarios are developed they can be included in the MCDA tool for screening and internal decision making required to identify and select the most sustainable solution for the South African sugar industry. By fully utilising the entire sugarcane plant, the sugar industry and farmers can be better prepared to face natural and economic challenges for the next 25 years.

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A1. Appendix A: Summary of profitable biorefinery scenarios

The internal rate of return (IRR), net present value (NPV), total capital investment (TCI) and total cost of production (TCOP) are summarized in Table A-1 below for the profitable scenarios. The profitability was determined using a real term discounted cash flow rate of return analysis for a discount rate of 9.7%.

Table A-1: Economic analysis results for the profitable biorefinery scenarios (IRR% ≥ 9.7%)

Profitable Biorefinery Scenarios	IRR (%)	NPV (million US$)	TCI (million US$)	TCOP (million US$)
Itaconic acid				
Itaconic acid and electricity co-production, with coal supplemented CHP plant	MRSP: 1740 US$/t	0	662.9	47.77
Succinic acid and PHB co-production				
100% glucose stream sent to PHB production, with the hemicellulose hydrolysate sent to succinic acid production,	12.50%	73.85	342.71	34.24
75% glucose split to PHB production, with 25% glucose and hemicellulose hydrolysate to succinic acid production,	14.80%	143.9	351.06	32.31
50% glucose split to PHB production, with 50% glucose and hemicellulose hydrolysate to succinic acid production,	16.70%	201.37	340	33.3
25% glucose split to PHB production, with 75% glucose and hemicellulose hydrolysate to succinic acid production	24.10%	447.25	352.2	33.16
Succinic acid				
Succinic acid and electricity co-production (ESS) (Dilute acid pretreatment with enzymatic hydrolysis)	21.60%	352.03	344.5	32.71
Electricity				
Electricity co-production in a CHP stand-alone plant	10.30%	6.1	130.11	13.16
Succinic acid (SA) co-production with alternative pretreatment methods				
Dilute acid pretreatment without enzymatic hydrolysis (EH)	17.43%	150.47	236.3	32.4
Alkaline delignification (NaOH)	12.27%	47.85	238.6	26
Ammonium Fibre Expansion (AFEX™) pretreatment with (EH)	21.33%	644.97	384.3	37.6
Steam explosion (STEX) pretreatment with EH	28.04%	644.97	384.2	37.6
SO_2 catalysed steam explosion (STEX with SO_2) pretreatment with EH	26.94%	106.27	258.3	32.6
STEX with subsequent alkaline delignification (STEX with NaOH) pretreatment with EH	14.82%	106.27	258.3	32.6

A2. Appendix B: Chapter 3 Supplementary information

Supplementary Information

Process design and economic analysis of a biorefinery co-producing itaconic acid and electricity from sugarcane bagasse and trash lignocelluloses

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The Aspen Plus® process flow diagrams PA-100 (Pretreatment, detoxification and enzymatic hydrolysis) and PA-400 (Evaporation and crystallisation downstream recovery) have been included in the supplementary information.

The PFD for PA-100 is provided in Figure S1 and the PFD for PA-400 is provided in Figure S2. The associated Aspen Plus® units' descriptions, operating conditions, electricity usage and installed costs are provided in Table S1and S2 for PA-100 and PA-400, respectively.

The mass and energy balances included in the stream tables have been generated for scenario A1. Scenario A2 and A3 follow the same process configuration for the DSP in PA-400, while scenario A3 follow the same pretreatment configuration for pretreatment, detoxification and enzymatic hydrolysis in PA-100. Scenario A2 does not have a pretreatment area (PA-100).

The seed train (PA-200) and fermentation areas (PA-300) are simulated as continuous processes with the *RStoic* Aspen Plus® unit, where the volumetric inlet flows were used to calculate the batch reactor schedule and installed equipment costs. The waste water treatment (WWT) plant in PA-500 and the combined heat and power (CHP) plant in PA-600 and PA-700 have been reported previously (Ali Mandegari et al., 2017; Steinwinder et al., 2011). Detailed PFDs and tables of operating and design conditions can be made available upon request for any plant area.

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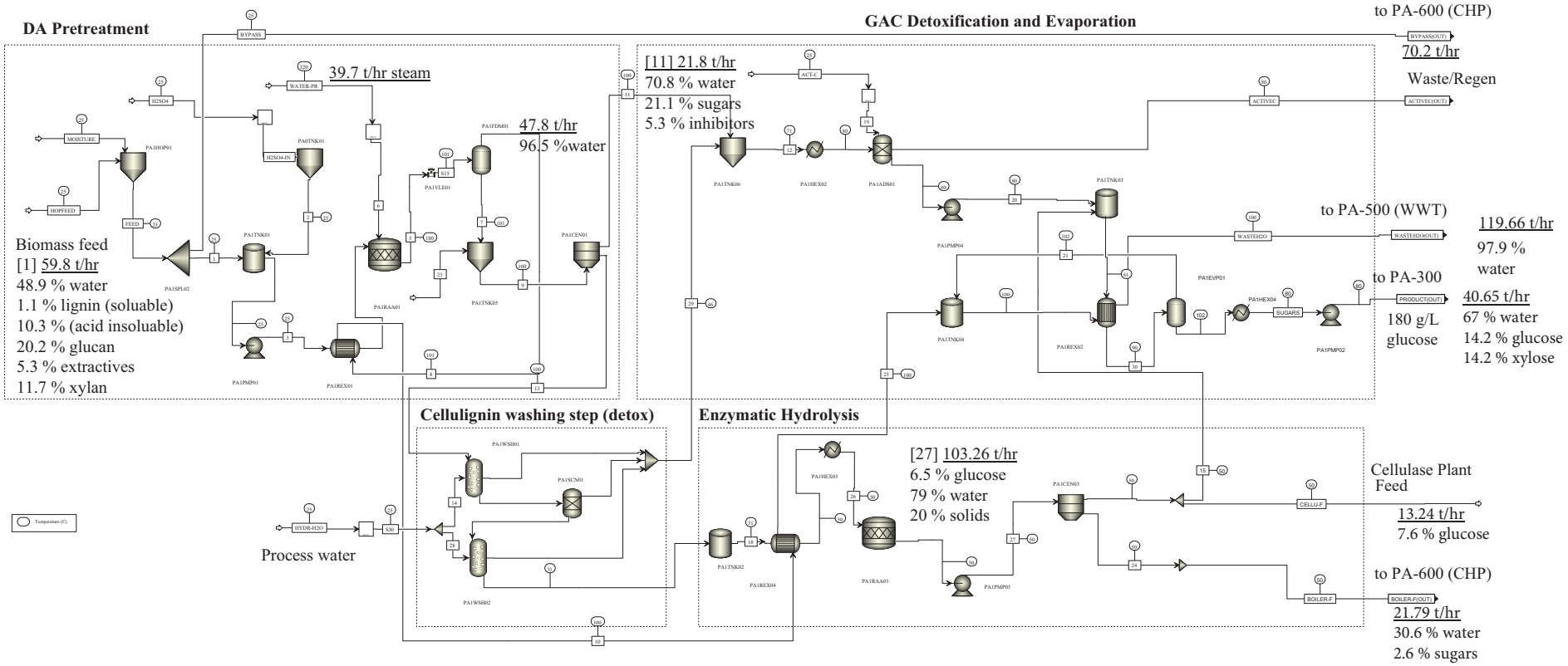
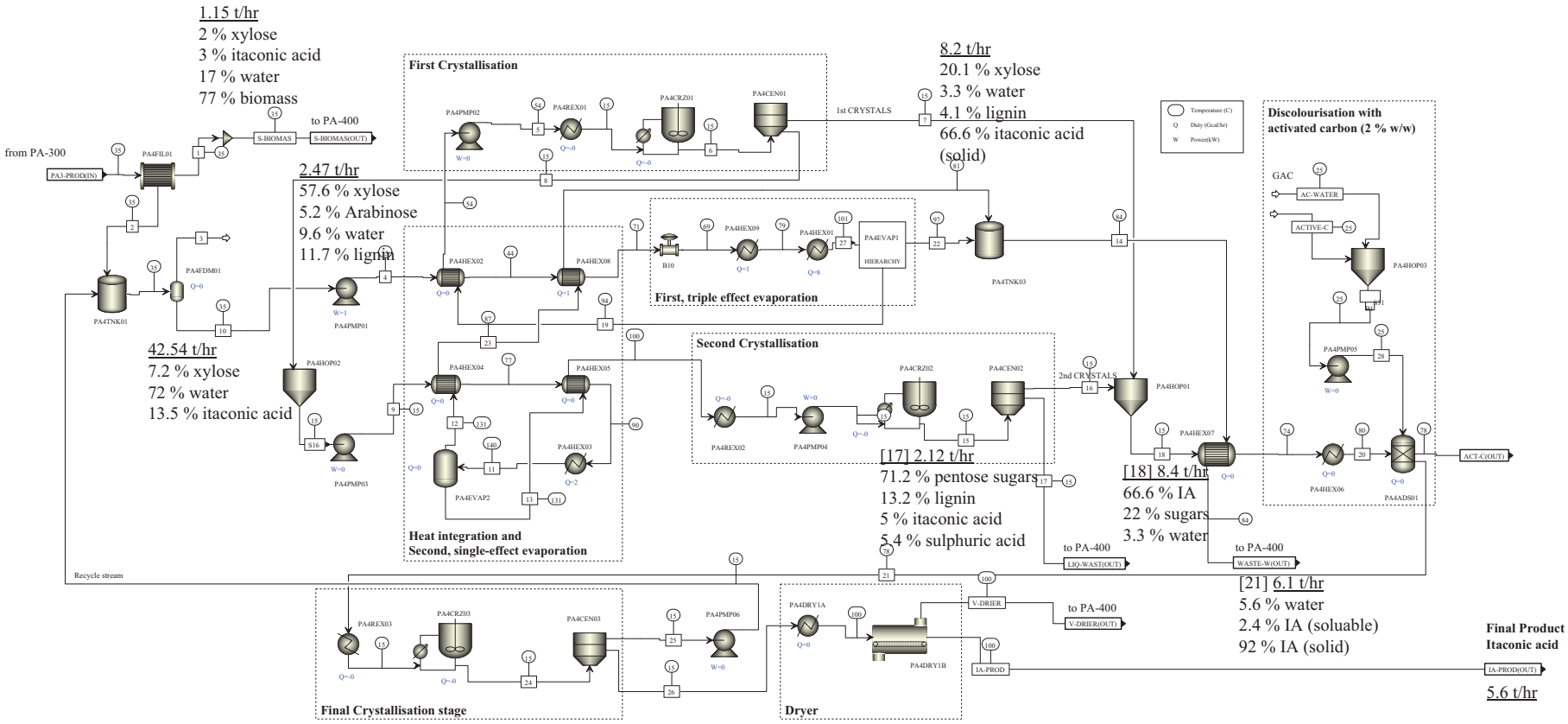


STREAM No.	1	2	3	5	6	7	8	9	10	11	12	13	14	15	16
PA-100	PA1TNK01	PA1TNK01	PA1REX01	PA1VLE01	PA1RAA01	PA1TNK05	PA1REX01	PA1CEN01	PA1REX04	PA1TNK06	PA1HEX02	PA1WSH01	PA1WSH01	PA1TNK03	PA1WSH02
	PA1SPL02	PA0TNK01	PA1PMP01	PA1RAA01	ACIDW-M	PA1FDM01	PA1FDM01	PA1TNK05	PA1REX01	PA1CEN01	PA1TNK06	PA1CEN01	PA1SPL01	B2	PA1SCN01
	LIQUID	LIQUID	LIQUID	MIXED	VAPOR	LIQUID	VAPOR	LIQUID	MIXED	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID
Substream: MIXED															
Mass Flow kg/hr															
Glucose	0	0	0	579.5	0	579.5	0.0	579.5	0.0	393.3	561.2	186.2	0	5202.7	18.4
Xylose	0	0	0	5841.0	0	5841.0	0.0	5841.0	0.0	3964.2	5656.8	1876.8	0	141.7	185.7
Arabinose	0	0	0	335.7	0	335.7	0.0	335.7	0.0	227.8	325.1	107.9	0	8.1	10.7
Cellobiose	0	0	0	38.4	0	38.4	0.0	38.4	0.0	26.1	37.2	12.3	0	43.7	1.2
Furfural	0	0	0	409.1	0	317.7	91.4	317.7	91.4	215.6	307.7	102.1	0	7.7	10.1
HMF	0	0	0	28.3	0	28.3	0.0	28.3	0.0	19.2	27.4	9.1	0	0.7	0.9
Acetic Acid	0	0	0	2951.2	0	1355.0	1596.2	1355.0	1596.2	919.6	1312.2	435.4	0	32.9	43.1
Water	29257.2	0	29257.2	68048.3	39725.86	21939.7	46109.8	22739.7	46109.8	15433.2	35649.9	7306.5	31422.6	62771.8	19158.0
Soluable lignin	642.9	0	642.9	642.9	0	642.2	0.6	642.2	0.6	435.9	622.0	206.3	0	15.6	20.4
Sulphuric acid	0	259.1	259.1	259.1	0	259.1	0.1	259.1	0.1	175.8	250.9	83.2	0	6.3	8.2
Activated carbon	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Flow kg/hr	29900.0	259.1	30159.1	79133.4	39725.9	31336.5	47798.2	32136.5	47798.2	21810.7	44750.3	10325.8	31422.6	68231.1	19456.7
Total Flow l/min	495.6	2.4	497.8	134348.0	184655.0	469.2	1315890.0	482.5	1192020.0	327.5	690.4	155.0	525.2	1065.3	325.3
Temperature K	298.2	298.2	298.2	453.3	593.2	374.6	374.6	372.9	373.4	372.9	343.9	372.9	298.2	323.6	319.5
Pressure atm	1	1	2	9.50	9.5	1	1	1	1	1	1	1	1	2	1
Vapor Frac	0	0	0	0.55	1	0	1	0	0.91	0	0	0	0	0	0
Liquid Frac	1	1	1	0.45	0	1	0	1	0.09	1	1	1	1	1	1
Density gm/cc	1.0	1.8	1.0	0.0	0.0	1.1	0.0	1.1	0.0	1.1	1.1	1.1	1.0	1.1	1.0
Average MW	18.4	98.1	18.5	20.4	18.0	24.2	18.5	24.0	18.5	24.0	21.8	24.0	18.0	19.4	18.3
Total Flow kg/hr	**59800.0**	**259.1**	**60059.1**	**99784.9**	**39725.9**	**51988.0**	**47798.2**	**52788.0**	**47798.2**	**21810.7**	**44750.3**	**30977.3**	**31422.6**	**68231.1**	**40108.2**
Substream: CIPSD															
Mass Flow kg/hr															
Glucan	12128.2	0	12128.2	8877.8	0	8877.8	0	8877.8	0	0	0	8877.8	0	0	8877.8
Extractives	3149.0	0	3149.0	3149.0	0	3149.0	0	3149.0	0	0	0	3149.0	0	0	3149.0
Ash	1051.7	0	1051.7	1051.7	0	1051.7	0	1051.7	0	0	0	1051.7	0	0	1051.7
Lignin	6175.7	0	6175.7	6175.7	0	6175.7	0	6175.7	0	0	0	6175.7	0	0	6175.7
Arabinan	363.8	0	363.8	68.4	0	68.4	0	68.4	0	0	0	68.4	0	0	68.4
Xylan	7031.7	0	7031.7	1329.0	0	1329.0	0	1329.0	0	0	0	1329.0	0	0	1329.0
Total Flow kmol/hr	206.9	0	206.9	141.4	0	141.4	0	141.4	0	0	0	141.4	0	0	141.4
Total Flow kg/hr	29900.0	0	29900.0	20651.5	0	20651.5	0	20651.5	0	0	0	20651.5	0	0	20651.5
Total Flow l/min	255.4	0	255.4	188.6	0	181.3	0	181.2	0	0	0	181.2	0	0	177.4

STREAM No.	18	19	20	21	23	24	25	26	27	28	29	30	BYPASS	CELLU-F	SUGARS	WASTEH2O
PA-100	PA1REX04	PA1ADS01	PA1TNK03	PA1TNK04	PA1TNK05	03-MIXER	PA1TNK04	PA1RAA03	PA1CEN03	PA1WSH02	PA1TNK06	PA1EVP01	$C-4		PA1PMP02	$C-1
	PA1TNK02	ACT-C-M	PA1PMP04	PA1EVP01		PA1CEN03	PA1REX04	PA1HEX03	PA1PMP03	PA1SPL01	B3	PA1REX02	PA1SPL02	B2	PA1HEX04	PA1REX02
	LIQUID	LIQUID	LIQUID	VAPOR	LIQUID	LIQUID	MIXED	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID	MIXED
Substream: MIXED																
Mass Flow kg/hr																
Glucose	18.3	0	561.2	0.0	0	553.8	0.0	18.3	6765.9	0	167.9	5763.9	0	1009.4	5763.9	0.0
Xylose	184.2	0	5656.8	0.1	0	15.1	0.0	184.2	184.2	0	1692.5	5798.4	0	27.5	5798.4	0.1
Arabinose	10.6	0	325.1	0.0	0	0.9	0.0	10.6	10.6	0	97.3	333.2	0	1.6	333.2	0.0
Cellobiose	1.2	0	37.2	0.0	0	4.7	0.0	1.2	56.9	0	11.1	80.9	0	8.5	80.9	0.0
Furfural	10.0	0	30.8	10.1	0	0.8	91.4	10.0	10.0	0	92.1	38.5	0	1.5	28.3	101.5
HMF	0.9	0	2.7	0.0	0	0.1	0.0	0.9	0.9	0	8.2	3.4	0	0.1	3.4	0.0
Acetic Acid	42.7	0	1312.2	799.1	0	3.5	1596.2	42.7	42.7	0	392.6	1345.1	0	6.4	546.0	2395.4
Water	82309.8	0	35486.3	71054.5	800	6681.8	46109.8	82309.8	81632.1	63797.4	20216.7	98258.0	34345.4	12178.5	27203.5	117164.0
Soluable lignin	20.3	0	622.0	0.8	0	1.7	0.6	20.3	20.3	0	186.1	637.5	754.7	3.0	636.7	1.4
Sulphuric acid	8.2	0	250.9	0.1	0	0.7	0.1	8.2	8.2	0	75.1	257.2	0	1.2	257.1	0.1
Activated carbon	0	834.1272	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0	0.0
Total Flow kg/hr	82606.1	834.1	44285.1	71864.7	800.0	7262.9	47798.2	82606.1	88731.8	63797.4	22939.6	112516.0	35100.0	13237.7	40651.6	119663.0
Total Flow l/min	1380.6	4.9	686.9	2013640.0	13.4	113.4	1105100.0	1391.0	1385.4	1066.3	364.6	1778.4	581.8	206.7	533.7	2954740.0
Temperature K	303.8	298.2	352.7	374.7	308.2	323.6	373.4	323.2	323.6	298.2	319.0	363.3	298.2	323.6	353.2	373.3
Pressure atm	1	1	3.99	1	1	2	1	1.48	2	1	1	1	1	2	1	1.00
Vapor Frac	0	0	0	1	0	0	0.84	0	0	0	0	0	0	0	0	0.89
Liquid Frac	1	1	0	0	1	1	0.16	1	1	1	1	1	1	1	1	0.11
Density gm/cc	1.0	2.9	1.1	0.0	1.0	1.1	0.0	1.0	1.1	1.0	1.0	1.1	1.0	1.1	1.3	0.0
Average MW	18.1	12.0	21.7	18.2	18.0	19.4	18.5	18.1	19.4	18.0	20.0	20.2	18.4	19.4	25.4	18.3
Total Flow kg/hr	**103258.0**	**834.1**	**44285.1**	**71864.7**	**800.0**	**21788.8**	**47798.2**	**103258.0**	**103258.0**	**63797.4**	**22939.6**	**112516.0**	**70200.0**	**13237.7**	**40651.6**	**119663.0**
Substream: CIPSD																
Mass Flow kg/hr																
Glucan	8877.8	0	0	0	0	2752.1	0	8877.8	2752.1	0	0	0	14237.4	0	0	0
Extractives	3149.0	0	0	0	0	3149.0	0	3149.0	3149.0	0	0	0	3696.6	0	0	0
Ash	1051.7	0	0	0	0	1051.7	0	1051.7	1051.7	0	0	0	1234.6	0	0	0
Lignin	6175.7	0	0	0	0	6175.7	0	6175.7	6175.7	0	0	0	7249.7	0	0	0
Arabinan	68.4	0	0	0	0	68.4	0	68.4	68.4	0	0	0	427.1	0	0	0
Xylan	1329.0	0	0	0	0	1329.0	0	1329.0	1329.0	0	0	0	8254.6	0	0	0
Total Flow kmol/hr	141.4	0	0	0	0	103.6	0	141.4	103.6	0	0	0	242.9	0	0	0
Total Flow kg/hr	20651.5	0	0	0	0	14525.9	0	20651.5	14525.9	0	0	0	35100.0	0	0	0
Total Flow l/min	176.4	0	0	0	0	160.7	0	177.6	160.7	0	0	0	299.8	0	0	0

