A TECHNO-ECONOMIC EVALUATION OF INTEGRATING FIRST AND SECOND GENERATION BIOETHANOL PRODUCTION FROM SUGARCANE IN SUB-SAHARAN AFRICA

By

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ABSTRACT

Climate change that results from greenhouse gases (GHG's) released from the burning of fossil fuels, together with the rising price of oil, have sparked interest in renewable biofuels. The production of biofuels also presents potential socio-economic benefits.

There are two types of technologies for bioethanol production:

- First generation bioethanol is produced from food feedstocks such as juice of sugarcane.
- Second generation bioethanol is produced from non-food feedstocks (lignocellulosic materials).

This project is concerned with 1st and 2nd generation bioethanol production from sugarcane juice and bagasse and the integration of these technologies. This project comprises a combination of experimental and process modelling work to assess energy efficiencies and the economic viability of integrated and stand-alone processes in the sub-Saharan African context.

First generation fermentation experiments were conducted and high ethanol concentrations of up to 113.7 g/L were obtained. It was concluded that a recombinant yeast strain may be able to replace a natural hexose fermenting yeast for 1st generation fermentations to reduce costs. 2nd generation fermentation experiments were performed and ethanol concentrations of close to 40 g/L were obtained. Combinations of 1st and 2nd generation fermentation experiments were performed to improve the 2nd generation fermentation. In one of the experiments it was concluded that the combination of 1st and 2nd generation fermentations significantly improved the 2nd generation fermentation with an overall ethanol concentration of 57.6 g/L in a shorter time than for the pure 2nd generation experiments.

It was determined from washing and pressing experiments that pressing the pre-hydrolysate liquor out of the pre-treated bagasse will sufficiently lower the levels of inhibitors in a 2nd generation fermentation when using a hardened yeast.

Some of the data from the 1st generation experiments were used along with literature data to model a first generation process in Aspen Plus® which processes 493 tons of cane per hour (tc/hr). Pinch heat integration was used to reduce the utility requirements. The process used the bagasse that was generated to co-produce steam and electricity. The excess electricity was sold for additional revenue. In one scenario the excess bagasse was determined at 57.5%. This bagasse was sold to a stand-alone

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2nd generation plant. The first generation process produced 85.5 litres of ethanol per ton of cane (L/tc), the integrated process produced 128 L/tc while the stand-alone 2nd generation process produced 185 litres of ethanol per ton of bagasse (50% moisture) or 25.5 L/tc. The amount of excess electricity that was produced ranged from 14.3 to 70.2 kWh/tc.

Economic analyses were performed using South African economic parameters to resemble the sub-Saharan African context. Data from the 1st generation process model and literature data for integrated 1st and 2nd generation and stand-alone 2nd generation processes were used for the analyses. It was found that the integrated plant is the most economically viable (IRR = 11.66%) while the 1st generation process basically broke even (IRR = 1.62%) and the 2nd generation process is unviable. This was as a result of high sugarcane prices and too few incentives for 2nd generation ethanol.

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OPSOMMING

Klimaatsverandering wat veroorsaak word deur kweekhuisgasse wat vrygestel word deur die verbranding van fossielbrandstowwe en die stygenede olieprys het belangstelling in hernubare biobrandstowwe laat opvlam. Die produksie van biobrandstowwe hou ook potensiële sosio-ekonomiese voordele in.

Daar is twee tegnologieë vir bioetanol produksie:

- Eerste generasie bioetanol word vanaf voedsel bronne soos suikersap geproduseer.
- Tweede generasie bioetanol word van nie-voedsel bronne (lignosellulose materiaal) geproduseer.

Hierdie projek handel oor 1ste en 2de generasie bioetanol produksie van suikersap en suikerriet bagasse en die integrasie van hierdie tegnologieë. Hierdie projek bestaan uit 'n kombinasie van eksperimentele- en prosesmodellering werk om die energiedoeltreffendheid en ekonomise vatbaarheid van geïntegreerde en alleenstaande prosesse in die sub-Sahara konteks te ondersoek.

Eerste generasie fermentasie eksperimente is uitgevoer en hoë etanol konsentrasies van tot 113.7 g/L is gekry. Dit was bepaal dat 'n rekombinante gisras 'n natuurilke heksose fermenterende gisras kan vervang vir 1ste generasie fermentasies om kostes te bespaar. 2de generasie fermentasie eksperimente is gedoen en etanol konsentrasies van amper 40 g/L is behaal. Kombinasies van 1ste en 2de generasie fermentasie-eksperimente was uitgevoer om die 2de generasie fermentasie te verbeter. In een van die eksperimente is dit bepaal dat die kombinasie van 1ste en 2de generasie fermentasie die 2de generasie fermentasie beduidend verbeter het met 'n etanol konsentrasie van 57.6 g/L en dít in 'n korter tyd as vir die suiwer 2de generasie eksperimente.