Figure S1: Pretreatment, enzymatic hydrolysis and detoxification PA-100 PFD



STREAM No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
PA-400	B2	PA4TNK01		PA4HEX02	PA4REX01	PA4CEN01	PA4HOP01	PA4HOP02	PA4HEX04	PA4PMP01	PA4EVAP2	PA4HEX04	PA4HEX03	PA4HEX07	PA4CEN02
	PA4FIL01	PA4FIL01	PA4FDM01	PA4PMP01	PA4PMP02	PA4CRZ01	PA4CEN01	PA4CEN01	PA4PMP03	PA4FDM01	PA4HEX03	PA4EVAP2	PA4EVAP2	PA4TNK03	PA4CRZ02
	MIXED	MIXED	VAPOR	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID	MIXED	VAPOR	LIQUID	MIXED	LIQUID
Substream: MIXED															
Mass Flow kg/hr															
Xylose	19.3	3076.2	0.0	3076.2	3075.8	3075.8	1652.3	1423.5	1423.5	3076.2	1423.5	0.0	1423.5	0.5	1423.5
Arabinose	1.8	279.5	0.0	279.5	279.5	279.5	150.1	129.3	129.3	279.5	129.3	3.33E-03	129.3	0.0	129.3
Furfural	0.2	28.2	0.0	28.2	7.1	7.1	3.8	3.3	3.3	28.2	3.3	0.7	2.5	21.9	2.5
HMF	0.0	3.4	0.0	3.4	3.3	3.3	1.8	1.5	1.5	3.4	1.5	0.0	1.5	0.1	1.5
Acetic Acid	3.4	542.2	6.21E-04	542.2	25.8	25.8	13.9	11.9	11.9	542.2	11.9	6.1	5.8	522.5	5.8
Water	191.0	30439.4	0.1	30610.9	511.6	511.6	274.8	236.8	236.8	30610.9	236.8	157.4	79.3	30256.8	79.3
Carbon dioxide	6.1	971.5	0.4	971.1	0.0	0.0	0.0	0.0	0.0	971.1	0.0	0.0	0.0	971.1	0.0
Soluable lignin	4.0	632.8	0.0	632.8	624.0	624.0	335.2	288.8	288.8	632.8	288.8	0.6	288.2	9.3	288.2
Micro-organism	883.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
α-keto-glucaric acid	0.2	25.9	0.0	25.9	24.7	24.7	13.3	11.4	11.4	25.9	11.4	0.1	11.4	1.3	11.4
Succinic acid	0.1	12.8	0.0	13.2	13.2	13.2	7.1	6.1	6.1	13.2	6.1	1.07E-03	6.1	0.0	6.1
Malic acid	0.0	5.9	0.0	6.1	6.1	6.1	3.3	2.8	2.8	6.1	2.8	0.0	2.8	7.29E-04	2.8
Itaconic acid	36.0	5733.7	0.0	5751.1	5744.4	271.1	145.6	125.5	125.5	5751.1	125.5	0.0	125.4	6.8	108.8
Sulphuric acid	1.6	255.5	0.0	255.5	254.6	254.6	136.8	117.8	117.8	255.5	117.8	0.1	117.8	1.0	117.8
Ammonia	0.2	24.4	8.36E-04	24.4	1.29E-03	1.29E-03	6.95E-04	5.99E-04	5.99E-04	24.4	5.99E-04	5.76E-04	2.28E-05	24.4	0.0
Oxygen	1.3	203.5	1.4	202.1	0.0	0.0	0.0	0.0	0.0	202.1	0.0	0.0	0.0	202.1	0.0
Nitrogen	1.57E-03	0.3	4.40E-03	0.2	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0	0	0.2	0
Activated carbon	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Flow kmol/hr	47.2	1802.3	0.1	1811.9	102.4	60.3	32.4	27.9	27.9	1811.9	27.9	8.9	19.1	1718.4	18.9
Total Flow kg/hr	1148.6	42235.2	1.9	42422.9	10570.0	5096.8	2737.9	2358.9	2358.9	42422.9	2358.9	165.1	2193.7	32018.0	2177.0
Total Flow l/min	90.3	655.0	24.1	653.1	113.9	47.6	25.5	22.0	22.0	653.1	4221.6	8090.6	20.8	3.73E+05	19.1
Temperature K	308.2	308.2	308.1	308.1	327.7	288.2	288.2	288.2	288.2	308.1	413.2	402.3	402.3	357.8	288.2
Pressure atm	1	1	1.0	1.5	1.5	1	1	1	1.5	1.0	1	0.6	0.6	0.8	1
Vapor Frac	0.0	0.0	1	0	0	0	0	0	0	0	0.3	1	0	0.3	0
Liquid Frac	0.2	1.0	0	1	1	1	1	1	1	1	0.7	0	1	0.7	1
Density gm/cc	0.2	1.1	1.30E-03	1.1	1.5	1.8	1.8	1.8	1.8	1.1	9.31E-03	3.40E-04	1.8	1.43E-03	1.9
Average MW	24.3	23.4	33.2	23.4	103.2	84.5	84.5	84.5	84.5	23.4	84.5	18.7	115.1	18.6	115.0
Total Flow kg/hr	**1148.6**	**42235.2**	**1.9**	**42537.2**	**10684.4**	**10684.4**	**8213.7**	**2470.6**	**2470.6**	**42537.2**	**2470.6**	**165.1**	**2305.5**	**32018.0**	**2305.5**
Substream: CIPSD															
Mass Flow kg/hr															
Biomass cells	3.69E-04	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Itaconic acid (solid)	0	0	0	114.3	114.3	5587.6	5475.8	111.8	111.8	114.3	111.8	0	111.8	0	128.4
Total Flow kmol/hr	1.50E-05	0	0	0.9	0.9	42.9	42.1	0.9	0.9	0.9	0.9	0	0.9	0	1.0
Total Flow kg/hr	3.69E-04	0	0	114.3	114.3	5587.6	5475.8	111.8	111.8	114.3	111.8	0	111.8	0	128.4
Total Flow l/min	3.60E-05	0	0	1.3	1.3	62.5	61.2	1.2	1.2	1.3	1.2	0	1.2	0	1.4

STREAM No.	16	17	18	19	20	21	22	23	24	25	26	27	28	IA-PROD	V-DRIER
PA-400	PA4HOP01	$C-31	PA4HEX07	PA4HEX02	PA4ADS01	PA4REX03	PA4TNK03	PA4HEX08	PA4CEN03	PA4PMP06	PA4DRY1A	$C-25	PA4ADS01	$C-27	$C-5
	PA4CEN02	PA4CEN02	PA4HOP01	$C-28	PA4HEX06	PA4ADS01	$C-18	PA4HEX04	PA4CRZ03	PA4CEN03	PA4CEN03	PA4HEX01	PA4PMP05	PA4DRY1B	PA4DRY1B
	LIQUID	LIQUID	LIQUID	MIXED	LIQUID	LIQUID	MIXED	MIXED	LIQUID	LIQUID	LIQUID	MIXED	LIQUID	SOLID	LIQUID
Substream: MIXED															
Mass Flow kg/hr															
Xylose	41.1	1382.3	1693.4	3075.8	1693.4	0	0.5	0.0	0	0	0	0	3076.2	0	0
Arabinose	3.7	125.6	153.9	279.5	153.9	0	0.0	3.33E-03	0	0	0	0	279.5	0	0
Furfural	0.1	2.4	3.9	7.1	3.9	0	21.1	0.7	0	0	0	0	28.2	0	0
HMF	0.0	1.5	1.8	3.3	1.8	0	0.1	0.0	0	0	0	0	3.4	0	0
Acetic Acid	0.2	5.6	14.0	25.8	14.0	0	516.4	6.1	0	0	0	0	542.2	0	0
Water	2.3	77.1	277.1	511.6	277.1	343.1	30099.3	157.4	343.1	171.6	171.6	30610.9	151.8	0	171.6
Carbon dioxide	0.0	0.0	0.0	0.0	0.0	0	971.1	0.0	0	0	0	971.1	0	0	0
Soluable lignin	8.3	279.9	343.6	624.0	343.6	0	8.7	0.6	0	0	0	632.8	0	0	0
Micro-organism	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
α-keto-glucaric acid	0.3	11.0	13.6	24.7	13.6	0	1.2	0.1	0	0	0	25.9	0	0	0
Succinic acid	0.2	5.9	7.3	13.2	7.3	0.7	0.0	1.07E-03	0.7	0.4	0.4	13.2	0	0	0.4
Malic acid	0.1	2.7	3.4	6.1	3.4	0.3	6.69E-04	5.97E-05	0.3	0.2	0.2	6.1	0	0	0.2
Itaconic acid	3.1	105.6	148.8	5744.4	148.8	148.8	6.8	0.0	35.0	17.5	17.5	5751.1	0	0	17.5
Sulphuric acid	3.4	114.3	140.2	254.6	140.2	0	0.9	0.1	0	0	0	255.5	0	0	0.0
Ammonia	0.0	2.21E-05	6.96E-04	1.29E-03	6.96E-04	0	24.4	5.76E-04	0	0	0	24.4	0	0	0
Oxygen	0.0	0.0	0.0	0.0	0.0	0	202.1	0.0	0	0	0	202.1	0	0	0
Nitrogen	0	0	0.0	0.0	0.0	0	0.2	0	0	0	0	0.2	0	0	0
Activated carbon	0	0	0	0	0	0	0	0	0	0	0	0	39.1	0	0
Total Flow kmol/hr	0.5	18.4	32.9	102.4	32.9	20.2	1709.5	8.9	19.3	9.7	9.7	1811.9	11.7	0	9.7
Total Flow kg/hr	62.9	2114.1	2800.8	10570.0	2800.8	493.0	31852.9	165.1	379.2	189.6	189.6	42422.9	190.9	0	189.6
Total Flow l/min	0.6	18.5	26.1	119.9	27.1	7.7	2.17E+05	1603.5	6.1	3.1	3.1	4.35E+05	2.8	0	3.2
Temperature K	288.2	288.2	288.2	396.0	353.2	350.7	370.9	359.6	288.2	288.2	288.2	374.5	298.2		373.2
Pressure atm	1	1	1	0.6	1	1	1.0	0.6	1.0	1	1	1	1.2		1.0
Vapor Frac	0	0	0	0.0	0	0	0.2	0.2	0	0	0	0.5	0		0
Liquid Frac	1	1	1	1	1	1	0.8	0.8	1	1	1	0.5	1		1
Density gm/cc	1.9	1.9	1.8	1.5	1.7	1.1	2.45E-03	1.72E-03	1.0	1.0	1.0	1.63E-03	1.2		1.0
Average MW	115.0	115.0	85.0	103.2	85.0	24.4	18.6	18.7	19.6	19.6	19.6	23.4	16.3		19.6
Total Flow kg/hr	**188.8**	**2116.7**	**8402.5**	**10684.4**	**8402.5**	**6094.7**	**31852.9**	**165.1**	**6094.7**	**303.9**	**5790.8**	**42537.2**	**190.9**	**5601.2**	**189.6**
Substream: CIPSD															
Mass Flow kg/hr															
Biomass cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Itaconic acid (solid)	125.8575	2.6	5601.7	114.3092	5601.7	5601.7	0	0	5715.5	114.3	5601.2	114.3	0	5601.2	0
Total Flow kmol/hr	1.0	0.0	43.1	0.9	43.1	43.1	0	0	43.9	0.9	43.1	0.9	0	43.1	0
Total Flow kg/hr	125.9	2.6	5601.7	114.3	5601.7	5601.7	0	0	5715.5	114.3	5601.2	114.3	0	5601.2	0
Total Flow l/min	1.4	0.0	62.7	1.3	62.7	62.7	0	0	63.9	1.3	62.7	1.3	0	62.7	0

Figure S2: Downstream recovery PA-400 (Evaporation and Crystallisation) PFD

Table S1: Equipment unit specifications and installed cost for PA-100

PA-100 Pretreatment, detoxification and enzymatic hydrolysis					
UNIT TAG	**DESCRIPTION**	**OPERATING CONDITIONS or SPECIFICATIONS**	**DESIGN COMMENT**	**ELECTRICITY**	**INSTALLED COST**
DA Pretreatment				kW	US$, 2016
PA1-REX-01	Pretreatment feed stream recovery heat exchanger	Model fidelity: Shortcut Shortcut flow direction: Countercurrent Calculation mode: Design Specification: Hot / cold outlet temperature approach Value: 10 K	Preheats feed stream no. 3 from 25 °C to 90 °C with pretreatment product waste stream no. 8 (101 °C to 100 °C)	-	116 900
PA1-RAA-01	Jacketed screw reactor	Pressure: 9.5 atm Duty: 0 cal/sec Reactions: see Table 1	Reactor is heated to the desired value of 320 °C using high pressure steam.	7	319 700
PA1-VLE-01	Pressure relieve valve	Outlet pressure: 1 atm			
PA1-FDM-01	Depressure flash drum	Pressure: 1 atm Duty: 0 cal/sec	Acetic acid and water is removed from the product stream via the vapour phase	-	193 600
PA1-CEN-01	Auto batch centrifuge	Solids separator Liquid load of solid outlet: 50 % Fraction of solids to solid outlet: 1 Pressure: 1 atm	Separates the hemicellulose hydrolysate from the cellulignin (WIS - water insoluable solids)	29.7	2 108 800
GAC Detoxification and Evaporation					
PA1-HEX-02	Heat exchanger	Temperature: 80 °C Pressure: 1 atm Utility: High pressure steam (HHP)	Heats hydrolysate and cellulignin wash water for GAC adsorption column	-	66 000
PA1-ADS-01	GAC Adsoprtion column	Removes 0.9 (split fraction) of furfural, HMF, Phenols, 0.00459 water and 1 carbon to the carbon stream outlet	Separation unit used in model, based on separation values used in literature, and costed as 2 adsorption columns: PA1-ADS-01 and PA1-ADS-02	-	345 000
PA1-REX-02	Recovery heat exchanger	Model fidelity: Shortcut Shortcut flow direction: Countercurrent Calculation mode: Design Specification: Hot / cold outlet temperature approach Value: 10 K	Preheats single effect evaporation feed stream no. 30 from 61 °C to 90 °C with evaporation product waste stream no. 21 (100 °C to 100 °C)	-	67 600
PA1-EVP-01	Single effect evaporator	Pressure: 0 atm Vapor fraction: 0.7122 Utility: HHP	Vapor fraction is varied until 180 g/L glucose is achieved in the SUGARS product stream	-	208 900
PA1-HEX-04	Heat exchanger	Temperature: 80 °C Pressure: 0 atm Utility: Cooling water (COOLW)	Reduced the temperature form 102 °C to 80 °C for safer transport of liquids	-	61 700
Cellulignin washing step					
PA1-WSH-01	Cellulignin washing station	Swash unit Liquid to solid mass ratio: 2		14	369 900
PA1-SCN-01		Sep unit Removes 0.9 split fraction of all MIXED stream components, except for 0.5 for water. No removal of CIPSD stream components	The removed components is mixed with the hemicellulose hydrolysate prior to GAC detoxification		
PA1-WSH-02	Enzymatic hydrolysis dilution	Swash unit Liquid to solid mass ratio: 4	Enzymatic hydrolysis feed stream is diluted to 20 %wt solids	-	122 400
Enzymatic hydrolysis					
PA1-REX-04	Recovery heat exchanger	Model fidelity: Shortcut Shortcut flow direction: Countercurrent Calculation mode: Design Specification: Cold stream outlet temperature Value: 50 °C	Waste stream no. 10 from the DA pretreatment step is used to preheat the EH feed stream no. 18 from 31 °C to 50 °C while the waste stream remains 100 °C	-	76 300
PA1-HEX-03	EH feed stream heat exchanger	Temperature: 50 °C Pressure: 1.5 atm Utility: HHP	Required during plant start-up, (and scenario initiation) before recycle streams are available for use in PA1-REX-04	-	58 100
PA1-RAA-03	Enzymatic hydrolysis reactos	Pressure: 1 atm Duty: 0 cal/sec Reactions: see Table 1	More than 1 EH reactor is required, based on the volumetrc flow rate and 72 hours residence tme, and therefore the EH reactor train is costed (x11 tanks)	70	57 262 137
PA1-CEN-03	Cellulignin solid bowl centrifuge	Decanter Ideal separation Residual moisture: 50 % (dry basis)	Removes glucose from residual cellulignin	6.6	898 900
Minor equipment					
PA1-PMP-01 PA1-PMP-02 PA1-PMP-03 PA1-PMP-04	Pumps	PA1-PMP-01/02/03: Discharge pressure: 2 atm, 75 % pump and 95 % driver efficiency Utility: Electricity (ELECT1) PA1-PMP-04: 3 atm pressure increase, 85 % pump and 75 % driver efficiency	Required to move liquid from one unit to another	2.4	188 000
PA1-TNK-01 PA1-TNK-02 PA1-TNK-03 PA1-TNK-04 PA1-TNK-05 PA1-TNK-06	Tanks	PA1-TNK-01/02/05/06: no specifications PA1-TNK-03/04: Pressure 1 atn	Used for plant maintenance and start-up/shut-downs, may also serve as buffer capacity Agitated, closed tanks	22.25	1 205 300
PA1-HOP-01	Feed hopper	No specification	Collection of biomass	-	1 934 300
Additional equipment (not modelled in Aspen Plus®, but included for the process flow sheet design)					
PA1-CNV-01/04/05/06 PA1-WWT-01/02 PA1-CNE-01 PA1-DST-01 PA1-PMP-05 PA1-TNK-07/08	Belt feeder/residual lignin conveyor/bypass conveyor/cellulignin screw conveyor Belt scale Overhead crane Dust collection system Sulphuric acid pump Sulphuric acid tank/GAC storage tank			12	1 062 744
TOTAL				163.95	66 666 281

Table S2: Equipment unit specifications and installed cost for PA-400

PA-400 Downstream recovery					
UNIT TAG	**DESCRIPTION**	**OPERATING CONDITIONS or SPECIFICATIONS**	**DESIGN COMMENT**	**ELECTRICITY**	**INSTALLED COST**
General units				kW	US$, 2016
PA4-FIL-01	Biomass tubular filter	Solids separator Liquid load of solid outlet: 30 % Fraction of solids to solid outlet: 1 Pressure: 1 atm	Separates biomass cells from fermentation broth	-	23 700
PA4-FDM-01	Deaeration drum	Pressure: 1 atm Duty: 0 cal/sec	Include to ensure only liquid phase for PA4-PMP-01	-	163 300
PA4-HEX-07	Discolourisation recovery heat exchanger	Model fidelity: Shortcut Shortcut flow direction: Countercurrent Calculation mode: Design Specification: Hot / cold outlet temperature approach Value: 10 K	Energy recovery from evaporation waste stream (85 °C to 85 °C) to heat discoulorisation step feed stream from 15 °C to 75 °C	-	64 100
First, triple-effect evaporation					
PA4-HEX-09	Preheater	Pressure: 0 atm (no change) Temperature: 10 °C increase Utility: High pressure steam (HHP)		-	64 400
PA4-HEX-01	Preheater	Temperature: 101.38 °C Pressure: 0 atm (no change) Utility: High pressure steam (HHP)		-	128 600
PA4-EVAP-01					
E-FDM-01/02/03	Evaporation module	01: Pressure: 0.99 atm (1 bar), 0 duty 02: Pressure: 0.79 atm (0.8 bar), 0 duty 03: Pressure: 0.59 atm (0.6 bar), 0 duty		-	389 400
E-HEX-01/02		01: Pressure: 1 atm, Vapor fraction: 0.5 02: Pressure: 0.79 atm (0.8 bar), heat from E-HEX-01		-	415 500
E-HEX-03/04		03: Pressure: 0.79 atm (0.8 bar), Vapor fraction: 0 04: Pressure: 0.59 atm (0.6 bar), heat from E-HEX-03		-	439 900
E-HEX-05		05: Pressure: 0.99 atm (1 bar), Vapor fraction: 0		-	-
E-PMP-02		Discharge pressure: 1.48 atm with 90 % pump and 70 % driver efficiency Utility: Electricity (ELECT1)		0.65	33 500
E-TNK-01		No specification		3	152 360
First Crystallisation					
PA4-REX-01	Crystalliser feed cooler	Temperature: 15 °C Pressure: 0 atm (no change) Utility: Chilled water (CHILLW)		-	69 300
PA4-CRZ-01	First Oslo Crystalliser	Temperature: 15 °C Pressure: 1 atm Components: Itaconic acid (MIXED) to solid (CIPSD) ITACO-01 --> ITACO-02 (CIPSD) Solubility: Concentration, 295 K for 95 gm/L PSD based on literature		-	1 044 700
PA4-CEN-01	Centrifuge	Solids separator Liquid load of solid outlet: 50 % Fraction of solids to solid outlet: 0.98 Pressure: 1 atm		13.2	527 000
Heat integration and Second, single-effect evaporation					
PA4-HEX-04/05	Recovery heat exchangers for PA4-EVAP-02	Model fidelity: Shortcut Shortcut flow direction: Countercurrent Calculation mode: Design Specification: Hot / cold outlet temperature approach Value: 10 K	PA4-HEX-04 uses EVAP-02 vapour outlet (from 129 °C to 86 °C) to preheat stream no. 9 from 15 °C to 76 °C and then PA4-HEX-05 further heats to 97 °C using EVAP-02's liquid stream (129 °C to 107 °C)	-	61 000
				-	54 100
PA4-HEX-02/08	Recovery heat exchangers for PA4-EVAP-01	Model fidelity: Shortcut Shortcut flow direction: Countercurrent Calculation mode: Design Specification: Hot / cold outlet temperature approach Value: 10 K	PA4-HEX-02 uses EVAP-01 liquid outlet (from 123 °C to 55 °C) to preheat stream no. 4 from 35 °C to 45 °C	-	61 600
				-	45 500
PA4-HEX-03	Evaporator preheater	Temperature: 140 °C Pressure: 1 atm Utility: High pressure steam (HHP)		-	66 600
PA4-EVAP-02	Single-effect evaporator	Pressure: 0.6 atm Duty: 0 cal/sec		-	118 100
Second Crystallisation					
PA4-REX-02	Crystalliser feed cooler	Temperature: 15 °C Pressure: 0 atm (no change) Utility: Chilled water (CHILLW)		-	60 300
PA4-CRZ-02	Second Oslo Crystalliser	Temperature: 15 °C Pressure: 1 atm Components: Itaconic acid (MIXED) to solid (CIPSD) ITACO-01 --> ITACO-02 (CIPSD) Solubility: Concentration, 295 K for 95 gm/L		-	111 400
PA4-CEN-02	Centrifuge	Solids separator Liquid load of solid outlet: 50 % Fraction of solids to solid outlet: 0.98 Pressure: 1 atm	Removes crystals from liquid phase	3.3	158 400
Discoulorisation with activated carbon					
PA4-HEX-06	Heat exchanger	Temperature: 80 °C Pressure: 1 atm Utility: High pressure steam (HHP)		-	49 900
PA4-ADS-01	Granular activated carbon (GAC) adsorption column	Sep unit, removes 100 % of all MIXED constituents, except 99 % of remaining glucose, 90 % of remaining succinic and malic acid 20 % of remaining water, 0 % of itaconic acid		-	102 100
Final Crystallisation stage and Dryer					
PA4-REX-03	Crystalliser feed cooler	Temperature: 15 °C Pressure: 0 atm (no change) Utility: Chilled water (CHILLW)		-	59 900
PA4-CRZ-03	Final Oslo Crystalliser	Temperature: 15 °C Pressure: 1 atm Components: Itaconic acid (MIXED) to solid (CIPSD) ITACO-01 --> ITACO-02 (CIPSD) Solubility: Concentration, 295 K for 95 gm/L		-	1 058 500
PA4-CEN-03	Centrifuge	Solids separator Liquid load of solid outlet: 50 % Fraction of solids to solid outlet: 0.98 Pressure: 1 atm	Removes crystals from liquid phase	6.6	229 100
PA4-DRY-1A	Dryer preheater	Temperature: 100 °C Pressure: 1 atm Utility: High pressure steam (HHP)		-	152 800
PA4-DRY-1B	Tray Dryer	Pressure: 1 atm Duty: 0 cal/sec Overall Entrainment: 0		-	56 500
Minor equipment					
PA4-HOP-01/02/03	Hopper	No specification		-	437 400
PA4-PMP-01/02/03/04/05/06	Centrifugal pumps	Discharge pressure: 1.5 atm (1.2 atm for PA4-PMP-05) with 75 % pump and 95 % driver efficiency Utility: Electricity (ELECT1)		3	177 900
PA4-TNK-01/03		Pressure estimate: 1 atm		10	390 300
Additional equipment (not modelled in Aspen Plus®, but included for the process flow sheet design)					
PA4-CNV-01 PA4-CNV-02 PA4-PMP-07/08/09				6.14	162 814
TOTAL				45.89	7 129 974