Dit was bepaal vanuit pers- en was eksperimente dat om die pre-hidrolisaat vloeistof uit die stoom-behandelde bagasse te pers, die vlak van inhibitore in 'n 2de generasie fermentasie voldoende verlaag vir die gebruik van 'n verharde gis.

Van die data van die 1ste generasie eksperimente was saam met literatuurdata gebruik om 'n 1ste generasie proses in Aspen Plus® te modelleer wat 493 ton suikerriet per uur prosesseer (tc/hr). Pinch hitte integrasie was gebruik om die dienste vereistes te verminder. In die proses word die bagasse gebruik om stoom en elektrisiteit te genereer. In een geval was die oortillge bagasse bepaal as 57.5%. Hierdie bagasse was verkoop aan 'n alleenstaande 2de generasie aanleg. Die eerste generasie proses

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het 85.5 liter etanol per ton suikerriet geproduseer (L/tc), die geïntegreerde proses het 128 L/tc geproduseer terwyl die 2de generasie proses 185 liter etanol etanol per ton bagasse (50% vog) of 25.5 L/tc geproduseer het. Die hoeveelhede oortillige elektrisiteit wat geproduseer is wissel van 14.3 tot 70.2 kWh/tc.

Ekonomiese analieses is gedoen met Suid-Afrikaanse ekonomiese parameters om die sub-Sahara Afrika-konteks uit te beeld. Data van die 1ste generasie prosesmodel en literatuurdata van geïntegreerde 1ste en 2de generasie en alleenstaande 2de generasie prosesse was vir die analieses gebruik. Dit is bepaal dat die geïntegreerde model die mees ekonomies vatbare model is (IRR = 11.66%) terwyl die 1ste generasie proses basies gelyk gebreek het (IRR = 1.62%) en die 2de generasie proses is ekonomies onvatbaar. Hierdie bevindinge is as gevolg van hoë suikerrietpryse en te min aansporings vir 2de generasie etanol.

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GLOSSARY

Term:	Definition:
Agricultural waste	Biomass produced by agriculture that currently has no use.
Anhydrous ethanol	Ethanol with a purity of 99.3 % or more.
Biodegradable	Breaking down of waste by biological factors present in the environment.
Biofuels	Fuels derived from biomass using a biological conversion route.
By-product	Additional products formed during a process.
Carbon neutral	The concept that no net CO_2 is released by burning biofuels.
Climate change	The change in weather patterns due to greenhouse gases (GHG's).
Co-product	Useful additional products formed during a process.
Detoxification	The specific removal of inhibitors.
Energy crops	Crops planted for the sole purpose of energy production.
Energy security	The ability of a country to meet its own energy demands without importing fossil fuels.
Enzyme	Bio-catalyst produced by a living organism.
Fermentation inhibitors	Chemical compounds that inhibit yeast cell growth and/or the fermentation process.
First generation biofuels	Biofuels derived from food crops.
Fossil fuels	Fuels derived from coal, oil and natural gas.
Greenhouse gases	Gases that trap the sun's heat in the atmosphere which causes a global rise in temperature.
Hemocytometer	Counting chamber used to determine cell counts.
Hexose sugars	Monomeric sugars with six carbon atoms.

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Hydrous ethanol	Ethanol with a 95.6 % ethanol content.
Lignocellulose	The fibrous non-food part of plants that consist of cellulose, hemicellulose and lignin.
Marginal lands	Lands that are not suited to the production of food crops.
Melle-Boinot fermentation	A fed-batch with yeast recirculation fermentation process.
Octane number	A measure of the pressure at which a fuel auto ignites.
Pentose sugars	Monomeric sugars with five carbon atoms.
Process modelling	Simulation of a chemical production process using process simulation software.
Rankine steam cycle	The process of generating electricity using steam
Recombinant yeast	A genetically manipulated yeast that is able to ferment hexose and pentose sugars.
Renewable fuels	Fuels derived from biomass.
Second generation biofuels	Biofuels derived from non-food crops.
Steam gun	Pressure reactor that is used for the pre-treatment of biomass with steam.
Sugarcane bagasse	Fibrous part of sugarcane that is obtained after the sucrose has been extracted.
Sugarcane juice	Sucrose containing juice obtained from the processing of sugarcane.
Sugarcane trash	The parts of the sugarcane that do not form part of the sucrose containing stalks such as the tops and the leaves of the sugarcane.
S. cerevisiae	*Saccharomyces cerevisiae*
Yeast propagation facility	Section in a plant where yeast is grown.