A3. Appendix C: Chapter 4 Supplementary information

Supplementary information

Process design and economic evaluation of integrated, multi-product biorefineries for the co-production of bio-energy, succinic acid and polyhydroxybutyrate (PHB) from sugarcane bagasse and trash lignocelluloses

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Contents:

1. Total operating costs for each scenario (Table 1)
2. Process Flow Diagram (PFD) stream tables for the succinic acid and PHB downstream recovery process areas (Table 2 and 3, respectively)
3. Equipment information tables for each plant area (i – iv as per Figure 1 in the main body of text)

List of Tables

Total operating costs

The variable, fixed and annual capital charge operating costs are provided in Table S1 for the total operating cost (TOC) for each scenario. The variable costs include the feedstock cost (10.79 US$/kg),[1] sulphuric acid cost (0.094 US$/kg),[2] PHB and SA growth media costs, ammonia (0.31 US$/kg),[3] reactive extraction solvent make-up costs, boiler chemicals, hydrazine, and the cost of ash disposal.[4]

The fixed operating costs is calculated by adding the labour cost,[4] labour overheads as 90 % of the labour cost, maintenance as 3 % of the NPP installed equipment cost and property taxes and insurance as 0.7 % of the FCI.[5] The annual capital charge is calculated as the activated carbon charge (1.2 US$/kg[6] for four (4) total batch replacements during the year), the enzyme nutrients (0.74 US$/kg),[5] and reactive extraction solvent costs for 4 total batch replacements during the year.

Table 1: Operating costs

SCENARIOS	**A**	**B**				**C**	**D**
	(PHB)	**(100)**	**(75)**	**(50)**	**(25)**	**(SA)**	**(CHP)**
Description	Million US$	Million US$	Million US$	Million US$	Million US$	Million US$	Million US$
Variable operating costs							
Feedstock cost[1]	7.90	7.90	7.90	7.90	7.90	7.90	7.90
Sulphuric acid[2]	0.33	0.24	0.25	0.26	0.26	0.26	-
SA nutrient media[3]	-	1.16	2.77	3.46	3.78	4.44	-
PHB nutrient media[3]	10.27	5.58	1.17	1.04	0.34	-	-
1-Octanol make-up	-	0.75	0.97	1.22	1.45	1.65	-
Trimethylamine make-up	-	0.10	0.14	0.16	0.19	0.21	-
Sodium Hydroxide	0.46	0.58	0.45	0.31	0.13	-	-
CHP plant chemicals	0.11	0.09	0.09	0.09	0.09	0.09	0.20
Make-up water	0.00	0.00	0.02	0.00	0.00	0.00	0.04
Ash waste disposal[4]	0.69	0.60	0.61	0.61	0.62	0.62	0.57
TOTAL	**19.79**	**17.30**	**14.37**	**15.05**	**14.77**	**15.17**	**8.71**
Fixed operating costs							
Total labour cost	3.31	3.44	3.72	3.76	3.76	2.83	0.92
Labour overheads	2.98	3.09	3.35	3.39	3.39	2.54	0.83
Maintenance	5.55	6.16	6.40	6.44	6.45	6.11	1.83
Property taxes and insurance	2.13	2.28	2.34	2.36	2.35	2.26	0.87
TOTAL	**14.00**	**14.97**	**15.81**	**15.95**	**15.95**	**13.75**	**4.44**
Annual capital charge							
Activated carbon	-	0.12	0.12	0.12	0.12	0.13	-
Enzyme nutrients	2.01	1.48	1.52	1.57	1.59	1.57	-
1-Octanol	-	0.34	0.44	0.55	0.65	0.74	-
Trioctylamine	-	0.00	0.01	0.01	0.01	0.01	-
Trimethylamine	-	0.04	0.05	0.06	0.07	0.08	-
TOTAL	**2.01**	**1.97**	**2.13**	**2.31**	**2.44**	**2.52**	**0.00**
Total Operating Cost (million US$)	**35.74**	**34.24**	**32.31**	**33.30**	**33.16**	**31.44**	**13.16**

Table 2: Downstream process recovery stream table for Scenario C: Succinic acid and electricity biorefinery

SCENARIO C (SA)																
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Mass Flow kg/h																
Sugars (Xylose, Arabinose, Cellobiose)	5.9	4759.1	0.0	4759.1	4759.1	4759.1	4759.1	4759.1	0	0	0	0	0	0	0	0
HMF and Furfural	0.2	188.1	0.0	188.1	188.1	188.1	188.1	188.1	0	0	0	0	0	0	0	0
Acetic acid	5.5	4458.7	0	4458.7	4458.7	4458.7	4458.7	4458.7	0	0	0	0	0	0	0	0
Water	259.7	210964.0	0	210964.0	210964.0	210964.0	210964.0	217502.0	0	0	0	0	41020.7	41020.7	23563.1	41010.1
Carbon dioxide	4.3	3455.5	0	3455.5	3455.5	3455.5	3455.5	3455.5	0	0	0	0	0	0	0	0
Lignin (soluable)	1.2	1001.2	0	1001.2	1001.2	1001.2	1001.2	1001.2	0	0	0	0	0	0	0	0
Micro-organism (cells)	90.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Succinic acid	16.9	13696.0	0	13696.0	1917.4	268.4	37.6	38.6	0	13658.43	0	154.0	15244.2	1739.7	0.5	0
Sulphuric acid	0.5	403.6	0	403.6	403.6	403.6	403.6	403.6	0	0	0	0	0	0	0	0
Ammonia	0.3	276.8	0	276.8	276.8	276.8	276.8	276.8	0	0	0	0	0	0	0	0
Oxygen	0	0.0	0	0	0	0	0	2.4	0	0	0	0	0	0	0	0
Nitrogen	0	0	0	0	0	0	0	7.4	0	0	0	0	0	0	0	0
Sodium bicarbonate	2.3	1886.7	0	1886.7	1886.7	1886.7	1886.7	1886.7	0	0	0	0	0	0	0	0
Formic acid	0.0	6.8	0	6.8	6.8	6.8	6.8	6.8	0	0	0	0	0	0	0	0
1-Octanol	0	0	0	0	350.0	336.8	333.8	335.1	484991.0	484992.0	335.0	484991.0	2241.3	2240.0	78.0	0
Trioctylamine	0	0	0	0	0	0	0	0	72469.9	72469.9	0	72469.9	0	0	0	0
Trimethylamine	0	0	0	0	0	0	0	0.3	0	0	0	0	13673.6	13673.6	12811.5	13673.0
Total Flow l/min	13.7	3976.5	0	3997.9	3860.2	3839.2	3836.0	3945.9	11696.7	11833.2	6.8	11705.8	1284.1	1134.5	772299.0	851219.0
Temperature K	311.2	311.2	0	323.2	322.9	322.7	322.6	322.2	322.2	322.8	298.2	322.8	322.8	323.2	373.2	369.6
Pressure atm	1.0	1.0	1.0	1.5	1.0	1.0	1.0	1.0	1.0	2.0	1.0	1.0	1.0	1.0	1.0	1.0
Substream: CIPSD (SOLID)																
Mass Flow kg/h																
Micro-organims (cells)	898.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Flow kg/h	**1285.9**	**241097.0**	**0.0**	**241097.0**	**229668.0**	**228006.0**	**227772.0**	**234323.0**	**557461.0**	**571121.0**	**335.0**	**557615.0**	**72179.7**	**58673.9**	**36453.2**	**54683.1**

SCENARIO F (SA)															
	17	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**E-1**	**E-2**	**E-3**
Mass Flow kg/h															
Water	8.3	17457.6	17457.6	10.6	17447.0	13148.2	13148.2	0	6600.0	13138.0	6538.0	0	0	0	0
Succinic acid	0	15243.6	1740.3	1.1	1739.2	2.1	2.1	0	0	1.1	1.1	0	0	0	0
Oxygen	0	0	0	0	0	2.4	422.4	420	0	2.4	2.4	0	0	0	0
Nitrogen	0	0	0	0	0	7.4	1587.4	1580	0	7.4	7.4	0	0	0	0
1-Octanol	0	2163.3	2163.3	1.3	2162.0	2.6	2.6	0	0	1.3	1.3	0	1.66E+05	1.60E+05	1.59E+05
Trioctylamine	0	0	0	0	0	0	0	0	0	0	0	0	24857.2	23915.1	23697.7
Trimethylamine	2.8	862.0	862.0	0.5	861.5	0.9	0.9	0	0	0.3	0.3	0	0	0	0
Total Flow l/min	0.2	559.7	376.7	0.2	376.4	220.7	438716.0	25558.4	110.3	220.5	110.3	0	4012.2	3860.1	3825.1
Temperature K	298.2	373.2	293.2	293.2	293.2	300.6	403.2	403.2	298.2	303.2	308.2		322.2	322.2	322.2
Pressure atm	1	1	1	1	1	1	1	1.5	1	2	1		1	1	1
Substream: CIPSD (SOLID)															
Mass Flow kg/h															
Succinic acid crystals		0	13503.4	13503.4	0	13503.4	0	0	0	0	0	13503.4	0	0	0
Total Flow kg/h		**35726.5**	**35726.5**	**13516.9**	**22209.7**	**26667.0**	**15163.7**	**2000.0**	**6600.0**	**13150.6**	**6550.6**	**13503.4**	**191209.0**	**183962.0**	**182290.0**

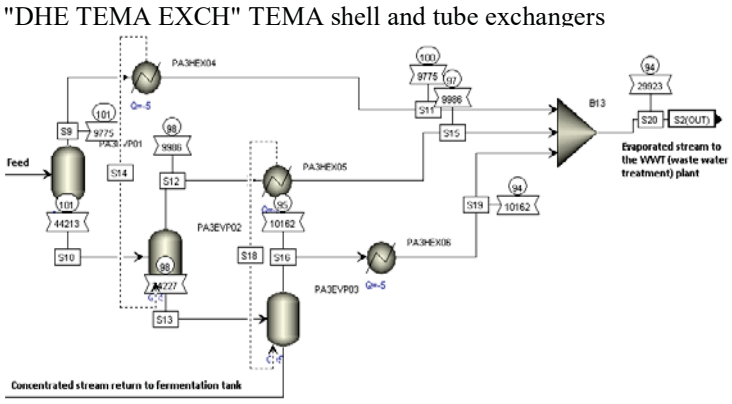
Table 3: Downstream process recovery stream table for Scenario A: PHB and electricity biorefinery

SCENARIO A (PHB)																				
STREAM NUMBER	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Mass Flow kg/h																				
Sugars (Xylose, Arabinose, Cellobiose)	77.9	25.9	52.0	0	71.5	6.4	77.9	19.5	0	6.4	0	6.4	5.7	0	0.6	0.6	0	0	0	0
Furfural and HMF	0.1	0.0	0.1	0	0.1	0.0	0.1	0.0	0	0.01	0	0.01	0.01	0	0.00	0.00	0	0	0	0
Acetic Acid	0.6	0.2	0.4	0	0.6	0.0	0.6	0.2	0	0.05	0	0.05	0.04	0	0.00	0.00	0	0	0	0
Water	22985.8	7860.5	15786.5	661.2	21701.5	1945.5	96401.4	5915.0	76560	78076.9	0	78076.9	74271.4	4350	8155.5	0.0	8155.5	8139.3	16.2	0
Carbon dioxide	1025.1	340.8	684.4	0	940.8	84.3	1025.1	256.4	0	83.9	0	83.9	75.6	0	8.3	8.3	0	0	0	0
Lignin (soluable)	0.3	0.1	0.2	0	0.2	0.0	0.3	0.1	0	0.02	0	0.02	0.0	0	0.00	0.00	0	0	0	0
Sulphuric acid	0.1	0.0	0.1	0	0.1	0.0	0.1	0.0	0	0.01	0	0.01	0.0	0	0.00	0.00	0	0	0	0
Ammonia	159.2	52.9	106.3	0	146.1	13.1	159.2	39.8	0	13.0	0	13.0	11.7	0	1.3	1.3	0	0	0	0
Oxygen	8.6	2.9	5.8	0	7.9	0.7	8.6	2.2	0	0.7	0	0.7	0.6	0	0.1	0.1	0	304.0	0	304.0
Magnesium sulphate	1.1	0.4	0.7	0	1.0	0.1	1.1	0.3	0	0.1	0	0.1	0.1	0	0.01	0.01	0	0	0	0
Nitrogen	55.2	18.3	36.8	0	50.7	4.5	55.2	13.8	0	4.5	0	4.5	4.1	0	0.4	0.4	0	1001.0	0	1001.0
Potassium phosphate	29.5	9.8	19.7	0	27.1	2.4	29.5	7.4	0	2.4	0	2.4	2.2	0	0.2	0.2	0	0	0	0
Citric acid	1.1	0.4	0.7	0	1.0	0.1	1.1	0.3	0	0.1	0	0.1	0.1	0	0.01	0.01	0	0	0	0
Diammonium phosphate	3.9	1.3	2.6	0	3.6	0.3	3.9	1.0	0	0.3	0	0.3	0.3	0	0.03	0.03	0	0	0	0
PHB	2740.9	2740.9	0	0	27.4	2713.5	81.7	27.4	0	2713.5	0	2713.5	0	0	2713.5	54.3	2659.2	0	0	0
Bio (CELLS - lysed)	0	0	0	0	0	0	1397.5	0	0	0	0	1401.7	0	0	1401.7	1397.5	4.2	0	0	0
Sodium Hydroxide	0	0	0	0	0	0	644.8	0	0	0	644.8	644.8	581.0	0	63.8	63.8	0	0	0	0
Total Flow l/min	449.4	177.8	282.6	11.1	388.9	71.5	1679.4	106.3	1279.6	1343.7	5.7	1390.1	1252.7	72.7	210.0	131.3	173.2	249658.0	0.3	11585.8
Temperature K	303.2	302.9	302.9	298.2	302.9	302.9	307.8	302.9	298.2	298.5	303.1	310.2	309.5	298.2	309.5	309.5	309.5	370.5	373.2	374.6
Pressure atm	1.5	1	1	1.5	2	1	1	1	1.5	1	1.5	1	1	1.5	1	1	1	1	1	2
Substream: CIPSD (SOLID)																				
Mass Flow kg/h																				
PHB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2659.2	0
Bio (CELLS)	1415.8	1415.8	0	0	14.2	1401.7	14.2	14.2	0	1401.7	0	0	0	0	0	0	0	0	4.2	0
Total Flow kg/h	**28505.2**	**12470.2**	**16696.2**	**661.2**	**22993.7**	**6172.8**	**99902.3**	**6297.5**	**76560.0**	**82303.6**	**644.8**	**82948.4**	**74952.9**	**4350.0**	**12345.5**	**1526.6**	**10819.0**	**9444.3**	**2679.6**	**1305.0**

Table 4: Pretreatment, Enzymatic Hydrolysis and Detoxification equipment information (operating conditions, Aspen model specifications, sizing and costing)

EQUIPMENT DESCRIPTION	OPERATING CONDITIONS	DESIGN COMMENT	ASPEN MODEL	SIZING and COST OF EQUIPMENT
Jacketed Screw Pretreatment Reactor	Temperature 173°C, Pressure: 8.5 atm, HPU steam	Removal of hemicellulose for improved enzymatic digestibility. 0.65 % H_2SO_4 at 1:2 solids to liquid ratio for 10 min	RSTOIC	Sizing and cost: Aspen Process Economic Analyzer Mapped as "DAT REACTOR" Agitated tank, enclosed and jacketed. Comment: It was assumed that the residence time of 10min could be achieved in a continuous operation using a plug flow reactor. This was later confirmed with a installed cost difference of 0.21 % (data not shown) using a similar approach to the sizing and cost of the enzymatic hydrolysis tanks with the cost data supplied by Medina et al., (2018)[7] for a dilute acid reactor.
Enzymatic hydrolysis tank(s)	Pressure: 1atm, Duty 0 cal/sec Slurry is diluted to 20 %wt and preheated to 50°C prior to entering the hydrolysis tank.	Cellulase enzymes hydrolyse cellulose into glucose Cellulase added at 20 mg protein per gram cellulignin.	RSTOIC	Sizing Total residence time (R, h) = [72 h[5]] + [12 h allowed to empty and clean tank (assumed value)] Total volume (V, m^3) = R x [Volumetric feed rate (m^3/h)] Number of tanks (N) = V / [30m^3][8]; Total installed cost = N x [installed cost per tank[8]] Comment: Installed cost per tank (30m^3) is based on a jacketed, glass lined reactor.[8]
Detoxification	Temperature 80°C, Pressure: 1 atm	Adsorption columns using granular activated carbon. Removal rate is based on Hodge *et al.,* (2009) at 0.7 for furfural, 1 for HMF, and 0.00459 for water in the SEP unit.[9]	SEP	Sizing: Contact time of 30 min per column (selected) and a total of 120 minutes residence time is required.[10,11] Therefore a configuration of 5 trains in parallel, each train has 4 columns in series. Thus resulting in a total of 40 columns. The diameter is set at 0,6 meters (assumed). The height is calculated based on the feed rate per train (i.e. total feed rate is divided by 5 to determine the feed rate per column train) using the method described in Towler and Sinnott, (2008) for adsorption columns.[12] Total installed cost: Installed cost per column (Stainless steel material of construction, based on cost calculated with the method provided in Towler and Sinnott, (2008) for a specific height) x 40 columns.[12]

Table 5: Succinic acid seed train, fermentation and downstream processing equipment information (operating conditions, Aspen model specifications, sizing and costing)

EQUIPMENT DESCRIPTION	OPERATING CONDITIONS	DESIGN COMMENT	ASPEN MODEL	SIZING and COST OF EQUIPMENT
Seed train reactors	Temperature 38°C Pressure 1 atm Utility: CHILLW (Chilled water)	The purpose of the seed train reactors is to produce an inoculum (micro-organism) to use in the fermentation tanks. A portion of the total sugar feed stream (10 %) is diverted to the seed train area.	RSTOIC	Sizing: The sizing is based on Aspen Process Economic Analyzer. Mapped as "DAT REACTOR" Agitated tank, eclosed and jacketed. Cost: The cost (C_5) provided by Aspen Process Economic Analyzer is used for the 5th Seed train reactor. The cost for the 1st, 2nd, 3rd and 4th seed train reactors are scaled from the C5 cost according to the scaling used in Humbird *et al.,* (2011). i.e. $C_1 = 0.214*C_5$; $C_2 = 0.331*C_5$, $C_3 = 0.448*C_5$ This method was used due to the small volume of the seed train relative to the fermentation tanks and thus the small capital cost contribution thereof.
Fermentation tanks	Temperature 38°C Pressure 1 atm Utility: CHILLW (Chilled water) pH 6 - 7 Required CO_2 aeration	The purpose of the fermentation tanks is to convert the sugar substrate into succinic acid using the micro-organism *Actinobacillus succinogenes.*	RSTOIC	Sizing Total residence time (R, h) = [38.8 h][13] + [12 h allowed to empty and clean tank (assumed value)] Total volume (V, m^3) = R x [Volumetric feed rate (m^3/h)] Number of tanks (N) = V / [30m^3][8]; Total installed cost = N x [installed cost per tank][8] Comment: Installed cost per tank (30m^3) is based on a jacketed, glass lined reactor.[8] Comment: A fed-batch approach is followed with initial feed stream at 85 g/L total sugars (85 %wt of total) and intermediate feed stream at 200 g/L (15 %wt of total feed stream).[14]
Triple effect evaporator	Evaporator 1: Pressure 1 atm and Vapour fraction 0.198, using HPU steam. Evaporator 2: Pressure 0.9 atm Evaporator 3: Pressure 0.8 atm Heat exchanger 1and 2: Pressure 0 atm, Vapour fraction 0 Heat exchanger 3: Pressure 0 atm, Vapour fraction 0, Utility: Cooling water	The purpose of this unit is to concentrate the sugar feed stream. Note: The heat from the vapour stream from the first evaporator is used as input energy to the second evaporator.	FLASH2 HEATER	Sizing and cost: Aspen Process Economic Analyzer Mapped as 3 "DVT CYLINDER" Vertical process vessel and 3 "DHE TEMA EXCH" TEMA shell and tube exchangers 
Reactive extraction columns	3 Columns in series. Succinic acid to organic phase: 0.86	Recovery of succinic acid from fermentation broth.	SEP	Sizing and cost: Aspen Process Economic Analyzer Mapped as "DVT CYLINDER" Vertical process vessel. Comment: The separation efficiencies is 'tested', and the energy