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NOMENCLATURE

Acronym:	Meaning:
BIGCC	Biomass integrated gasification combined cycle
CBP	Consolidated bioprocessing
CCFD	Cumulative cash flow diagram
CEPCI	Chemical engineering plant cost index
CFD	Cash flow diagram
CHP	Co-generation of heat and power
COFIT	Co-generation feed-in tariff
COM	Cost of manufacture
DPBP	Discounted payback period
FCI	Fixed capital investment
FPU	Filter paper unit
GGE	Gasoline gallon equivalent
GHG	Greenhouse gas
HEN	Heat exchanger network
HMF	Hydroxymethylfurfural
HPLC	High performance liquid chromatography
HSF	Hybrid saccharification fermentation
IRR	Internal rate of return
MC	Moisture content
MEE	Multi-effect evaporator
MESP	Minimum ethanol selling price

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MUMNE	Minimum utilities minimum number of exchangers
NBS	New Brunswick Scientific
NERSA	National energy regulator of South Africa
NPV	Net present value
OD	Optical density
PVR	Present value ratio
SASA	South African sugar association
SHF	Separate hydrolysis and fermentation
SSF	Simultaneous saccharification and fermentation
USM	Umfolozi Sugar Mill
WIS	Water insoluble solids
YPD	Yeast extract, peptone powder and dextrose
ZAR	South African Rand (currency)

Symbol:	**Meaning:**
ΔT_{min}	Minimum approach temperature
η_{elec}	Efficiency of electricity generation
$\eta_{liquid\ fuel}$	Efficiency of producing ethanol
$\eta_{overall}$	Overall plant efficiency
A	Cost attribute used in the six tenths rule
C	Capital cost
C_{OL}	Cost of operating labour
C_{RM}	Cost of raw materials
C_{UT}	Cost of utilities

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C_{WT}	Cost of waste water treatment
COM_d	Cost of manufacture excluding depreciation
dO_2	Dissolved oxygen content in fermentation
E_{Elec}	Electrical energy
E_{th} *biomass*	Thermal energy of biomass
E_{th} *fuel*	Thermal energy of fuel
f	Inflation
N_{OL}	Number of operators required per shift

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1. INTRODUCTION

Ethanol as a fuel for motor vehicles has been around for a long time. It was used in Otto's first internal combustion engine in 1897 (Taherzadeh & Karimi, 2008). However, the use of ethanol as a fuel has been suppressed due to the availability of cheap fossil fuels during the last century. This is all about to change due to environmental concerns regarding the use of fossil fuels and rising fossil fuel prices that have sparked new world wide interest in the use of more environmentally benign biofuels such as bioethanol, biobutanol, biodiesel and biogas.

This project is concerned with the production of bioethanol from whole sugarcane. More direct and simpler alcohol production processes using whole sugarcane are needed (Felipe, 2010). This project consists of a combination of experimental and process- and economic modelling work. A selection of novel aspects of the integration of first and second generation bioethanol production were investigated, both with experimental and modelling approaches. The novel aspect of the experimental part of the project is the combination of 1st and 2nd generation technologies in the same fermentation. The novel aspect of the modelling comes in with using South African parameters to perform an economic analysis on bioethanol production from sugarcane to represent the sub-Saharan African context.

The experimental part of this project is concerned with fermentation- and other supporting experiments. Different fermentation strategies were investigated to determine their effects on the ethanol production process.

Some of the experimental data generated from the experimental part of this project was used to simulate a first generation bioethanol production process that produces ethanol from the sucrose contained in the sugarcane and that uses the sugarcane bagasse (fibrous part of sugarcane) to generate steam and electricity. This was compared to an integrated 1st and 2nd generation process and a stand-alone 2nd generation process from literature.

The process model that was developed in this project and the models from literature were used to perform economic analyses to assess the economic viability of these processes in a sub-Saharan African context. The economic analysis was used to determine what should be done to improve the viability of such projects. Literature states government support is needed to make such processes viable (Sims, 2002b).

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1.1 BACKGROUND INFORMATION

First generation biofuels are biofuels that are produced from food crops such as the juice of sugarcane and ear-corn. First generation bioethanol is being widely produced around the world from corn, sugarcane and sugar beet in the United States, Brazil and Europe, respectively (Taherzadeh & Karimi, 2008; Gnansounou, 2009; Sims, 2002).

The problem with first generation bioethanol is that sugary and starchy materials are used as feedstocks to produce these fuels. This means that the production of first generation bioethanol competes directly with the production of food. This has sparked the on-going, worldwide food versus fuel debate. Sources indicate that the use of food for fuel feedstock causes the food prices to increase and this affects the food security of the poor across the globe. Another school of thought is that it may stabilise the food market and it gives producers more security. (Timilsina & Shrestha, 2010)

Second generation bio-fuels is seen as a partial solution to the food versus fuel debate. This technology uses non-food biomass as a feedstock such as agricultural wastes and by-products (Blaschek et al, 2010). Dedicated energy crops such as switch grass and Jatropha (Dorado, 2008) can also be used as a feedstock for this technology. Second generation biofuels produced from energy crops is only seen as a partial solution to the food versus fuel debate because the cultivation of energy crops may compete indirectly with food sources for available land on which food sources can be cultivated. However, fuel produced from residues from existing agriculture/forestry does not compete with food for available land. Second generation biofuels from energy crops may also compete directly with animal feed and fertiliser production meaning that it may compete with resources for food production. However, some dedicated energy crops can be grown on marginal lands that are not suited to the production of food crops (Blaschek et al., 2010b).

Second generation biofuels include cellulosic ethanol that is produced from lignocellulosic feedstocks (Leibbrandt, 2010). Other second generation biofuels include bio-butanol (Ezeji & Blaschek, 2010), bio-oil and char from the pyrolysis of lignocellulose and Fischer-Tropsch fuels produced from lignocellulosic materials (Leibbrandt, 2011). As mentioned in the previous paragraph, lignocellulosic feedstocks may include agricultural wastes and by-products such as sugarcane bagasse, corn stover, sorghum bagasse, triticale straw, etc. Agricultural by-products and biomass in municipal solid waste can play an important role in making biofuel production sustainable (Blaschek et al., 2010a).

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The use of biofuels has the potential to be carbon neutral (Wyman et al., 1992; Liu et al., 2010). This is because of the assumption that the greenhouse gases (GHG's) that are emitted from burning these fuels are recycled by the next crop that is cultivated as a biomass feedstock. Many literature sources state that the use of biofuels is essential to reduce GHG emissions to mitigate climate change (Dias et al., 2009; Liu et al., 2010; Seabra & Macedo, 2011; Nass et al., 2007; Timilsina & Shrestha, 2010; Gomez et al., 2008). Biofuels will help to mitigate GHG emissions even if they are not completely carbon neutral, but have a positive effect on the life cycle GHG emissions, compared to fossil fuels. The use of biofuels will increase energy security around the world by decreasing the dependence on fossil fuels by displacing fossil fuels with renewable biofuels (Liu et al., 2010; Seabra & Macedo, 2011; Timilsina & Shrestha, 2010). Climate change serves as a driver for the development of biofuels as a replacement for fossil fuels or, more likely, as an additive to fossil fuels (Dias et al., 2009; Felipe, 2010; Dias et al., n.d.; Timilsina & Shrestha, 2010).

1.2 Main project aims

The primary aims of this project are:

- Determine the effect of different fermentation strategies on the ethanol production process from sugarcane juice and bagasse.
- Determine the effect of process integration between first and second generation biofuel production technologies on the economic viability of such projects.
- Determine what financial incentives and market changes are necessary to make biofuels more economically viable and attractive to investors.

The main project aims are expanded upon in section 2.10.

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2. LITERATURE REVIEW

2.1 THE CASE FOR BIOENERGY AND BIOFUELS

Currently 10 % of the total global energy need is met by biomass. 10 % of the energy from biomass (1% of the total global energy need) is produced from modern bioenergy in the form of power, heat and fuel. Biofuels for transport account for 2.2 % of all bioenergy (however this percentage is rapidly increasing). See Figure 1 for a visual explanation of the global energy needs. The total sustainable technical potential of bio-energy is estimated to be 25 % (80 EJ) of the current global energy use. There is great room for improvement but research is needed to determine how much of this technical potential can be utilised in a cost effective and sustainable manner (Blaschek et al., 2010b). The impending oil shortage and environmental concerns has created space for the return of fuel ethanol (Felipe, 2010).