	1-Octanol to organic phase: 0.9979 Trioctylamide to organic phase: 1 based on reported values.[15]	Overall Purity: 99.5 wt% and Recovery 98 wt%		requirement was determined using a Decanter unit (Pressure 1 atm, temperature 50°C, Electricity utility, UNIF-LL Property method, i.e. UNIFAC for liquid-liquid systems and Redlich-Kwong equation of state and Henry's law) for a mixture of succinic acid water only. The decanter model could not be used on the fermentation broth due to limited information on the broth constituents.
Back extraction	1 Column Succinic acid to aqueous phase: 0.99 1-Octanol to aqueous phase: 0.0046 Trimethylamine to aqueous phase: 1	Succinic acid migrates from the organic phase back into an aqueous phase prior to evaporation and crystallisation.	SEP	Sizing and cost: Aspen Process Economic Analyzer Mapped as "DVT CYLINDER" Vertical process vessel
Evaporation drum	Temperature: 100°C Pressure: 1 atm HPU steam	Purpose is to concentrate the succinic acid stream prior to crystallisation.	FLASH2	Sizing and cost: Aspen Process Economic Analyzer Mapped as "DVT CYLINDER" Vertical process vessel Property method used: NRTL-NTH
Crystallisation	Temperature: 20°C Pressure: 1 atm Solubility is Concentration data type with 77 gm/L at 295 K.[16] PSD taken from Miller (1978) for water-soluble crystals in aqueous slurries.[17]	Purpose is to crystallise the succinic acid from the aqueous slurry. The unit is preceded by a heat exchanger that cools the stream down to 20°C using chilled water utility.	CRYSTALLIZER	Sizing and cost: Aspen Process Economic Analyzer Mapped as "ECRYOSLO" Oslo growth type crystallizer
Drying	Shortcut method No change in pressure Temperature: 130°C using HPU steam Moisture specification basis: WET	Purpose is to dry the succinic acid crystals to ensure the product is ready for packaging and distribution.	DRYER	Sizing and cost: Aspen Process Economic Analyzer Mapped as "ED ATMOS TRAY" Atmospheric tray batch dryer

Table 6: Cellulase plant, PHB and downstream processing equipment information (operating conditions, Aspen model specifications, sizing and costing)

EQUIPMENT DESCRIPTION	OPERATING CONDITIONS	DESIGN COMMENT	ASPEN MODEL	SIZING and COST OF EQUIPMENT
Cellulase plant	Some of the glucose rich stream is diverted as cellulase plant feed, calculated at 3.9 * cellulase protein (kg/h), based on ratio: 2418 kg/h glucose required to produce 620 kg/h protein.[5]	A modular unit is included based on the mass flow rate of cellulase protein required (based on the feed mass flow rate of cellulignin entering the enzymatic hydrolysis tanks).	n/a	Sizing and cost: Scaled from base cost (10 730 186 US$) and scaled to size based on the cellulase protein required (scaling exponent: 0.6) and scaled to time based on CEPCI values (585.7 for 2011 and 536.5 for 2016), Installation factor used was 1.707 based on the report by Humbird *et al.*, (2011).
Growth train	Temperature: 30°C Pressure: 1 atm Chilled water utility Diluted to <20 g/L total sugars[18]	Functions similar to the succinic acid seed train. During the growth phase the micro-organism utilises the sugar and nutrients to grow in size, without significant PHB production.	RSTOIC	Sizing and cost: Aspen Process Economic Analyzer Mapped as "DAT REACTOR" Agitated tank, enclosed and jacketed.
Synthesis train	Temperature: 30°C Pressure: 1 atm Chilled water utility Concentrated to 700 g/L[18]	PHB is produced intracellularly during the growth phase.	RSTOIC	Sizing Total residence time (R, h) = [44.016 h][18] + [12 h allowed to empty and clean tank (assumed value)] Total volume (V, m^3) = R x [Volumetric feed rate (m^3/h)] Number of tanks (N) = V / [30m^3][8]; Total installed cost = N x [installed cost per tank] Comment: Installed cost per tank (30m^3) is based on a jacketed, glass lined reactor[8]
Blending tank	Temperature: 37°C Pressure: 1 atm Steam LPU Cell concentration: 50 g/L[19]	The micro-organism cells are lysed with 0.2 M NaOH solution to expose the PHB contained within the cells. The PHB becomes crystalline upon exposure.	RSTOIC	Sizing and cost: Aspen Process Economic Analyzer Mapped as "DAT REACTOR" Agitated tank, enclosed and jacketed. Comment: Due to the residence time of 5h required,[20] the cost provided from Aspen Economic Analyzer is multiplied with 3, for a total of 3 blending tanks.
Spray dryer	Spray dryer Pressure: 1 atm Height: 2.36 meter, Diameter: 0.8 meter. PSD: Solid particle formation using Rosin-Rammier-Sperling-Bennet function	The spray dryer atomises the crystalline PHB into particles for packaging and distribution.	DRYER	Sizing and cost: Aspen Process Economic Analyzer Mapped as "ED SPRAY" Continuous spray drying system

Table 7: Waste water treatment (WWT) plant equipment information (operating conditions, Aspen model specifications, sizing and costing)

EQUIPMENT DESCRIPTION	OPERATING CONDITIONS	DESIGN COMMENT	ASPEN MODEL	SIZING and COST OF EQUIPMENT
Based on the process flow diagram from the NREL report on the process design of a WWT for the cellulosic ethanol model by Steinwinder, Gill and Gerhardt (2011).[21] The amount of methane produced, based on the feed stream's COD levels, is comparable to the value used in Humbird et al., (2011) at 228g CH_4 per kg COD removed.[5]				
Biodigester	Temperature 35°C Pressure 1.1 atm	Mesophilic anaerobic biodigester to break down the organic constituents of the waste water streams and thus lower the COD demand of the water (chemical oxygen demand).	The biodigester is simulated as 3 reactors (RSTOIC)	Sizing and cost: Aspen Process Economic Analyzer Comment: The set of Anaerobic digestion stoichiometric reactions are based on the work by Peris, (2011), Cheng *et al.,* (2012), Rajendran *et al.,* (2014) and Tenneti, (2015).[22–25] The CHP boiler is equipped with a limestone absorbent to reduce sulphur oxide emissions.[21] The biogas line is equipped with an emergency flare, to which the vapour from the evaporation unit is also sent if the methane is within the flammability limits (5 - 15 %vol). Sludge is simulated as $C_5H_7NO_2$ (Ethyl cyanoacetate).
	Hydrolysis stage	3 Xylose → 7.5 Acetic Acid [Fractional conversion, (X) = 1] Glucose + 0.1115 NH_3 → 0.1115 Sludge + 0.744 Acetic Acid + 0.5 Propionic Acid + 0.4409 n-Butyric Acid + 0.6909 CO_2 + 1.0254 H_2O [X=1] Itaconic acid + 2 H_2O → Acetic Acid + 2 CO_2 + H_2 + CH_4 ; [X = 1] 20 Biomass cells + 10 H_2O → 10 Acetic Acid + 2 H_2 + 4 NH_3 ;[X = 1] 3 Succinic acid + 3 H_2O → 4.5 Acetic Acid + 3 CO_2 + 3 H_2 ;[X = 1] Cellobiose + H_2O → 4 Ethanol + 4 CO_2 ;[X = 0.95] 3 Arabinose → Propionic Acid + n-Butyric Acid + Iso-Valeric Acid + 3 CO_2 + 3 H_2O ;[X = 0.95] H_2SO_4 → H_2S + 2 O_2 ;[X = 0.08]	
	Acidogenesis and Acetogenesis stages	Iso-Valeric Acid + 0.0653 NH_3 + 0.5543 CO_2 + 0.8044 H_2O → 0.0653 Sludge + 0.8912 Acetic Acid + 0.4454 CH_4 + Propionic Acid + 0.0006 H_2 ;[X = 1] n-Butyric Acid + 0.0653 NH_3 + 0.8038 H_2O + 0.0006 H_2 + 0.5543 CO_2 → 0.0653 Sludge + 1.8909 Acetic Acid + 0.446 CH_4 ;[X = 1] Propionic Acid + 0.06198 NH_3 + 0.314336 H_2O → 0.06198 Sludge + 0.9345 Acetic Acid + 0.660412 CH_4 + 0.160688 CO_2 + 0.00055 H_2 ;[X = 1] Ethanol + H_2O → Acetic Acid + 2 H_2 ;[X = 1] 2 CO_2 + 4 H_2 → Acetic Acid + 2 H_2O ;[X = 1]	
	Methanogenesis stage	14.497 H_2 + 0.0836 NH_3 + 3.8334 CO_2 → 3.4154 CH_4 + 7.4996 H_2O + 0.0836 Sludge ;[X = 0.98] Acetic Acid + 0.022 NH_3 → 0.022 Sludge + 0.945 CH_4 + 0.066 H_2O + 0.945 CO_2 ;[X = 1]		
	Gas - liquid separation	Pressure 1 atm 0 cal/sec Duty	FLASH2	Not included in the equipment cost Property method: NRTL with Ideal gas law and Henry's law
Clarifier	Solids separator Solids to solid outlet: 0.9 Liquid load of solids outlet: 0.5	Aerobic digestion	CFUGE	Sizing and cost: Scaled from base cost (174 385 US$) and scaled to size based on the feed stream (scaling exponent: 0.51) and scaled to time based on CEPCI values (585.7 for 2011 and 536.5 for 2016), Installation factor used was 1.96 based on Humbird *et al.,* (2011).
Reverse Osmosis Plant	Water fraction to purified water outlet: 0.9.[26,27]	Water purification unit. Purified water is used as process water in the biorefinery.	SEP	Sizing and cost: Scaled from base cost (2 210 979 US$) and scaled to size based on the feed stream (scaling exponent: 0.6) and scaled to time based on CEPCI values (585.7 for 2011 and 536.5 for 2016), Installation factor used was 1.0 based on Humbird *et al.,* (2011).
Evaporation Modular Plant	Same configuration as the triple effect evaporator.	Removes water from slurry. Concentrated slurry is used as fuel source for the CHP plant.	FLASH2 HEATER	Sizing and cost: Aspen Process Economic Analyzer Mapped as 3 Vertical process vessels and 3 heat exchangers (TEMA shell and tube exchanger)

Table 8: Combined heat and power (CHP) plant equipment information (operating conditions, Aspen model specifications, sizing and costing)

EQUIPMENT DESCRIPTION	OPERATING CONDITIONS	DESIGN COMMENT	ASPEN MODEL	SIZING and COST OF EQUIPMENT
Boiler	Simulated using various blocks			Sizing and cost: Scaled from base cost (28 550 000 US$) and scaled to size based on the mass flow rate of superheated steam generated at 600°C (scaling exponent: 0.6) and scaled to time based on CEPCI values (585.7 for 2011 and 536.5 for 2016), Installation factor used was 1.8 based on Humbird *et al.*, (2011). Cost includes a baghouse (air filtration) unit. The amount of air will vary to keep the furnace section output stream at 870°C which is taken as the maximum temperature for a biomass boiler, using a Design Specification block. Property method: RKS-BM (Redlich-Kwong-Soave equation of state with Boston-Mathias modifications)
	Furnace section	Pressure -0.034 atm Duty 0 cal/sec Combustion reactions [X = 0.99]	RSTOIC	
	Heat transfer to boiler section	Temperature 278°C No Pressure change 10 % Heat loss to atmosphere	HEATER	The temperature of the furnace output stream decreases from 870°C to 278°C. Property method: IAPWS-95
	Heat transfer to incoming air	Temperature 149°C No Pressure change	HEATER	The temperature of the furnace output stream decreases from 278°C to 149°C. Property method: IAPWS-95
	Cyclone and Baghouse	Removes solid particles from the furnace output stream	SSPLIT	Cost is included in the Boiler cost.[5]
	Boiler tube section	Pressure 0 atm Heat received from furnace	FLASH2	Inlet stream pressure is 63 atm (Pump is used to increase pressure).[4] The amount of boiler water entering the boiler will vary to keep the boiler output stream (super-heated steam) at 600°C, using a design specification block. Property method: IAPWS-95
Condensing Extraction Steam Turbines (CEST)	Isentropic type turbine with isentropic efficiency of 0.85 per stage. Each stage is a separate Aspen block (Compr). Stage 1 discharge pressure: 32 atm (De-superheated to 12.83 atm for HPU) Stage 2 discharge pressure: 6.415 atm (LPU) Stage 3 discharge pressure: 0.1 atm Stage 3 has Vapour-liquid convergence	The turbine utilises the superheated steam for electricity generation, while the intermediate streams are extracted and used for direct steam injection (Pretreatment reactor) as well as providing the sugar mill with steam and the HPU and LPU steam.	COMPR	Sizing and cost: Scaled from base cost (9 500 000 US$) and scaled to size based on the mass flow rate of inlet stream into the 1st CEST stage (scaling exponent: 0.6) and scaled to time based on CEPCI values (585.7 for 2011 and 536.5 for 2016), Installation factor used was 1.8 as per Humbird *et al.*, (2011). Property method: STEAMNBS (NBS/NRC Steam tables)
Desuperheating station	Pressure: specified as required Design specification block used to control boiler feed water added to the station for required temperature	Desuperheating stations are used to maximise the steam usage. They are energy destructive and should not be used if the aim is to optimise electricity generation.	MIXER	Sizing and cost: Not included in capital cost - factored in as part of piping. Property method: STEAMNBS (NBS/NRC Steam tables)

Table 9: Other, minor equipment unit information

EQUIPMENT DESCRIPTION	OPERATING CONDITIONS	DESIGN COMMENT	ASPEN MODEL	SIZING and COST OF EQUIPMENT
Pumps	Discharge pressure: 2 atm Utility: Electricity (kW)	Pump efficiency: 75%, Driver efficiency: 95%	PUMP	Mapped as "DCP CENTRIF" Centrifugal single or multi-stage pump
Holding tanks	n/a	n/a	MIXER	Mapped as "DAT REACTOR" Agitated tank, eclosed and jacketed
Flash drums	Varies from unit to unit	n/a	FLASH2	Mapped as "DVT Cylinder" Vertical process vessel
Separating equipment	Centrifuges Solids separator	Fraction of solids to solid outlet: 1 Liquid load of solid outlet: 0.5	CFUGE	Mapped as "ECT Solid Bowl" Solid bowl centrifuge OR "ECT Batch Auto" Auto batch filtering centrifuge. Property method: SOLIDS
Heat exchangers (using another stream)	Shortcut, Countercurrent	Hot/Cold temperature approach: 10°C	HEATX	Mapped as "DHE TEMA EXCH" TEMA shell and tube exchanger
Heat exchanger (using an utility)	Temperature: unit specific Pressure: 0 atm (no change)	Utility: HPU, LPU or Elec depending on requirement	HEATER	Mapped as "DHE TEMA EXCH" TEMA shell and tube exchanger
Filtration	Solids separator Solids to solid outlet: 1 Liquid load of solids outlet: 0.3	Removal of micro-organism cells in succinic acid downstream processing	CFFILTER	Mapped as "EF TUBULAR" Tubular fabric filter (bank of 3) Property method: SOLIDS
Washing	Liquid to solid mass ratio: 1	Washing of succinic acid crystals with water prior to drying.	SWASH	Mapped as "DF ROTY DRUM" Rotary drum filter

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A4. Appendix D: Chapter 5 Supplementary information

Economic evaluation and comparison of succinic acid and electricity co-production from sugarcane bagasse and trash lignocelluloses in a biorefinery, using different pretreatment methods: Dilute acid (H_2SO_4), Alkaline (NaOH), Organosolv, Ammonia Fibre Expansion (AFEX™), Steam explosion (STEX), and Wet oxidation.

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Supplementary information

Mass and Energy balance results for Case Study 1 (Table 1) and 2 (Table 2). Moreover, DCF analysis results for Case Study 1 (Table 3) and 2 (Table 4).

Overview of the equipment units' operating conditions, selection, sizing and costing are provided in in Tables 5 - 14. Although a block flow diagram is provided in Figures 2 and 3, a screenshot of the Aspen Plus® simulations are provided in Figure 1 - 9 as supporting information.

Table of Tables

Table of Figures

Table 1: Mass and Energy balance results for Case study 1: Impact of enzymatic hydrolysis solids loadings on profitability

Pretreatment methods	DAT	AFEX™	STEX	STEX with SO_2
	5 % Solids loading			
Bypass ratio (%)	64	76	68	67
Succinic acid produced (t/h)	7.0	5.3	9.6	9.4
Fermentable sugar stream concentration (g/L)	30.6	23.8	47.9	37.8
Saturated steam (t/h)	31.1	n/a	7.0	18.0
LPU (t/h)	12.5	16.8	19.4	7.0
HPU (t/h)	95.7	110.5	77.8	115.5
Electricity Produced (MWh)	8.8	9.5	16.2	7.7
Electricity Required (MWh)	2.7	4.2	14.0	2.8
Sellable electricity (MWh)	6.1	5.3	2.2	4.9
	12.5 % Solids loading			
Bypass ratio (%)	33	62	41	39
Succinic acid produced (t/h)	12.8	8.3	17.6	17.3
Fermentable sugar stream concentration (g/L)	63.0	38.1	121.7	68.8
Saturated steam (t/h)	57.9	-	13.0	31.0
LPU (t/h)	6.7	17.8	21.0	13.3
HPU (t/h)	64.3	99.7	64.1	58.4
Electricity Produced (kWh)	7.8	12.0	10.1	9.1
Electricity Required (kWh)	3.2	5.3	4.5	2.8
Sellable electricity (kWh)	4.6	6.7	5.6	6.3

Table 2: Mass and Energy balance results for Case study 2: Impact of solids to liquid ratio on profitability

Pretreatment methods	DAT	NaOH	STEX with NaOH
Actual S:L ratio	1:20	1:15	1:10
Bypass ratio (%)	78	79	68
Succinic acid produced (t/h)	4.1	3.5	5.3
Fermentable sugar stream concentration (g/L)	17.5	110.1	112.7
Saturated steam (t/h)	n/a	n/a	6.2
LPU (t/h)	3.0	14.1	5.8
HPU (t/h)	142.2	29.6	48.1
Electricity Produced (kWh)	7.9	9.1	7.9
Electricity Required (kWh)	6.0	1.4	1.5
Sellable electricity (kWh)	1.9	7.7	6.4
Mid-way S:L ratio	1:11	1:8.5	1:6
Bypass ratio (%)	62	74	60
Succinic acid produced (t/h)	7.1	4.3	6.6
Fermentable sugar stream concentration (g/L)	29.9	105.5	113.0
Saturated steam (t/h)	n/a	n/a	7.8
LPU (t/h)	4.3	11.8	4.6
HPU (t/h)	131.1	32.1	53.0
Electricity Produced (kWh)	8.2	9.0	7.4
Electricity Required (kWh)	6.1	1.5	1.7
Sellable electricity (kWh)	2.1	7.5	5.7

Table 3: Economic evaluation results for Case study 1: Impact of solids loading on profitability

Pretreatment methods	DAT	AFEX™	STEX	STEX with SO2
	5 % Solids loading			
TCI (million US$)	415.2	426.4	411.0	442.7
TOC (million US$)	29.7	27.2	30.5	32.5
NPV (million US$)[a]	(91.7)	(206.2)	59.0	15.4
IRR (%)[a]	6.7	2.6	11.6	10.2
MRSP[b] (US$/t)	1 802	2 380	1 335	1 461
	12.5 % Solids loading			
TCI (million US$)	406.2	446.3	411.0	410.9
TOC (million US$)	37.0	32.6	37.0	39.4
NPV (million US$)[a]	232.5	(49.8)	521.8	491.8
IRR (%)[a]	16.7	8.2	24.1	23.4
MRSP[b] (US$/t)	1 067	1 639	779	810

a) Net present value (NPV) and Internal rate of return (IRR) for a succinic acid selling price of 1500 US$/t and electricity selling price of 0.08 US$/kWh. b) Minimum required selling price of succinic acid for a NPV of 0 US$ and electricity selling price of 0.08 US$/kWh

Table 4: Economic evaluation outcome for Case Study 2: Impact of solids to liquid ratio on profitability

Pretreatment methods	DAT	NaOH	STEX with NaOH
Actual S:L ratio	1:20	1:15	1:10
TCI (million US$)	297.7	217.9	228.4
TOC (million US$)	28.4	23.9	24.4
NPV (million US$)[a]	(5.8)	(102.3)	(2.0)
IRR (%)[a]	9.4	2.9	9.6
MRSP[b] (US$/t)	2 500	2 158	1 509
Mid-way S:L ratio	1:11	1:8.5	1:6
TCI (million US$)	333.2	222.4	244.8
TOC (million US$)	33.6	24.2	25.5
NPV (million US$)[a]	(5.4)	(52.1)	58.4
IRR (%)[a]	9.5	6.5	12.7
MRSP[b] (US$/t)	1 659	1 777	1 290

a) Net present value (NPV) and Internal rate of return (IRR) for a succinic acid selling price of 1500 US$/t and electricity selling price of 0.08 US$/kWh. b) Minimum required selling price of succinic acid for a NPV of 0 US$ and electricity selling price of 0.08 US$/kWh