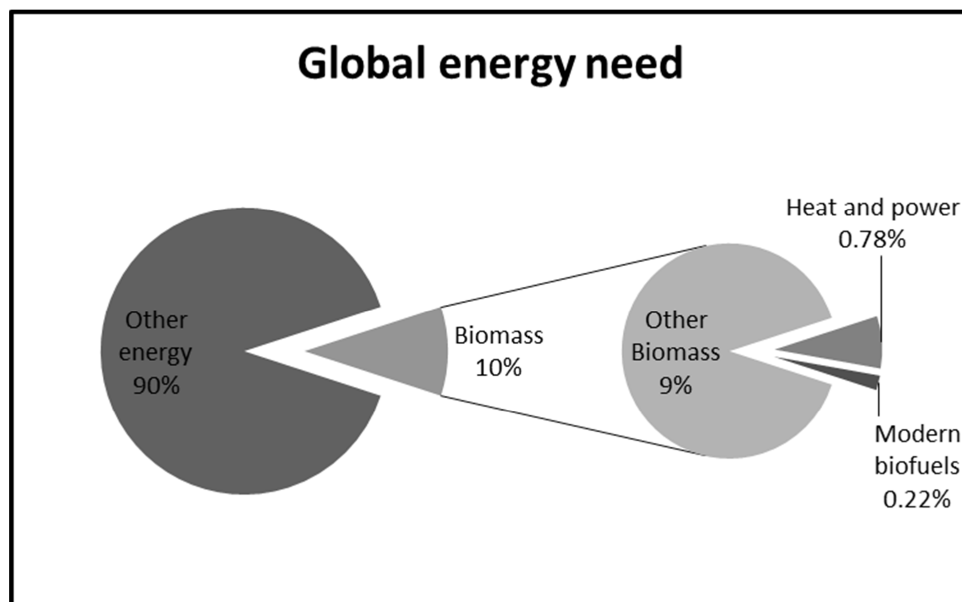

Figure 1: Pie chart of the global energy supply as described by Blaschek et al (2010b)

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2.1.1 ENVIRONMENTAL BENEFITS

In recent years renewable energy resources have received a lot of interest to supplement fossil fuel resources, due to the adverse effects that the burning of fossil fuels have on the environment (Blaschek et al., 2010b). The burning of fossil fuels causes greenhouse gases (GHG) to be released into the atmosphere. The problem with GHG's released into the atmosphere by burning fossil is that not all of the GHG's are recaptured by crops and other plants causing the GHG levels to increase. The emissions from the burning of fossil fuels causes pollution in urban areas and climate change. Renewable energy sources will help to reduce GHG emissions and to slow down the effects of climate change by displacing fossil fuels and recycling carbon through the growing of new crops. (Taherzadeh & Karimi, 2008; Leal, 2010; Sims, 2002b; Gnansounou, 2009)

The transport sector consumes large amounts of fossil fuels and is responsible for about 30 – 33 % of the total GHG emissions worldwide (Sims, 2002b) and it is the largest contributor to CO2 emissions in the USA (Yang et al., 2010). This has led to significant research efforts focused on renewable fuels such as liquid biofuels for use in internal combustion engines.

Biofuels can significantly reduce pollution. Biofuels contain lower sulphur levels than fossil fuels that when burned emits lower levels of sulphuric compounds that will cause less acid rain. Fossil fuels causes water pollution due to oil spills and groundwater contamination from underground tanks and runoff from road surfaces due to fuel leakages on the roads. It is estimated that about one quarter of underground fuel storage tanks contribute to underground water contamination. Biofuels are more biodegradable and this will help alleviate groundwater contamination due to fuels. The burning of fossil fuels causes ozone formation at ground-level. Ozone is toxic gas and powerful oxidising agent. Biofuels can reduce ground-level ozone formation since biofuels emit fewer ozone-forming pollutants. (Sims, 2002b)

Biofuels have favourable properties when blended with other fuels. Biofuels are known as "oxygenates" due to the oxygen atoms present in their molecular structure. The oxygen present in biofuels leads to improved combustion and cleaner exhaust emissions when blended with other fuels. Biofuels that are blended with fuels serve to displace some of the fossil fuels causing less irreversible emissions. Bioethanol has a very high octane number, typically 105 – 120, and it can be used as an "octane enhancer" when blended with gasoline. Ethanol can be blended into gasoline to form gasohol (up to 15% ethanol). Engines can use gasohol without being modified. Ethanol enhances octane number and it has no significant effect on the lower heating value of the fuel when blended with fuels in small amounts (Gnansounou, 2009). (Sims, 2002b)

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2.1.2 Socio-economic benefits

Many countries import oil, for example the United States import 60 % of their oil supplies (Blaschek et al., 2010b), and this makes their economy dependent on the oil price. Biofuels will help to lessen this dependence of economies on the oil price. In 2007 16.4 billion gallons of biofuel was produced with bioethanol being the main biofuel (Blaschek et al., 2010b; Sims, 2002b).

Biofuels have the advantages that it is renewable, potentially sustainable and it can utilise waste biomass streams that currently have little value or need to be disposed.

Biofuels also have the following socio-economic benefits (Sims, 2002b):

- The feedstock is usually manufactured in the country of use which helps create more jobs in rural areas where agriculture is practised.
- The local production of biofuels also helps to improve energy security.
- Biofuel production will help to strengthen the agricultural sector by providing new options for land use and creating new revenue streams from streams that were previously regarded as waste streams.

Economic drivers such as government incentives will be required to make ethanol competitive with gasoline (Gnansounou, 2009; Timilsina & Shrestha, 2010).

2.2 First Generation bioethanol production from sugarcane

First generation bioethanol is produced from food stocks. 48 % of world ethanol production in 2006 was from sugarcane juice (Gnansounou, 2009). Sucrose contained in sugarcane was considered in this study. As a result of increasing gasoline prices, the use of first generation bioethanol as a biofuel has been introduced on a large scale in Brazil, the United States and various European countries (Blaschek et al., 2010b).

In this section for first generation bioethanol production from sugarcane the general process was divided into the following sectors according to Leal (2010):

2.2.1 SUGARCANE RECEPTION, PREPARATION AND JUICE EXTRACTION

These sections are very important as it requires high capital investment, operation and maintenance costs. It also has high energy consumption and a large impact on the plant efficiency (Leal, 2010).

Cane reception consists of the spiller (hilo) that dumps cane onto the feeding table by overturning the transport container to tip the cane out onto a conveyor belt (Leal, 2010). The cane is then transported by conveyer belts to the cane preparation section.

Cane preparation is very important to the efficiency of juice extraction. Cane is prepared first by washing and then shredding the cane. In the present study a dry-cleaning system is used for the removal of dirt from the cane, instead of a wet washing stage, to reduce water usage (Dias et al., 2009). After the cane has been washed it is shredded by a set of heavy duty rotating knives. The quality of cane preparation is measured by the percentage of open cells in the cane known as the cane preparation index. A minimum value of 80 % is required for the cane preparation index. By using heavy duty knives values of 90 to 92 % can be achieved for the cane preparation index. (Leal, 2010)

The prepared cane can then either be fed to a mill or a diffuser, for the extraction of sucrose from the prepared cane in the form of mixed juice. A mill extracts the sugarcane juice by compressing the cane between large cylinders (Modesto et al., 2009). A diffuser uses hot water to leach the sucrose from the prepared cane (Modesto et al., 2009). Traditionally Brazilian sugar mills make use of the mill to extract the sucrose (Leal, 2010). However in the present study a diffuser is considered for the sucrose extraction process due to the advantages of using a diffuser that are listed below:

- Higher extraction efficiency (up to 99 % for diffusers versus a 97 % maximum for mills) (Pellegrini & De Oliveira Junior, 2011; Modesto et al., 2009; Bosch Projects, n.d.). However, to reach such high extraction efficiencies in diffusers, heavy duty knives must be used for cane preparation to reach open cell values of 90 – 92 % and sand and dust must be effectively removed from the cane (Modesto et al., 2009).
- Reduced capital costs (Bosch Projects, n.d.).
- A mill requires medium pressure steam (approximately 20 bar) whereas a diffuser only requires low pressure steam (2 – 2.5 bar) (Modesto et al., 2009; Bosch Projects, n.d.)
- Diffusers require less mechanical energy (10 kWh/tc for diffusers versus 15 kWh/tc for mills) (Pellegrini & De Oliveira Junior, 2011) and they have lower power consumption (Bosch Projects, n.d.).

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- Diffuser maintenance costs are 50 – 60 % (Bosch brochure) or even up to 70 % (Modesto et al., 2009) lower than milling maintenance costs.
- Easy operation with fewer operators. Only three operators are required for diffusion instead of eight required for milling (Modesto et al., 2009) or even as few as one operator could be required (Bosch Projects, n.d.).
- Diffusers require less mechanical energy than mills (2.2 kWh/tc vs 8.2 kWh/tc), thus one can subtract 6 kWh/tc from the plant power demand when replacing a mill with a diffuser (Bosch Projects, n.d.).

The sucrose containing juice that exits from the diffuser is termed mixed juice. The fibrous material that is left over after sucrose extraction is called bagasse. The bagasse that leaves the diffuser goes through two dewatering mills (Modesto et al., 2009) to reduce the moisture content (MC) to approximately 50 % so that the bagasse can be burned in the boilers to generate heat and power.