Table 5: General, minor equipment unit information

EQUIPMENT DESCRIPTION	OPERATING CONDITIONS	DESIGN COMMENT	ASPEN MODEL	EQUIPMENT MAPPING (Sizing and cost: Aspen Process Economic Analyzer)
Pumps	Discharge pressure: 2 atm Utility: Electricity (kW)	Pump efficiency: 75%, Driver efficiency: 95%	PUMP	Mapped as "DCP CENTRIF" Centrifugal single or multi-stage pump
Holding tanks	n/a	n/a	MIXER	Mapped as "DAT REACTOR" Agitated tank, enclosed and jacketed
Flash drums	Varies from unit to unit	n/a	FLASH2	Mapped as "DVT Cylinder" Vertical process vessel
Separating equipment	Centrifuges Solids separator	Fraction of solids to solid outlet: 1 Liquid load of solid outlet: 0.5	CFUGE	Mapped as "ECT Solid Bowl" Solid bowl centrifuge OR "ECT Batch Auto" Auto batch filtering centrifuge. Property method: SOLIDS
Heat exchangers (using another stream)	Shortcut, Countercurrent	Hot/Cold temperature approach: 10°C	HEATX	Mapped as "DHE TEMA EXCH" TEMA shell and tube exchanger
Heat exchanger (using an utility)	Temperature: unit specific Pressure: 0 atm (no change)	Utility: HPU, LPU or Elec depending on requirement	HEATER	Mapped as "DHE TEMA EXCH" TEMA shell and tube exchanger
Filtration	Solids separator Solids to solid outlet: 1 Liquid load of solids outlet: 0.3	Removal of micro-organism cells in succinic acid downstream processing	CFFILTER	Mapped as "EF TUBULAR" Tubular fabric filter (bank of 3) Property method: SOLIDS
Washing	Liquid to solid mass ratio: 1	Washing of succinic acid crystals with water prior to drying.	SWASH	Mapped as "DF ROTY DRUM" Rotary drum filter

Table 6: DAT Pretreatment, Enzymatic Hydrolysis and Detoxification equipment information (operating conditions, Aspen model specifications, sizing and costing)

EQUIPMENT DESCRIPTION	OPERATING CONDITIONS	DESIGN COMMENT	ASPEN MODEL	SIZING and COST OF EQUIPMENT
Jacketed Screw Pretreatment Reactor	Temperature 173°C, Pressure: 8.5 atm, HPU steam	Removal of hemicellulose for improved enzymatic digestibility. 0.65 % H_2SO_4 at 1:2 solids to liquid ratio for 10 min	RSTOIC	Sizing and cost: Aspen Process Economic Analyzer Mapped as "DAT REACTOR" Agitated tank, enclosed and jacketed. Comment: It was assumed that the residence time of 10min could be achieved in a continuous operation using a plug flow reactor. This was later confirmed with an installed cost difference of 0.21 % (data not shown) using a similar approach to the sizing and cost of the enzymatic hydrolysis tanks with the cost data supplied by Medina et al., (2018)[1] for a dilute acid reactor.
Enzymatic hydrolysis tank(s)	Pressure: 1atm, Duty 0 cal/sec Slurry is diluted to 20 %wt and preheated to 50°C prior to entering the hydrolysis tank.	Cellulase enzymes hydrolyse cellulose into glucose Cellulase added at 20 mg protein per gram cellulignin.	RSTOIC	Sizing Total residence time[2] (R, h) = [72 h] + [12 h allowed to empty and clean tank (assumed value)] Total volume (V, m^3) = R x [Volumetric feed rate (m^3/h)] Number of tanks (N) = V / [30m^3][3]; Total installed cost = N x [installed cost per tank[3]] Comment: Installed cost per tank (30m^3) is based on a jacketed, glass lined reactor.[3]
GAC Detoxification	Temperature 80°C, Pressure: 1 atm	Adsorption columns using granular activated carbon. Removal rate is based on Hodge *et al.,* (2009) at 0.7 for furfural, 1 for HMF, and 0.00459 for water in the SEP unit.[4]	SEP	Sizing: Contact time of 30 min per column (selected) and a total of 120 minutes residence time is required.[5,6] Therefore a configuration of 5 trains in parallel, each train has 4 columns in series. Thus resulting in a total of 40 columns. The diameter is set at 0,6 meters (assumed). The height is calculated based on the feed rate per train (i.e. total feed rate is divided by 5 to determine the feed rate per column train) using the method described in Towler and Sinnott, (2008) for adsorption columns.[7] Total installed cost: Installed cost per column (Stainless steel material of construction, based on cost calculated with the method provided in Towler and Sinnott, (2008) for a specific height) x 40 columns.[7]

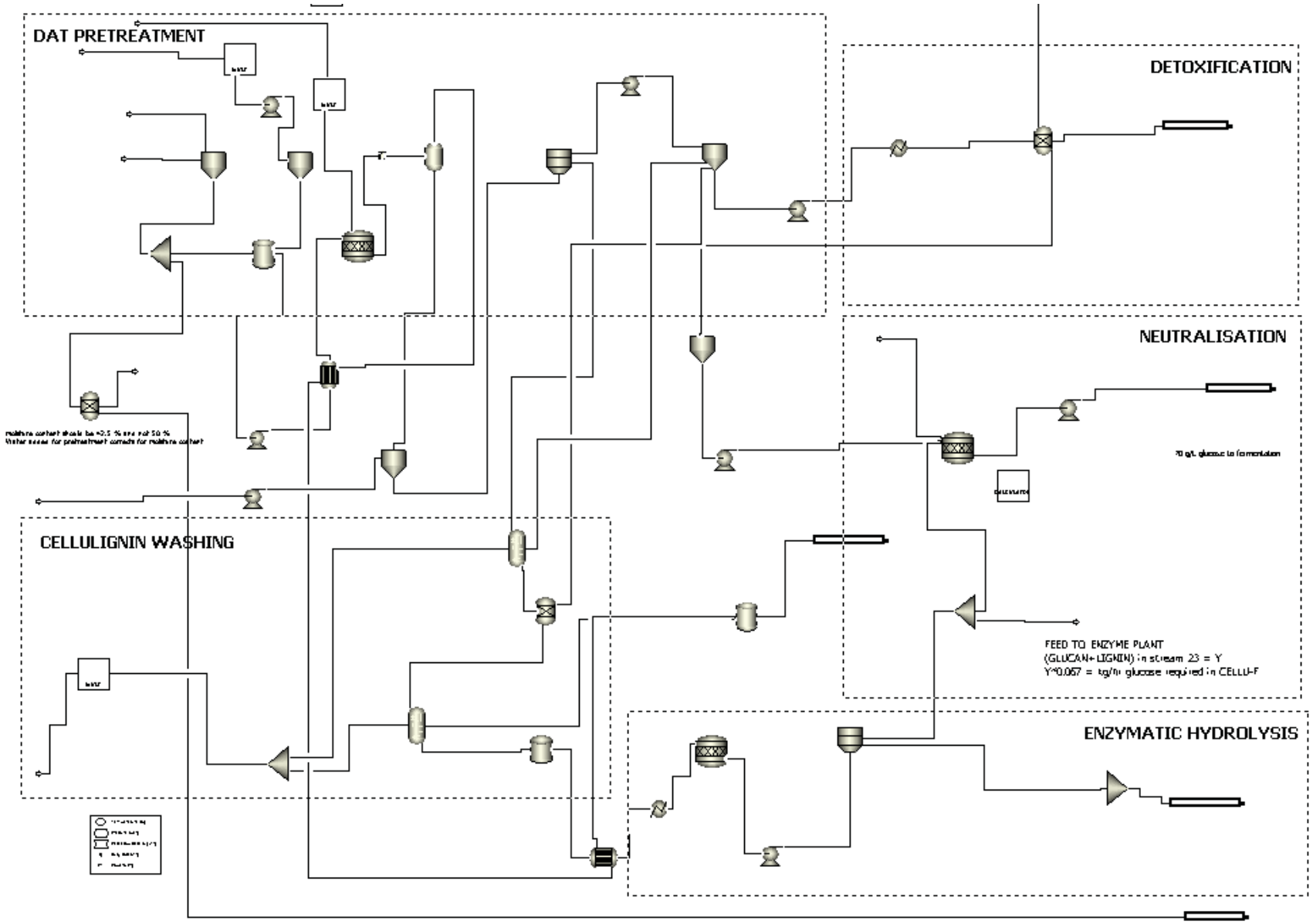

Figure 1: Screenshot of DAT witht EH Aspen Plus® pretreatment simulation

Table 7: DAT with no EH, Pretreatment and Detoxification equipment information (operating conditions, Aspen model specifications, sizing and costing)

EQUIPMENT DESCRIPTION	OPERATING CONDITIONS	DESIGN COMMENT	ASPEN MODEL	SIZING and COST OF EQUIPMENT
Jacketed Screw Pretreatment Reactor	Temperature 165°C, Pressure: 7.0 atm, HPU steam	Removal of hemicellulose without sugar degradation 0.5 % H_2SO_4 at 1:2 solids to liquid ratio for 15 min	RSTOIC	Volumetric feed flow rate: 139.9 m^3/h (from simulation) Reactor schedule: 3 Tanks required Acid pretreatment reactor: 106 079 USD (2009) from Medina *et al.,* (2018)[1]. E-2 Adjusted for year: 148 777 USD (2016 with 556.8 CEPCI value) E-1 Adjusted for capacity from 35m^3 to 139.9 m^3: 130809.5 USD Total for 3 tanks: 392 428.5 USD Note: At more than 10 minutes residence time, the cost difference as calculated from Medina *et al.,* (2018) and Aspen Process Economic Analyzer becomes significant. For example there is a 7.8 % difference between this cost and the cost reported by Aspen Process Economic Analyzer of 364100 USD
Neutralisation step	Temperature 25°C, Pressure: 1 atm, No utility required	Neutralisation reaction prior to detoxification of hemicellulose hydrolysate	RSTOIC	<u>Sizing and cost</u>: Aspen Process Economic Analyzer Ca(OH)2 and NaOH are added in stoichiometric quantitates using a Design specification block. H_2SO_4 + Ca(OH)2 → $CaSO_4$ + 2H_2O; (Fractional conversion, X = 1) Acetic acid + NaOH → Sodium Acetate + H_2O; (X = 1)
GAC Detoxification	Temperature 80°C, Pressure: 1 atm	Adsorption columns using granular activated carbon. Removal rate is based on Hodge *et al.,* (2009) at 0.7 for furfural, 1 for HMF, and 0.00459 for water in the SEP unit.[4]	SEP	<u>Sizing:</u> Contact time of 30 min per column (selected) and a total of 120 minutes residence time is required.[5,6] Therefore a configuration of 5 trains in parallel, each train has 4 columns in series. Thus resulting in a total of 40 columns. The diameter is set at 0,6 meters (assumed). The height is calculated based on the feed rate per train (i.e. total feed rate is divided by 5 to determine the feed rate per column train) using the method described in Towler and Sinnott, (2008) for adsorption columns.[7] <u>Total installed cost:</u> Installed cost per column (Stainless steel material of construction, based on cost calculated with the method provided in Towler and Sinnott, (2008) for a specific height) x 40 columns.[7]

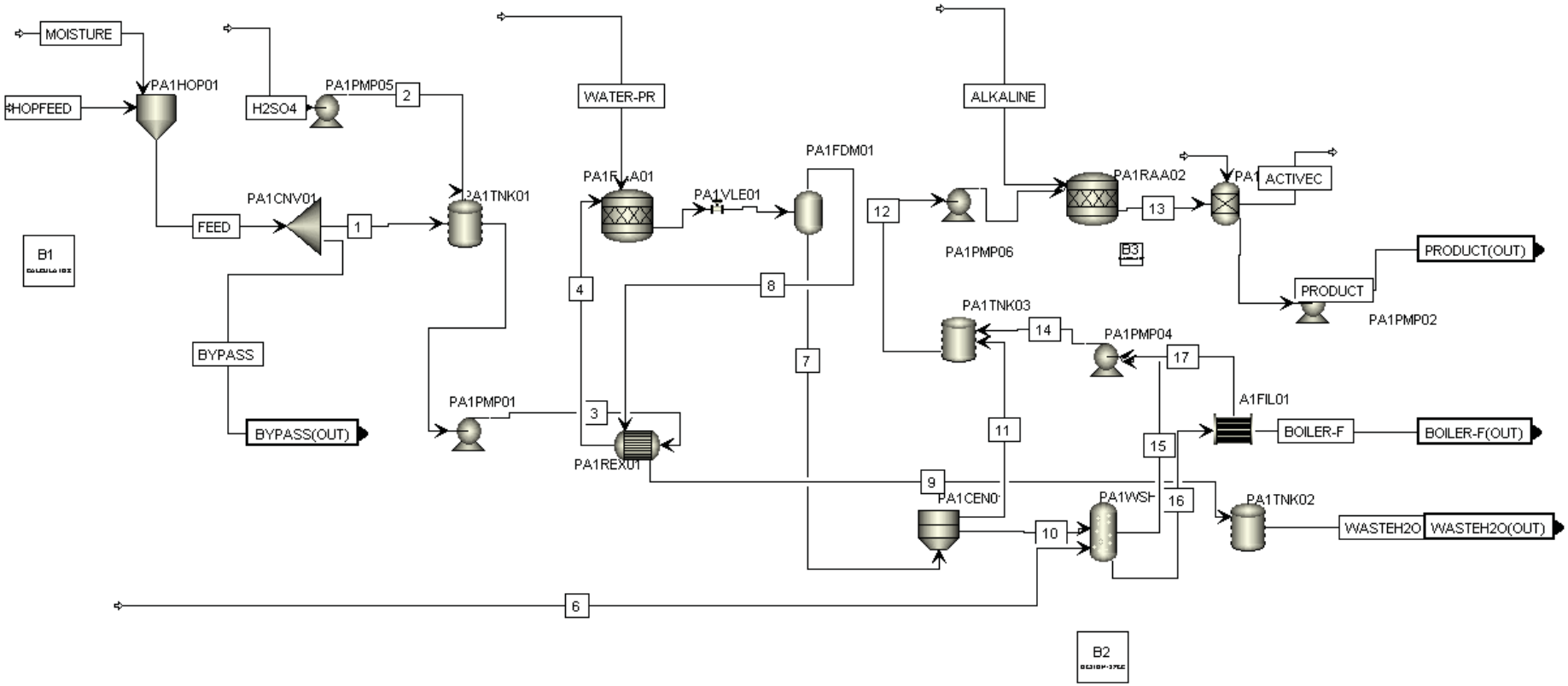

Figure 2: Screenshot of DAT without EH Aspen Plus® pretreatment simulation

Table 8: NaOH Pretreatment, Enzymatic Hydrolysis and Detoxification equipment information (operating conditions, Aspen model specifications, sizing and costing)

EQUIPMENT DESCRIPTION	OPERATING CONDITIONS	DESIGN COMMENT	ASPEN MODEL	SIZING and COST OF EQUIPMENT
Delignification Pretreatment Reactor	Temperature 121°C, Pressure: 2.07 atm, HPU steam	Removal of lignin and hemicellulose for improved enzymatic digestibility. 0.25 M NaOH at 1:2 solids to liquid ratio for 2h.	Mixer RSTOIC	Volumetric feed flow rate: 339.2 m^3/h (from simulation) Reactor schedule: 3 Tanks required Time | 1h | 2h | 3h | 4h | 5h | 6h | … Tank 1 | Fill | Residence time | Fill | Residence time | Fill Tank 2 | Fill | Residence time | Fill | … | … Tank 3 | Fill | Residence time | Fill | … Alkaline reactor: 106 079 USD (2009) from Medina *et al.,* (2018)[1]. E-2 Adjusted for year: 148 777 USD (2016 with 556.8 CEPCI value) E-1 Adjusted for capacity from 35m^3 to 339.2 m^3: 463 158.56 USD Total for 3 tanks: 1 389 475.7 USD
Lignin Recovery	Duty: 0 cal/sec Pressure: 1 atm	Lignin precipitation and filtration. H_2SO_4 is added to obtain a pH of 2. Lignin recovery of 48.2 %wt obtained.	RSTOIC	Sizing and cost: Aspen Process Economic Analyzer Comment: Assumed no residence time was required.
Enzymatic hydrolysis tank(s)	Pressure: 1atm, Duty 0 cal/sec Slurry is diluted to 20 %wt and preheated to 50°C prior to entering the hydrolysis tank.	Cellulase enzymes hydrolyse cellulose into glucose Cellulase added at 20 mg protein per gram cellulignin.	RSTOIC	Sizing Total residence time[8] (R, h) = [30 h] + [12 h allowed to empty and clean tank (assumed value)] Total volume (V, m^3) = R x [Volumetric feed rate (m^3/h)] Number of tanks (N) = V / [30m^3][3]; Total installed cost = N x [installed cost per tank[3]] Comment: Installed cost per tank (30m^3) is based on a jacketed, glass lined reactor.[3]

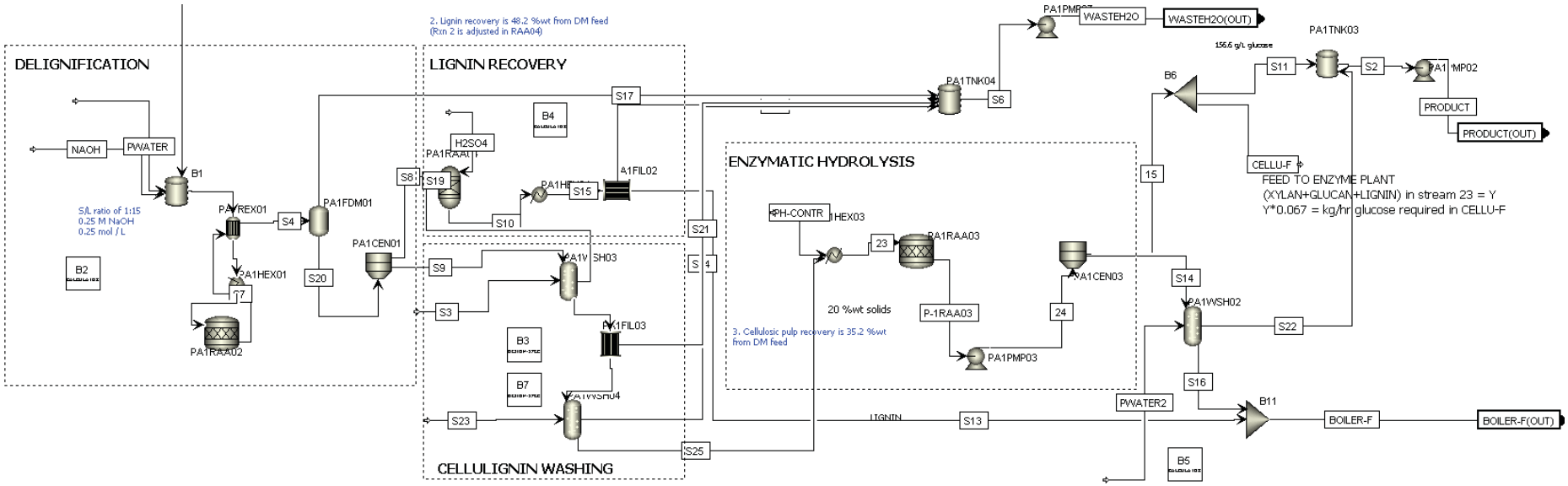

Figure 3: Screenshot of NaOH Aspen Plus® pretreatment simulation

Table 9: Organosolv Pretreatment, Enzymatic Hydrolysis and Detoxification equipment information (operating conditions, Aspen model specifications, sizing and costing)

EQUIPMENT DESCRIPTION	OPERATING CONDITIONS	DESIGN COMMENT	ASPEN MODEL	SIZING and COST OF EQUIPMENT
Organosolv Pretreatment Reactor	Steam: Temperature 208°C, Pressure 18 atm Quantity is determined by design specification for outlet stream conditions of 175°C RStoic: Pressure: 9.77 atm, No Duty	Removal of lignin for improved enzymatic digestibility. Aqueous solvent (50 %v/v ethanol) at 1:5 solids to liquid ratio for 60 min 1.25 wt% H_2SO_4 added as catalyst	RSTOIC	Volumetric feed flow rate: 268.75 m^3/h (from simulation) Reactor schedule: 3 Tanks required Pretreatment reactor: 106 079 USD (2009) from Medina *et al.*, (2018)[1]. E-2 Adjusted for year: 148 777 USD (2016 with 556.8 CEPCI value) E-1 Adjusted for capacity from 35m^3 to 268.75 m^3: 619772.21 USD Total for 3 tanks: 1 859 316.6 USD
Solvent Recovery	Number of stages: 40 Reflux ratio: 0.3 (Mass) Distillate to feed ratio: 0.55 (Mass)	Feed streams: Stage 20 Pressure Stage 1: 4.9 atm Condenser: Total	RADFRAC	Sizing and cost: Aspen Process Economic Analyzer
Enzymatic hydrolysis tank(s)	Pressure: 1atm, Duty 0 cal/sec Slurry is diluted to 20 %wt and preheated to 50°C prior to entering the hydrolysis tank.	Cellulase enzymes hydrolyse cellulose into glucose Cellulase added at 20 mg protein per gram cellulignin.	RSTOIC	Sizing Total residence time[9] (R, h) = [24 h] + [12 h allowed to empty and clean tank (assumed value)] Total volume (V, m^3) = R x [Volumetric feed rate (m^3/h)] Number of tanks (N) = V / [30m^3][3]; Total installed cost = N x [installed cost per tank[3]] Comment: Installed cost per tank (30m^3) is based on a jacketed, glass lined reactor.[3]
Lignin Recovery	Duty: 0 cal/sec Pressure: 1 atm	Lignin precipitation and filtration. H_2SO_4 is added to obtain a pH of 2. Lignin recovery of 48.2 %wt obtained.	RSTOIC	Sizing and cost: Aspen Process Economic Analyzer Comment: Assumed no residence time was required.
Neutralisation step	Temperature 25°C, Pressure: 1 atm, No utility required	Neutralisation reaction prior to detoxification of xylose rich stream.	RSTOIC	Sizing and cost: Aspen Process Economic Analyzer Ca(OH)2 are added in stoichiometric quantitates using a Calculator block. $H_2SO_4 + Ca(OH)2 \rightarrow CaSO_4 + 2H_2O$; (X = 1) The xylose rich stream is sent separately from the glucose rich stream, directly to the triple effect evaporator in the fermentation area to remove any residual ethanol which may act as an inhibitor.

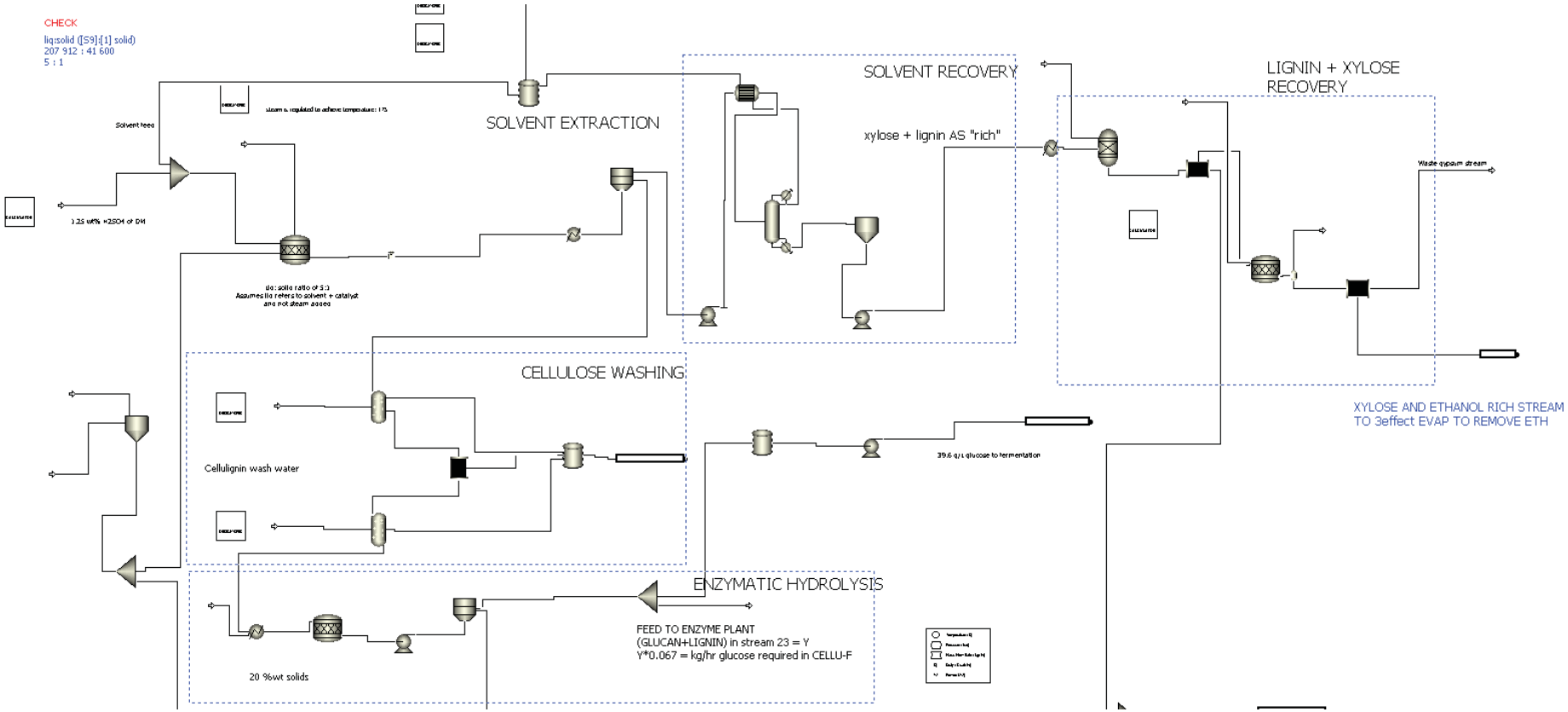

Figure 4: Screenshot of the Organosolv Aspen Plus® pretreatment simulation

Table 10: AFEX™ Pretreatment, Enzymatic Hydrolysis and Detoxification equipment information (operating conditions, Aspen model specifications, sizing and costing)

EQUIPMENT DESCRIPTION	OPERATING CONDITIONS	DESIGN COMMENT	ASPEN MODEL	SIZING and COST OF EQUIPMENT
AFEX™ Pretreatment Reactor	RStoic: Duty: 0 cal/sec Pressure: 0 atm (no change) Feed: Pressure: 1.7 MPa Temperature: 144°C due to heat of mixing.	Disruption of lignocellulose bonds for improved enzymatic digestibility. Pressurised ammonia at 1:1 solid to liquid ratio for 30 min.[10,11]	RSTOIC	Volumetric flow rate: 310.91 m^3/h (from simulation) Reactor schedule: 3 Reactors required. 15 minutes included to charge reactor and 15 min residence time. Pretreatment reactor: 106 079 USD (2009) from Medina *et al.*, (2018)[1]. E-2 Adjusted for year: 148 777 USD (2016 with 556.8 CEPCI value) E-1 Adjusted for capacity from 35m^3 to 268.75 m^3: 619772.21 USD Total for 3 tanks: 1 859 316.6 USD
Ammonia Recovery	Number of stages: 9 Reboiler: None Condenser: None Feed streams: Stage 1 and 9 Pressure Stage 1: 5 atm	High pressure steam (266°C and 13 atm) is depressurised to 5atm (259°C) using a valve and fed to the distillation column to supply it with energy.	RADFRAC	Sizing and cost: Aspen Process Economic Analyzer
Enzymatic hydrolysis tank(s)	Pressure: 1atm, Duty 0 cal/sec Slurry is diluted to 20 %wt and preheated to 50°C prior to entering the hydrolysis tank.	Cellulase enzymes hydrolyse cellulose into glucose Cellulase added at 20 mg protein per gram cellulignin.	RSTOIC	Sizing Total residence time (R, h) = [72 h^2] + [12 h allowed to empty and clean tank (assumed value)] Total volume (V, m^3) = R x [Volumetric feed rate (m^3/h)] Number of tanks (N) = V / [30m^3][3]; Total installed cost = N x [installed cost per tank[3]] Comment: Installed cost per tank (30m^3) is based on a jacketed, glass lined reactor.[3]

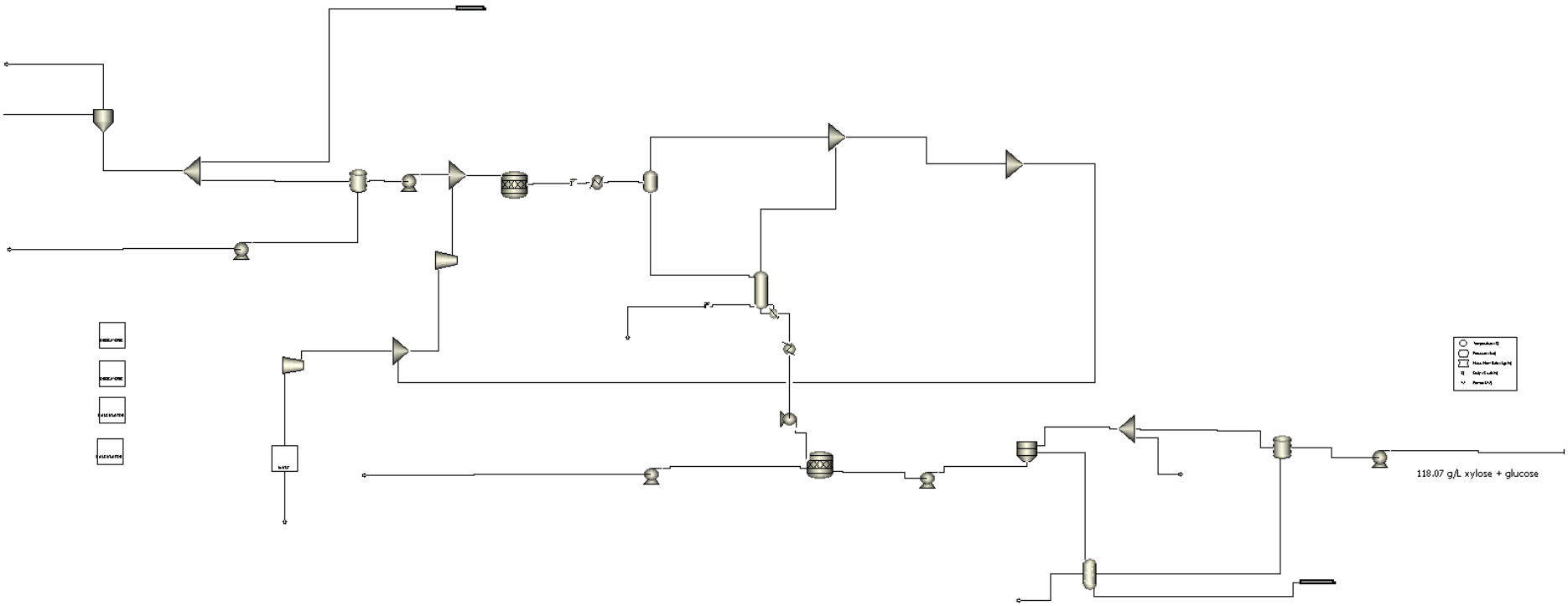

Figure 5: Screenshot of the AFEX™ Aspen Plus® pretreatment simulation

Table 11: STEX Pretreatment, Enzymatic Hydrolysis and Detoxification equipment information (operating conditions, Aspen model specifications, sizing and costing)

EQUIPMENT DESCRIPTION	OPERATING CONDITIONS	DESIGN COMMENT	ASPEN MODEL	SIZING and COST OF EQUIPMENT
STEX Pretreatment Reactor	RStoic Pressure: 13.82 atm (1.4 MPa) Duty: 0 cal/sec Steam is controlled via a Design Spec for outlet stream: Temperature 195°C, Pressure: 13.82 atm,	Autohydrolysis disrupts the biomass and breakdown of hemicelluloses lead to increased enzymatic digestibility. Heated to 195°C under pressure for 7.5 minutes using direct steam injection (208°C and 1.8 MPa (17.8 atm))	RSTOIC	Sizing and cost: Aspen Process Economic Analyzer Mapped as Agitated tank, enclosed and jacketed. Comment: The residence time of 7.5min could be achieved in a continuous operation using a plug flow reactor. The fraction "lost" during steam explosion (26.2 g per 100 g DM) is simulated using a flash drum (Pressure 1 atm and no duty) followed by a centrifuge (100 % solids to solid outlet, 15 % liquid load of solid outlet).
Enzymatic hydrolysis tank(s)	Pressure: 1atm, Duty 0 cal/sec Slurry is diluted to 20 %wt and preheated to 50°C prior to entering the hydrolysis tank.	Cellulase enzymes hydrolyse cellulose into glucose Cellulase added at 20 mg protein per gram cellulignin.	RSTOIC	Sizing Total residence time[12] (R, h) = [96 h] + [12 h allowed to empty and clean tank (assumed value)] Total volume (V, m^3) = R x [Volumetric feed rate (m^3/h)] Number of tanks (N) = V / [30m^3][3]; Total installed cost = N x [installed cost per tank[3]] Comment: Installed cost per tank (30m^3) is based on a jacketed, glass lined reactor.[3]

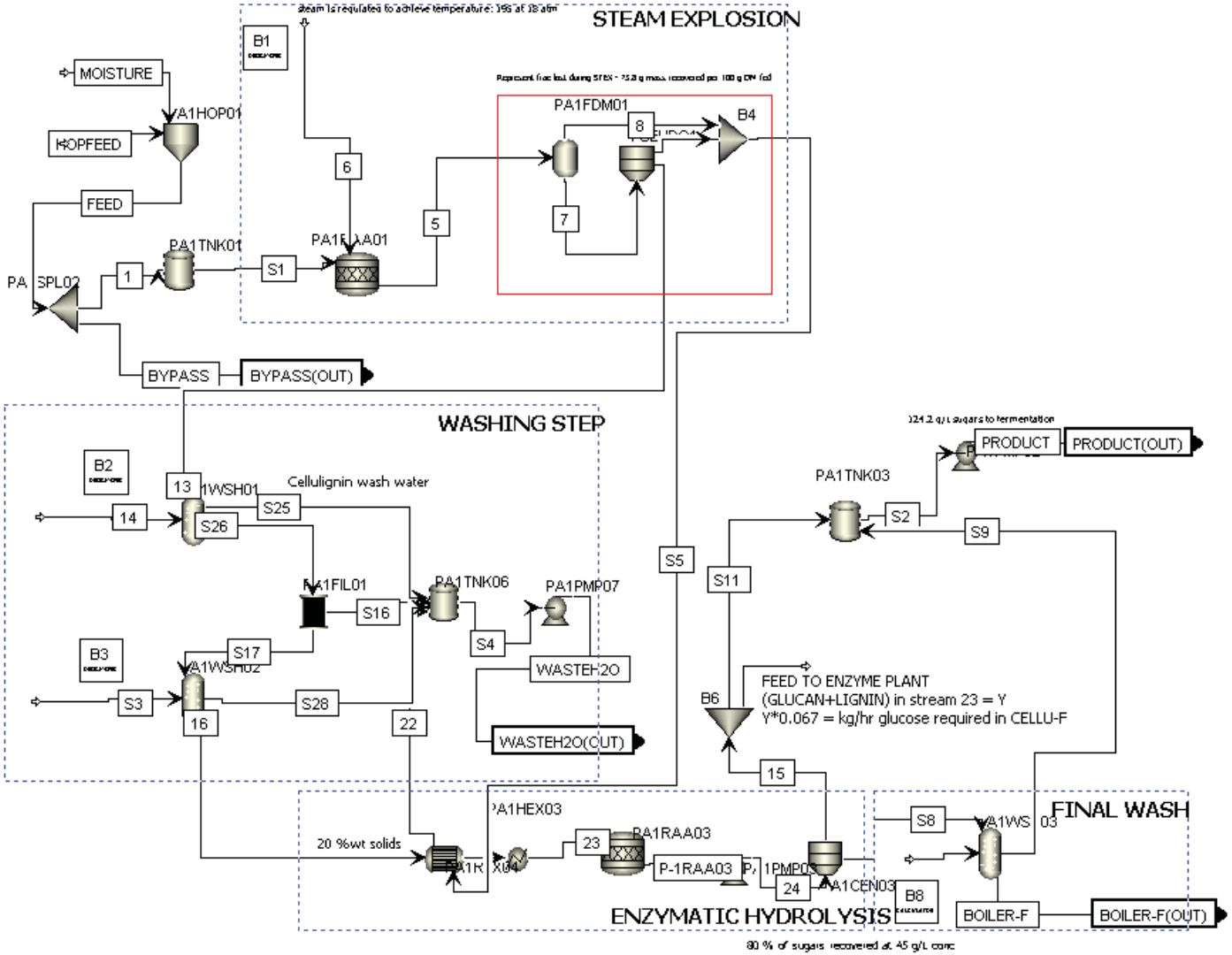

Figure 6: Screenshot of the STEX Aspen Plus® pretreatment simulation

Table 12: STEX with SO_2, Pretreatment, Enzymatic Hydrolysis and Detoxification equipment information (operating conditions, Aspen model specifications, sizing and costing)

EQUIPMENT DESCRIPTION	OPERATING CONDITIONS	DESIGN COMMENT	ASPEN MODEL	SIZING and COST OF EQUIPMENT
SO_2 catalysed STEX Pretreatment Reactor	RStoic Pressure: 16.78 atm (1.7 MPa) Duty: 0 cal/sec Steam (208°C and 1.8 MPa (17.8 atm)) is controlled via a Design Spec for outlet stream: Temperature 190°C, Pressure: 16.78 atm	The pretreatment severity is increased from autohydrolysis in STEX pretreatment by the addition of an acid-generating compound such as sulphur dioxide (SO_2).[12] Biomass is heated to 190°C for 7.5 minutes.	RSTOIC	Sizing and cost: Aspen Process Economic Analyzer Mapped as Agitated tank, enclosed and jacketed. Comment: The residence time of 5min could be achieved in a continuous operation using a plug flow reactor. The feedstock moisture content was increased to 75 wt% and mixed with the SO2 catalyst at 2% mass per mass water content of the feedstock.[13]
SO_2 Reactor	Pressure: 1 atm Duty: 0 cal/sec	Production of SO_2 releases heat, which is used to preheat the biomass slurry for STEX pretreatment.		Sizing and cost: Aspen Process Economic Analyzer $S + O2 \rightarrow SO_2$; (X=1)
Enzymatic hydrolysis tank(s)	Pressure: 1atm, Duty 0 cal/sec Slurry is diluted to 20 %wt and preheated to 40°C prior to entering the hydrolysis tank.	Cellulase enzymes hydrolyse cellulose into glucose Cellulase added at 20 mg protein per gram cellulignin.	RSTOIC	Sizing Total residence time[13] (R, h) = [72 h] + [12 h allowed to empty and clean tank (assumed value)] Total volume (V, m^3) = R x [Volumetric feed rate (m^3/h)] Number of tanks (N) = V / [30m^3][3]; Total installed cost = N x [installed cost per tank[3]] Comment: Installed cost per tank (30m^3) is based on a jacketed, glass lined reactor.[3]
GAC Detoxification	Temperature 80°C, Pressure: 1 atm	Adsorption columns using granular activated carbon. Removal rate is based on Hodge *et al.,* (2009) at 0.3 for furfural, and 0.99541 for water in the SEP unit.[4]	SEP	Sizing: Contact time of 30 min per column (selected) and a total of 120 minutes residence time is required.[5,6] Therefore a configuration of 5 trains in parallel, each train has 4 columns in series. Thus resulting in a total of 40 columns. The diameter is set at 0,6 meters (assumed). The height is calculated based on the feed rate per train (i.e. total feed rate is divided by 5 to determine the feed rate per column train) using the method described in Towler and Sinnott, (2008) for adsorption columns.[7] Total installed cost: Installed cost per column (Stainless steel material of construction, based on cost calculated with the method provided in Towler and Sinnott, (2008) for a specific height) x 40 columns.[7]

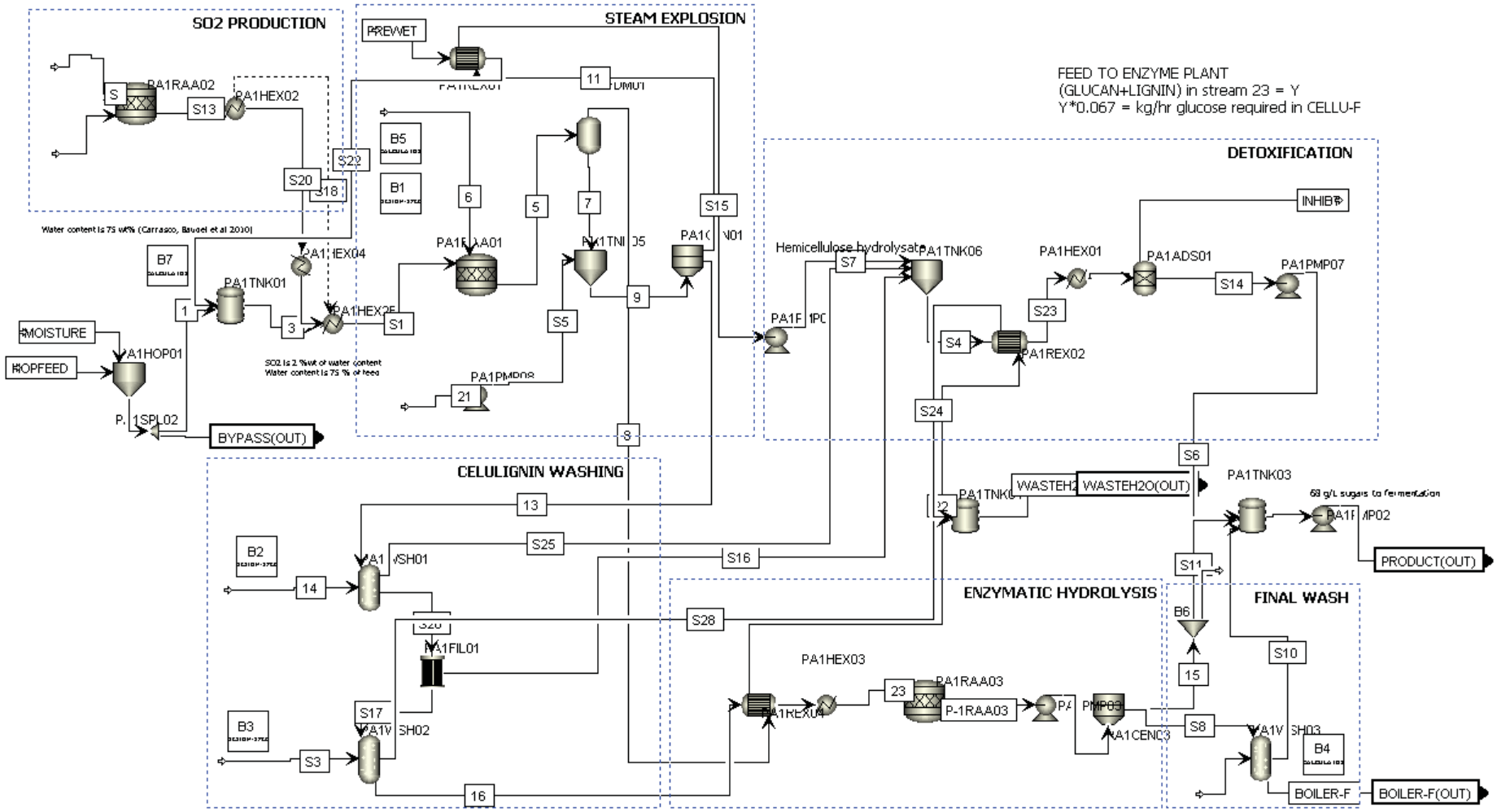

Figure 7: Screenshot of the STEX with SO2 Aspen Plus® pretreatment simulation

Table 13: STEX with NaOH, Pretreatment, Enzymatic Hydrolysis and Detoxification equipment information (operating conditions, Aspen model specifications, sizing and costing)