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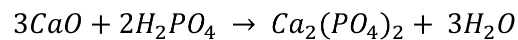
2.2.2 JUICE TREATMENT AND CLARIFICATION

The mixed juice is physically treated first by screens and hydro-cyclones to remove solid particles such as fine bagasse fibres and sand. The mixed juice is then clarified to remove the remaining dirt and other contaminants before it is fed to the fermentation section. This is done by first adding phosphoric acid (H3PO4) to the juice and then heating the juice to 70 °C. After the initial heating step lime (CaO) is added and then the juice is heated further to 105 °C. After the final heating step a flocculant polymer is also added and the juice is fed to a clarifier. The lime and phosphoric acid reacts to form a calcium phosphate according to Equation 1 that flocculates due to the added flocculant and settles out in the clarifier. The settling solids drag the impurities down with them to the bottom of the clarifier. The clarifier produces clear juice from its overflow and mud from its underflow. The mud still contains some sugars. The mud is filtered and the filtrate is recycled to the point where the lime is added to minimise sugar losses. (Dias et al., 2009; Leal, 2010)



$$3CaO + 2H_2PO_4 \rightarrow Ca_2(PO_4)_2 + 3H_2O$$

Equation 1

2.2.3 JUICE CONCENTRATION AND STERILISATION

The clear juice from the clarifier overflow is sent to the concentration section to be concentrated up to a higher sugar-content for fermentation. The clear juice initially contains about 15 % sucrose. Part of this stream is concentrated up to above 65 % sucrose content using a 5-stage multiple effect evaporator (MEE). A bypass stream of un-concentrated juice is then blended with the concentrated juice to produce juice with a final concentration of approximately 22 %, which must then be sterilised. This juice is known as the concentrated juice (Dias et al., 2009).

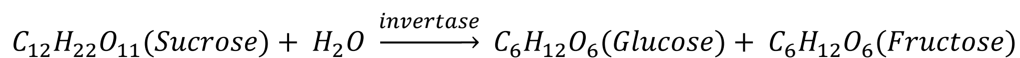
The concentrated juice is sterilised by heating it up to 130 °C and then rapidly cooling it down to fermentation temperature (32 °C in this study) (Dias et al., 2009). The juice must be sterilised after it has been concentrated to reduce chances of contamination during fermentation. Since only a portion of the juice have been concentrated (and sterilised in that way) the bypass stream of juice may still contain contaminants, thus necessitating the sterilisation process.

2.2.4 Sugar syrup fermentation

The most widely used process in Brazil is the fed-batch method with yeast recirculation process known as the Melle-Boinot fermentation process (Dias et al., 2011; Leal, 2010). For yeast recirculation, flocculation of the yeast is a good property since it enhances yeast recovery and recirculation (Senthilkumar & Gunasekaran, 2009). Continuous fermentation still has many problems that must be solved, but it also holds many advantages and it is expected to be the way of the future (Leal, 2010).

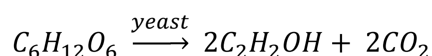
In Brazilian distilleries the fermentation time is about 8 hours, with a fermentation yield of approximately 91 % and an ethanol concentration in the fermented broth of above 13 % (w/v) (Leal, 2010; Gnansounou, 2009).

The yeast S. cerevisiae can't ferment sucrose directly to ethanol although it can take up small amounts of sucrose for other purposes such as cell growth (Stambuk et al., 2000). The sucrose that is to be fermented must first be broken up into its monomeric sugar building blocks namely glucose and fructose according to Equation 2, before it can be fermented to ethanol according to Equation 3. The maximum theoretical fermentation yield is 0.511g ethanol per gram of hexose sugar (fructose and glucose). The hydrolysis of sucrose is achieved by an enzyme called invertase (Gnansounou, 2009) that is produced by the yeast so that the yeast can utilise the sucrose. Earlier literature claims that the enzyme invertase is associated with the cell wall of the yeast S.cerevisiae (Demis et al., 1954; Burger et al., 1961; Islam & Lampen, 1962), but later research claims that invertase is an extracellular enzyme (Stambuk et al., 2000). The invertase activity of S. cerevisiae is approximately 300 times that of its fermentation capacity (Demis et al., 1954).



$$C_{12}H_{22}O_{11}(Sucrose) + H_2O \xrightarrow{invertase} C_6H_{12}O_6(Glucose) + C_6H_{12}O_6(Fructose)$$

Equation 2



$$C_6H_{12}O_6 \xrightarrow{yeast} 2C_2H_2OH + 2CO_2$$

Equation 3

It is interesting to note that there is also some invertase present in sugar cane (Del Rosario & Santisopasri, 1977) that inverts sucrose causing glucose and fructose to be present in the sugar syrup before fermentation.