EQUIPMENT DESCRIPTION	OPERATING CONDITIONS	DESIGN COMMENT	ASPEN MODEL	SIZING and COST OF EQUIPMENT
STEX Pretreatment Reactor	RStoic Pressure: 12.83 atm (1.3 MPa) Duty: 0 cal/sec Steam is controlled via a Design Spec for outlet stream: Temperature 190°C, Pressure: 12.83 atm,	Autohydrolysis breaks down hemicelluloses for increased enzymatic digestibility. Heated to 190°C under pressure for 15 minutes using direct steam injection (208°C and 1.8 MPa (17.8 atm))	RSTOIC	Volumetric flow rate: 5.25 m^3/h (from simulation) Reactor schedule: 5 Reactors required. STEX Pretreatment reactor: 106 079 USD (2009) from Medina *et al.*, (2018)[1]. E-2 Adjusted for year: 148 777 USD (2016 with 556.8 CEPCI value) E-1 Adjusted for capacity from 35m^3 to 5.25 m^3: 38 367.5 USD Total for 5 tanks: 191 837.5 USD
Delignification Reactor	Temperature 100°C, Pressure: 1 atm, HPU steam	Alkaline delignification removes lignin for increased enzymatic digestibility. 1 % w/v NaOH at 1:2 solids to liquid ratio for 60min.	Mixer RSTOIC	Volumetric feed flow rate: 95.44 m^3/h (from simulation) Reactor schedule: 3 Reactors required. Alkaline reactor: 106 079 USD (2009) from Medina *et al.*, (2018)[1]. E-2 Adjusted for year: 148 777 USD (2016 with 556.8 CEPCI value) E-1 Adjusted for capacity from 35m^3 to 95.44 m^3: 245 678.4 USD Total for 3 tanks: 737 035.23 USD
Post Hydrolysis Reactor	Temperature 121°C Pressure 2.07 atm HPU steam	The oligomers are treated with H_2SO_4 at 121°C for 30 minutes and converted to monosaccharides.[14]		Sizing and cost: Aspen Process Economic Analyzer Cellulose (soluble) + H_2O → Glucose; (X=1) Xylan (soluble) + H_2O → Xylose; (X=1)
Neutralisation step	Temperature 25°C, Pressure: 1 atm, No utility required	After post hydrolysis, the added acid is neutralised prior to fermentation to avoid inhibition caused by low pH	RSTOIC	Sizing and cost: Aspen Process Economic Analyzer $Ca(OH)_2$ are added in stoichiometric quantitates using a Calculator block. $H_2SO_4 + Ca(OH)_2$ → $CaSO_4 + 2H_2O$; (X = 0.9)
Enzymatic hydrolysis tank(s)	Pressure: 1atm, Duty 0 cal/sec Slurry is diluted to 20 %wt and preheated to 50°C prior to entering the hydrolysis tank.	Cellulase enzymes hydrolyse cellulose into glucose Cellulase added at 20 mg protein per gram cellulignin.	RSTOIC	Sizing Total residence time[2] (R, h) = [72 h] + [12 h allowed to empty and clean tank (assumed value)] Total volume (V, m^3) = R x [Volumetric feed rate (m^3/h)] Number of tanks (N) = V / [30m^3][3]; Total installed cost = N x [installed cost per tank[3]] Comment: Installed cost per tank (30m^3) is based on a jacketed, glass lined reactor.[3]
Lignin Recovery	Duty: 0 cal/sec Pressure: 1 atm	Lignin precipitation and filtration using H_2SO_4	RSTOIC	Sizing and cost: Aspen Process Economic Analyzer Comment: Assumed no residence time was required.

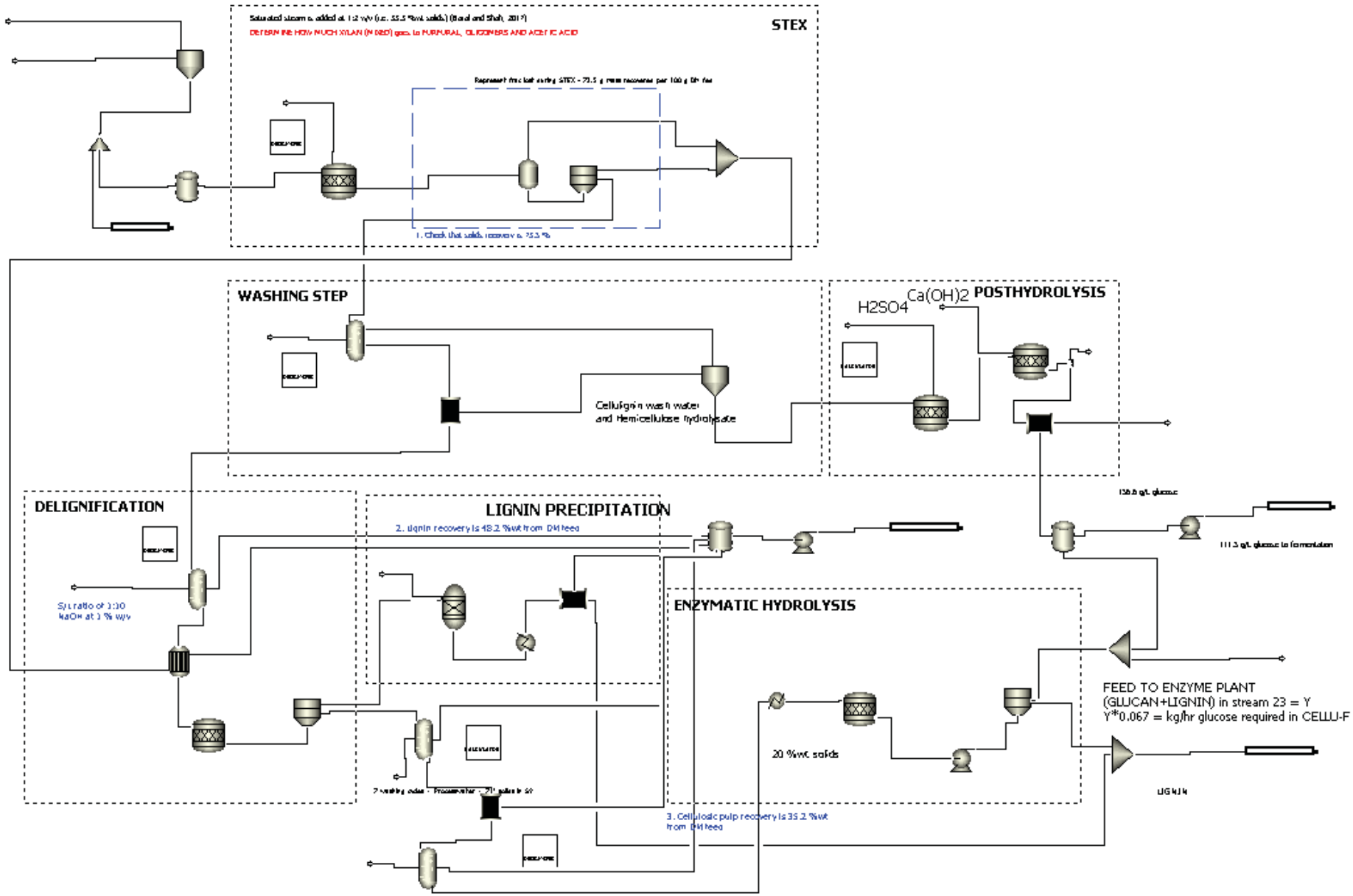

Figure 8: Screenshot of the STEX with NaOH Aspen Plus® pretreatment simulation

Table 14: WO Pretreatment, Enzymatic Hydrolysis and Detoxification equipment information (operating conditions, Aspen model specifications, sizing and costing)

EQUIPMENT DESCRIPTION	OPERATING CONDITIONS	DESIGN COMMENT	ASPEN MODEL	SIZING and COST OF EQUIPMENT
Wet Oxidation Pretreatment Reactor	Temperature 185°C, Pressure: 18.75 atm, HPU steam	Oxygen is used as a catalyst to reduce temperatures and pretreatment time required to solubilise the hemicellulose and lignin fractions for increased enzymatic digestibility.[15] Charged with O_2 at 0.6 MPa (Calculated as 77.45 kg/10 L) Residence time of 10 min.	RSTOIC	Sizing and cost: Aspen Process Economic Analyzer Mapped as Agitated tank, enclosed and jacketed. Comment: The reactor would be filled with O_2 first, sealed, and then heated to 185°C and 1.9 MPa (18.75 atm)
Oxygen Modular plant	Not included in simulation, only in capital cost estimate.			2 183 366 USD Refercence: https://www.slideshare.net/Rahul_Ghalme/cryogenic-air-separation-plant-design. Accessed 19 July 2018.
Enzymatic hydrolysis tank(s)	Pressure: 1atm, Duty 0 cal/sec Slurry is diluted to 20 %wt and preheated to 50°C prior to entering the hydrolysis tank.	Cellulase enzymes hydrolyse cellulose into glucose Cellulase added at 20 mg protein per gram cellulignin.	RSTOIC	Sizing Total residence time[16] (R, h) = [96 h] + [12 h allowed to empty and clean tank (assumed value)] Total volume (V, m^3) = R x [Volumetric feed rate (m^3/h)] Number of tanks (N) = V / [30m^3][3]; Total installed cost = N x [installed cost per tank[3]] Comment: Installed cost per tank (30m^3) is based on a jacketed, glass lined reactor.[3]

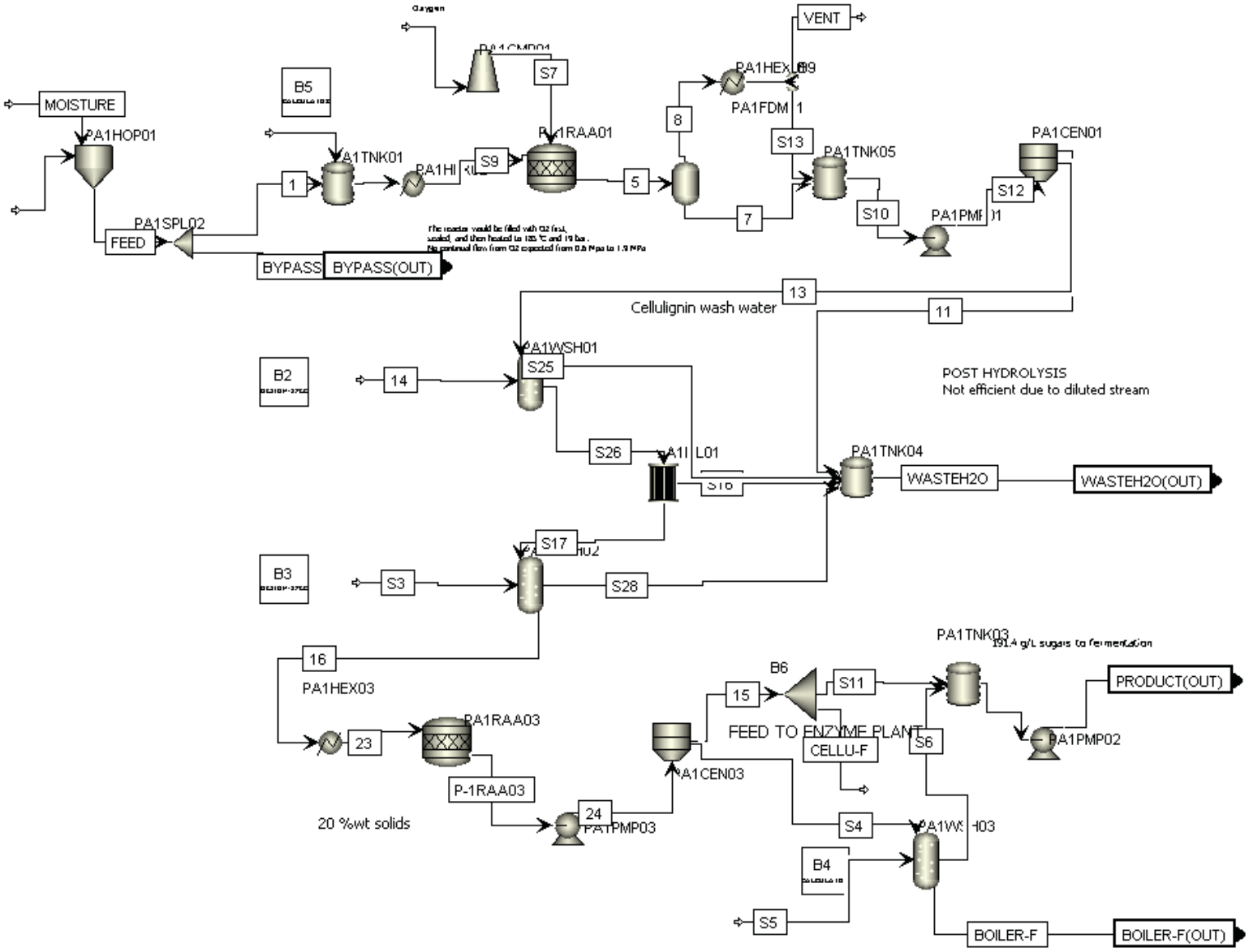

Figure 9: Screenshot of the WO Aspen Plus® pretreatment simulation

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A5. Appendix E: Chapter 6 Supplementary information

Supplementary information

Life cycle assessment and multi-criteria analysis of sugarcane biorefinery scenarios: finding a sustainable solution for the South African sugar industry

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1. Allocation sensitivity analysis

The ISO 14044 standard recommends that the use of allocation should be avoided. Ideally, 100 % of the environmental burden should be attributed to a single product. However this is not always possible due to multiple products resulting from a process[1]. In that case, the environmental burden can be allocated across products based on some physical value, with the most common options being the products' mass or economic value[1].

Sugar processing produces sugar, bagasse, molasses and filter cake, but these products differ in mass and value with bagasse and filter cake waste by-products having an apparent value of 0 US$/t. Therefore, if the allocation is based on economic value, the environmental burden associated with sugarcane cultivation is only allocated to sugar and molasses and none to the bagasse and trash feedstock. To this end, the environmental impact of the scenarios are compared between mass and economic allocation in an allocation sensitivity analysis using the IMPACT 2002+ V2.14 Single score method for a functional unit of 1 kWh generated.

The economic and mass allocation for the sugarcane cultivation and sugar production (mill and refinery) are provided in Table 1. The same approach is applied for each process area, for the economic allocation, where 0 % is attributed to the waste or by-product streams and 100 % is allocated to the valuable or main product, such as fermentable sugars in the pretreatment area (PA-100) or fermentation broth (PA-300) in the fermentation process area.

Table 1: Mass and Economic allocation for sugarcane cultivation and sugar processing

Process stage	Products	Economic allocation	Mass allocation
Sugarcane cultivation	Harvested cane	100 %	93.75 %
	Tops and trash	0 %	6.25 %
	Total	100 %	100 %
Sugar Production	Sugar	67.4 %	27.86 %
	Molasses	32.6 %	11.42 %
	Filter cake	0 %	18.94 %
	Bagasse	0 %	41.78 %
	Total	100 %	100 %

The mass allocated results are higher (33 – 66 %) than the economic allocation due to the relative cost of bagasse to sugar (Table 4). The impact on ecosystem quality, climate change and resources are also underestimated compared to the mass allocated results due to the negligible impact attributed to sugarcane cultivation for the production of 1 kWh generated electricity.

Overall, the relative environmental contributions of the scenarios is similar between the two allocation methods as seen in Figure 12, and either could be used. The economic allocation could be improved by assigning monetary value to the bagasse and trash, and thereby attribute environmental burdens to these by-products for the economic allocation.

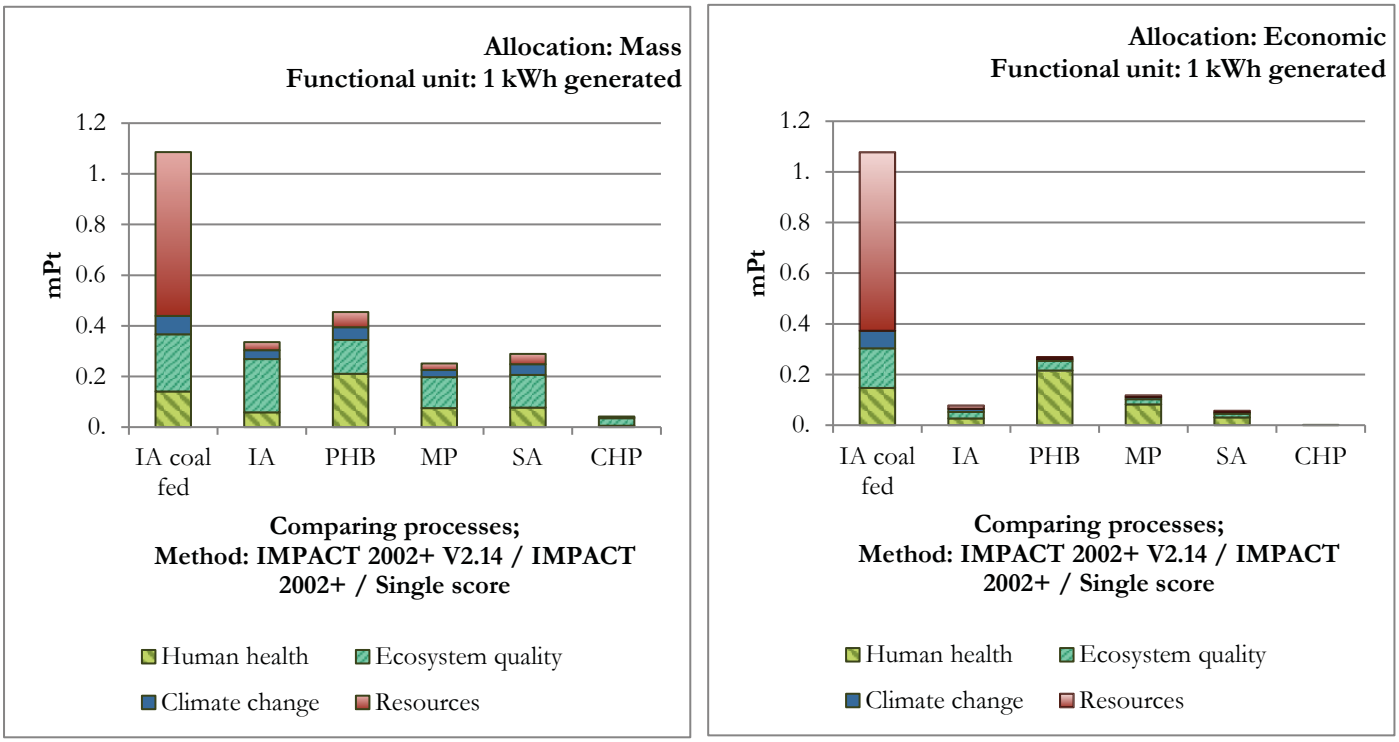

Figure 1: Single score results for mass (left) and economic (right) allocation used

2. LCIA and comparison of scenarios using damage orientated method

The results for the end-point, damage orientated IMPACT 2002+ V2.14 methodology were used for the comparison of biorefinery scenarios based on 1 kg bio-product (Figure 2a) and 1 kWh generated electricity (Figure 2b). Due to the small PHB production volume of the biorefinery in Scenario 3 (PHB), the environmental damage caused by 1 kg PHB is substantial. However, when the functional unit of 1 kWh is considered, the impact of the coal used on the CHP plant for Scenario 1 become significant across all impact categories, with 1 kWh generated in Scenario 6 (CHP) causing the least amount of environmental damage.

The marine eco-toxicity impact category breakdown for 65 t/h lignocellulosic feedstock (bagasse and trash) is shown in Figure 3.

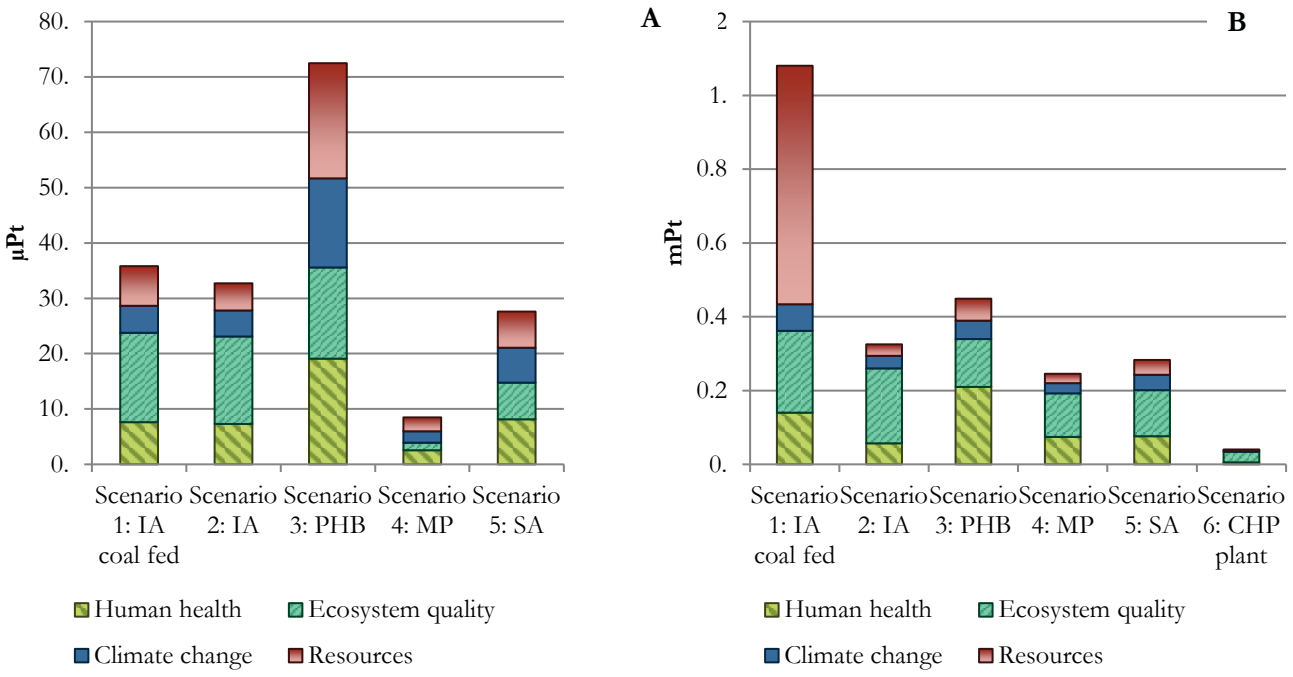

Figure 2: LCIA comparison of biorefinery scenarios (LEFT: based on 1 kg bio-product; RIGHT: based on 1 kWh generated)

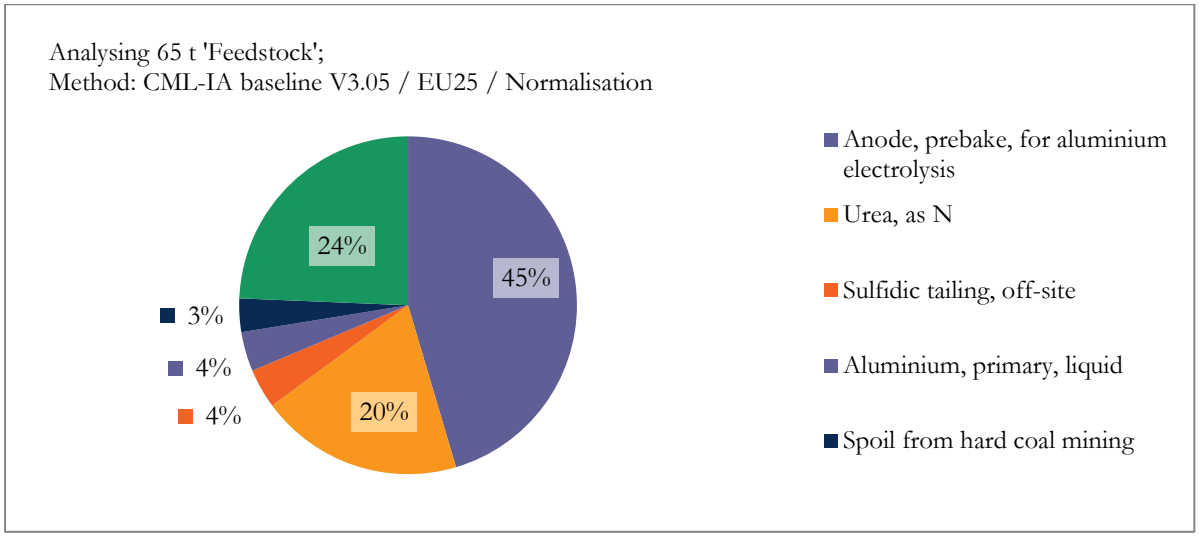

Figure 3: Marine eco-toxicity impact category breakdown for 65 t/h sugarcane lignocellulosic feedstock

3. Technical readiness level (TRL) selection of each plant area

The reported TRL for the production of itaconic acid, succinic acid and PHB from glucose is high, with PHB production at TRL 7, succinic acid at TRL 8 and itaconic acid at a TRL 8-9 [2]. However sugarcane lignocelluloses pretreatment and the production of these bio-products from pretreated sugarcane lignocelluloses are still in the research to pilot scale phase with a TRL 1 - 5. The various plant areas are thus discussed to identify the areas where additional research and development work are required most.

3.1 Pretreatment, detoxification and enzymatic hydrolysis (PA-100) TRL

Dilute acid treatment (DAT) and steam explosion (STEX) pretreatment technologies are in the preliminary design and pilot scale operation stage with a TRL ≥5 [3]. A TRL of 5 has been assigned to STEX pretreatment. The DAT received a slightly lower TRL of 4 due to the equipment design consideration required for a higher solids to liquid ratio (Chapter 5). Likewise, a TRL of 3 was assigned to enzymatic hydrolysis due to the simulation assumption made for a high (20 %) enzymatic hydrolysis solids loading [3]. A TRL of 9 was assigned to the cellulase modular plant based on the commercial Novozymes® facility.

Although commercial fermentation of these bio-products exist, they are based on glucose and not pretreated lignocelluloses. Therefore the upstream conditions of the experiments that the Aspen Plus® simulations are based on differ slightly from the upstream conditions reported in literature. These differences include the feed stream particle size distribution, the enzyme cocktail used (i.e. combination of different cellulases: endoglucanase, exo-glucanase and β-glucosidase) and the measured impact of the equipment design on the enzymatic hydrolysis yields and residence time.

3.2 Itaconic acid production TRL

The itaconic acid fermentation received a TRL of 2 for the proof of concept phase [4]. The technical readiness of itaconic acid fermentation can be improved by conducting bench scale experiments used to investigate the yield of itaconic acid from detoxified hemicellulose hydrolysate, such as the recent study by Saha *et al.,* (2018) for pretreated wheat straw [4]. The crystallisation and evaporation downstream process (DSP) received a TRL of 8 since it is technically mature [5], with the same itaconic acid recovery scheme reported in literature from 1952 to 2014 [6–8]. Nonetheless the DSP scheme should be verified for the specific fermentation broth and is outdated with regards to more energy efficient technologies available such as membrane-integrated hybrid bio-reactor systems (Pal, Dekonda and Kumar, 2015).

3.3 Succinic acid production TRL

Various succinic acid producing micro-organisms and fermentation strategies have been investigated to improve fermentation conditions and demonstrate succinic acid production. Therefore the biochemical fermentation step is considered to be within the process development to pre-pilot study phase [9,10], with a TRL of 4. The solvent extraction used in the DSP should be tested on the simulated fermentation broth to confirm the product recovery and purity [11,12], and ensure that no by-product migrates into the organic solvent phase which will require additional processing steps to remove. Therefore the succinic acid DSP has a TRL of 7.

3.4 PHB production TRL

The PHB fermentation and DSP are within the process development to pre-pilot study phase with a TRL of 4. The fermentation results could be verified for the specific pretreated sugarcane lignocelluloses simulated, since the characteristics of the PHB produced are sensitive to the feedstock and fermentation conditions. However the technical readiness of the alkaline DSP can be considered high, and therefore receives the same TRL of 7 as the overall commercial PHB process [2], since the same micro-organism is used as reported in literature (Recombinant *E. coli* XL1-Blue).[13,14]. Therefore no significant changes in the DSP feed stream are expected.

Finally, the co-firing of biomass with coal is in the demonstration phase and received a TRL of 7, although the CHP plant is proven technology. The TRL of the plant areas for the respective scenarios are provided in Table 8

4. LCI parameters and values for sugarcane cultivation, sugar production and Scenario 1 - 6

The LCI values for sugarcane cultivation and transportation are provided in Table 2. The LCI values for sugar production is provided in Table 3. These values are similar across scenarios since all the scenarios are based on a typical sugar cane mill and 65 t/h (dry mass) biomass feedstock.

The Input and Output parameters used in SimaPro® v8.0 for each scenario as shown in Figure 4 are provided in Table 4.

Table 2: Sugarcane cultivation and transportation LCI values [15,16]

Sugar cane cultivation and Transportation	Value	Values used in LCI: Scaled for a typical sugar mill
Cultivation area	40 000 ha	5 ha
Average cane harvest	60 t/ha	300 t
Tops and trash (50 % of total available) [17]	7.5 % [17]	22.5 t (20 t dry) [17]
Irrigation water (20 % of total area)	8000 m^3/ha	8000 m^3
Irrigation electricity consumption	108 kWh/ha	108 kWh
Nitrous oxide (NO_2) emissions to air	1.25 % of Nitrogen input	7.5 kg
NO_x emissions to air	0.5 % of Nitrogen input	3 kg
Fertilizer used (per ha)	120 kg Urea (Nitrogen),	600 kg
	30 kg Di ammonium phosphate,	150 kg
	125 kg Potassium oxide (KCl)	625 kg
Herbicide used	26.9 g/t sugar cane	8.07 kg
Pesticide used	2.21 g/t sugar cane	0.66 kg
Transportation by road (94 % of cane)	Average distance: 25 km Truck: 1.08 MJ/tkm	7614 MJ
Transportation by rail (6 % of cane)	Average distance: 50 km Train: 0.68 MJ/tkm	612 MJ
Collection of trash, leaves and tops [b]	Average distance: 50 km Truck: 1.08 MJ/tkm	1350 MJ

a) 27.8 % of sugarcane; 45 t/h dry tonnes; b) Based on assumption for Green cane harvesting

Table 3: Sugar production LCI values [15]

Sugar production	Value	Values used in LCI: Scaled for a typical sugar mill
Sugar produced	6 t/ha	30 t
Bagasse produced	27.8 % of cane	83.4 t (45 t dry)
Molasses produced	4.1 % of cane	12.3 t
Filter cake produced	6.8 % of cane	20.4 t
Water use for cane processing [16]	0.6 m^3/t cane	180 m^3
Pollutant loadings of COD [16]	3320 g/t cane	996 kg
Pollutant loadings of BOD5 [16]	1590 g/t cane	477 kg

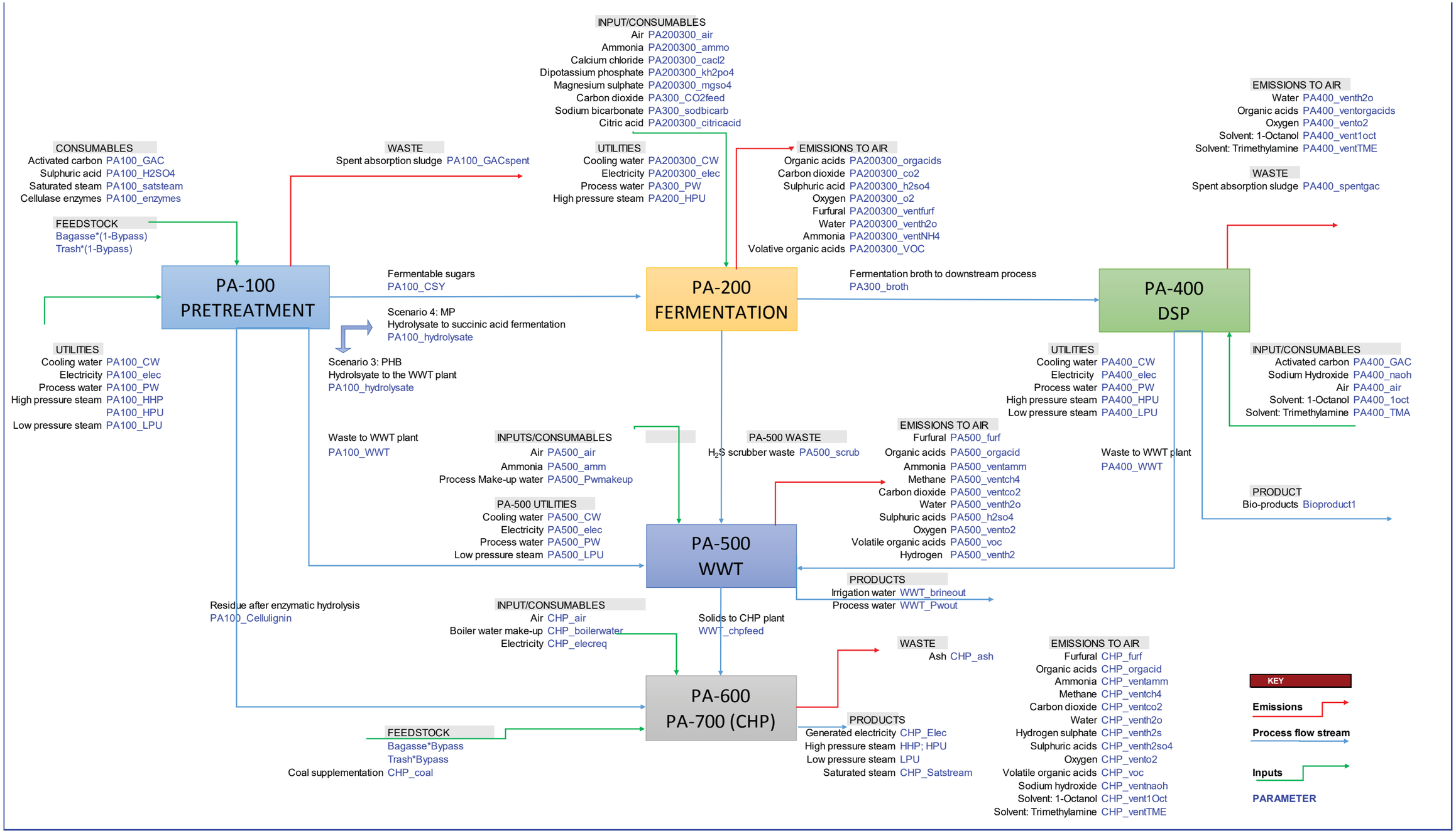

Figure 4: Generic PFD (process flow diagram) with variables for the parameter values used in the SimaPro calculation setup

Table 4: Parameters names and values of each scenario used in SimaPro calculation setup

Parameters	Scenario 1: IA coal	Scenario 2: IA	Scenario 3: PHB	Scenario 4: MP	Scenario 5: SA	Scenario 6: CHP
PA400_CW	84796.4	39943.1	0.0	2097558.0	3598030.0	0.0
PA400_elec	2.9	1.4	36.8	456.6	883.3	0.0
PA400_GAC	0.6	0.3	0.0	0.0	0.0	0.0
PA400_PW	330.0	151.8	81571.0	18791.1	9696.1	0.0
PA400_spentgac	5449.1	2498.5	0.0	0.0	0.0	0.0
PA400_venth2o	324.8	0.1	8139.3	73.3	104.7	0.0
PA400_ventorgacids	34.3	0.0	0.0	0.0	0.0	0.0
PA100_CW	180621.0	83074.7	0.0	0.0	0.0	0.0
PA100_elec	24.2	11.2	28.5	26.9	10.3	0.0
PA100_GAC	13.9	6.5	0.0	17.0	0.0	0.0
PA100_GACspent	1006.8	465.0	0.0	920.0	0.0	0.0
PA100_H2SO4	563.3	259.0	490.0	389.0	0.0	0.0
PA100_HHP	86360.6	64674.8	0.0	0.0	0.0	0.0
PA100_PW	207800.0	96020.0	196550.0	142113.0	210424.0	0.0
PA200300_orgacids	0.1	0.0	0.0	14.1	0.0	0.0
PA200300_air	24469.4	2763.0	19366.0	5672.0	0.0	0.0
PA200300_ammo	340.0	147.0	696.0	415.9	882.5	0.0
PA200300_cacl2	348.0	188.3	0.0	0.0	0.0	0.0
PA200300_co2	396.5	229.2	0.0	0.0	0.0	0.0
PA200300_CW	81352.1	840525.0	3057711.2	1889707.3	770301.3	0.0
PA200300_elec	34.7	16.9	336.9	37.1	236.5	0.0
PA200300_h2so4	0.0	0.0	0.0	0.0	0.0	0.0
PA200300_kh2po4	55.9	30.2	5383.3	1001.3	995.2	0.0
PA200300_mgso4	69.6	37.7	171.8	33.5	399.4	0.0
PA200300_o2	1351.4	826.9	0.0	0.0	0.0	0.0
PA200300_ventfurf	0.0	0.0	0.0	0.5	0.0	0.0
PA200300_venth2o	62.0	35.3	0.0	532.2	0.0	0.0
PA200300_ventNH4	1.0	0.5	0.0	0.0	0.0	0.0
PA200300_VOC	0.0	0.0	0.0	0.0	0.0	0.0
CHP_air	875814.0	984112.0	1075547.0	1017312.0	866660.0	1178457.0
CHP_ash	3065.6	3023.0	3592.0	3249.0	3151.0	2982.0
CHP_boilerwater	86360.6	39726.0	75133.0	59811.0	14293.0	0.0
CHP_Elec	7291.5	7276.8	7437.3	7687.2	8875.8	54378.2
CHP_elecreq	111.9	895.2	1040.0	984.3	847.0	1169.5
CHP_furf	1.4	1.0	1.3	0.8	0.0	0.0
CHP_orgacid	0.4	0.2	0.2	11.4	1.3	0.0
CHP_ventamm	1.9	0.8	71.7	17.0	7.4	0.0
CHP_centch4	20.3	9.4	2.2	23.9	12.4	0.0
CHP_ventco2	76817.2	100550.0	8721.2	95298.1	83287.3	119513.0
CHP_venth2o	44903.4	79133.7	1244.9	58753.9	57256.5	85743.3
CHP_venth2s	1.6	0.8	7.6	5.9	0.0	0.0
CHP_venth2so4	228.5	108.4	450.6	356.6	0.0	0.0
CHP_vento2	137146.0	146408.0	168534.0	158510.0	131287.0	179235.0
CHP_voc	38.1	25.2	24.9	23.3	604.1	10.3
HHP	288949.0	133586.0	0.0	0.0	0.0	0.0
HPU	0.0	0.0	66473.0	70494.0	64815.0	15050.0
LPU	0.0	0.0	5300.0	4670.0	13970.0	0.0
PA300_sodbicarb	0.0	0.0	0.0	1531.5	3929.1	0.0
CHP_ventsb	0.0	0.0	0.0	212.7	3929.1	0.0
CHP_vent1Oct	0.0	0.0	0.0	180.4	432.4	0.0
CHP_ventTME	0.0	0.0	0.0	0.1	0.3	0.0
PA100_HPU	0.0	0.0	12054.4	9563.2	0.0	0.0

Parameters	Scenario 1: IA coal	Scenario 2: IA	Scenario 3: PHB	Scenario 4: MP	Scenario 5: SA	Scenario 6: CHP
PA500_air	4359.3	1160.0	70664.1	5202.0	10219.0	0.0
PA500_amm	100.0	50.0	0.0	0.0	0.0	0.0
PA500_cod	1763.4	629.4	1236.8	895.8	2211.9	0.0
PA500_CW	613660.0	143706.0	541675.0	38726.4	530414.6	0.0
PA500_elec	848.3	400.9	1058.3	660.5	1105.7	0.0
PA500_furf	0.0	0.1	3.4	0.0	0.0	0.0
PA500_orgacid	0.0	0.0	0.2	0.0	0.9	0.0
PA500_Pwmakeup	0.0	0.0	0.0	0.0	15261.1	0.0
PA500_scrub	0.7	0.6	4.0	3.2	0.0	0.0
PA500_ventamm	5.9	2.6	17.6	18.7	0.0	0.0
PA500_ventch4	1.3	1.0	2.2	1.2	4.8	0.0
PA500_ventco2	1045.6	688.5	8721.2	1869.5	3671.2	0.0
PA500_venth2o	597.2	355.3	1244.9	326.7	1404.0	0.0
PA500_venth2so4	0.0	0.0	0.0	0.0	0.0	0.0
PA500_vento2	471.1	225.3	787.0	745.6	417.5	0.0
PA500_voc	0.0	0.0	0.0	0.0	0.0	0.0
Bagasse	40000.0	40000.0	40000.0	40000.0	40000.0	40000.0
Bioproduct1	12178.8	5601.2	2680.0	11774.0	19309.0	0.0
Bypass	0.0	0.5	0.1	0.3	0.4	1.0
CHP_Satstream	86360.6	39726.0	75133.0	59811.0	14315.0	0.0
PA100_Cellulignin	47362.2	21789.0	41209.0	32683.0	22639.0	0.0
PA100_CSY	88384.8	40652.0	128998.0	25591.0	143580.0	0.0
PA100_WWT	259185.0	119663.0	22542.0	18706.0	120451.0	0.0
PA300_broth	93142.8	43384.0	28505.0	172466.0	335156.0	0.0
PA400_WWT	75485.9	35474.0	99902.0	183657.0	323934.0	0.0
Trash	25000.0	25000.0	25000.0	25000.0	25000.0	25000.0
WWT_brineout	52456.3	21085.0	40726.0	27208.0	10600.0	0.0
WWT_chpfeed	11300.1	8154.0	36454.0	18724.0	15943.0	0.0
WWT_Pwout	270450.0	125264.0	359330.0	218442.0	380664.0	0.0
CHP_coal	25089.6	0.0	0.0	0.0	0.0	0.0
PA100_satsteam	0.0	0.0	75133.0		14293.0	0.0
PA100_hydrolysate	0.0	0.0	167439.0	193505.0	0.0	0.0
PA200_HPU	0.0	0.0	44468.5	22137.7	2533.7	0.0
PA500_LPU	0.0	0.0	3601.4	744.4	6253.2	0.0
PA500_venth2	0.0	0.0	0.0	0.0	0.0	0.0
PA400_vento2	0.0	0.0	304.0	411.7	605.4	0.0
PA400_naoh	0.0	0.0	644.8	213.0	0.0	0.0
PA400_HPU	0.0	0.0	9886.5	38754.2	62232.6	0.0
PA400_LPU	0.0	0.0	1674.5	3914.3	7708.1	0.0
PA400_air	0.0	0.0	1305.0	5918.0	2900.0	0.0
CHP_ventnaoh	0.0	0.0	644.8	0.0	0.0	0.0
CHP_venth2s	0.0	0.0	0.0	0.0	0.0	0.0
PA200300_WWT	0.0	0.0	0.0	63891.0	398.0	0.0
PA300_PW	0.0	0.0	0.0	0.0	176513.4	0.0
PA300_CO2feed	0.0	0.0	0.0	2139.0	10649.0	0.0
PA100_LPU	0.0	0.0	2.8	2.2	1.9	0.0
PA400_1oct	0.0	0.0	0.0	223.4	467.8	0.0
PA400_TMA	0.0	0.0	0.0	2.4	5.4	0.0
PA400_vent1oct	0.0	0.0	0.0	0.0	0.0	0.0
PA400_ventTME	0.0	0.0	0.0	0.0	0.3	0.0
PA200300_citricacid	0.0	0.0	171.8	7.5	0.0	0.0
PA100_enzymes	525.5	241.8	457.2	362.6	373.7	

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