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Ethanol production from molasses is very similar to ethanol production from sugar syrup. Ethanol production from molasses usually has a fermentation temperature of 25 °C, a residence time of 48 to 80 hours, and 6 – 8 % ethanol concentration in the fermented broth (Senthilkumar & Gunasekaran, 2009). Continuous fermentation is preferred over batch due to better yields (89 – 90 % compared to 80 – 84 % for batch), higher productivity, less water usage and ease of operation (Senthilkumar & Gunasekaran, 2009) and the initial lag phase of batch and fed-batch fermentation is avoided (Taherzadeh & Karimi, 2008).

The method for first generation fermentation used in this study was adapted from the method used by NCP Alcohols and it is described here. NCP Alcohols produces potable ethanol from the fermentation of molasses. First water is added to the molasses to obtain a molasses syrup with approximately 22 % sugar content. Before fermentation the diluted molasses syrup is pasteurised (heated to above 80 °C and then cooled to fermentation temperature) to reduce the risk of contamination. (In this study the syrup was sterilised at 130 °C for 30 min using an autoclave.) The pasteurised juice is pumped into a fermenter and an inoculum culture of S.cerevisiae yeast is added. The fermentation process takes place at approximately 32 °C under anaerobic conditions. More molasses syrup is added after inoculation in a fed-batch manner similar to the Melle-Boinot fermentation process. After a residence time of about 55 to 60 hours an alcohol content of approximately 80 – 90 g/L is reached with a maximum residual sugar concentration of 5 g/L. The 80 – 90 g/L in this case is higher than the 60 to 80 g/L obtained by Senthilkumar & Gunasekaran (2009), but it is much less than the 130 g/L produced by Brazillian distilleries which will negatively impact distillation costs, however NCP Alcohols is more concerned with the taste of the ethanol (potable ethanol) than producing high concentrations (at high concentrations the yeast may become stressed and affect the taste). The alcohol containing mash or beer is then sent to the ethanol recovery section. One can see that the parameter values given here are very similar to that given in the previous paragraph by Senthilkumar & Gunasekaran (2009), except for the temperature. The temperature difference may be attributed to the high cooling costs that would result from keeping fermenters at 25 °C, since refrigeration will be required to keep the fermenters at 25 °C in the subtropical climate of Durban where NCP Alcohols is situated (Kitching, 2011). Lithium bromide absorption refrigeration was used by Dias et al (2009) to produce cooling water to cool the fermenters.

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2.3 Second Generation or cellulosic bioethanol

"Especially promising is cellulosic ethanol that can capitalise on microbial engineering and biotechnology to reduce costs" (Blaschek et al., 2010b).

Second generation bioethanol is ethanol that is produced from a non-food biological feedstock. (Qureshi et al., 2010) states that it is essential to utilise lignocellulosic biomass to meet global biofuel demands. Examples of such feedstocks are sugarcane bagasse, corn stover, corn cobs, switch grass, etc. In this project sugarcane bagasse that is produced by the first generation bioethanol plant is the lignocellulosic feedstock that will be used for the second generation ethanol plant.

There are a lot of challenges to produce ethanol from lignocellulosic feedstocks. These challenges include the production of cheap feedstock and the effective conversion of feedstock to ethanol.

Cellulosic feedstock is the most abundant renewable energy resource on earth and thus it is cheap. In some cases lignocellulosic biomass can be obtained for free such as agricultural and industrial wastes. However, one must still pay for the harvest/collection and transport of the material. Only in the case where lignocellulose is produced on-site by a co-located plant as a zero cost by-product or negative cost waste stream (due to disposal costs) can it be seen as completely free. This is the case for a sugar mill or ethanol distillery with a cellulosic ethanol facility annexed to the original plant. In other cases one can receive money in the form of a tipping fee for the removal of lignocellulosic biomass, such as garden clippings from urban areas, however one must still pay for transportation.

Lignocellulosic feedstocks can be grown on agriculturally marginal lands and this will help to reduce the competition for land between food and fuel crops (Blaschek et al 2010). Agricultural by-products (such as sugarcane bagasse) can play an important role in triggering the transition to sustainable biofuels (Blaschek et al 2010). Table 1 shows the prices of some lignocellulosic feedstocks compared to corn (2010 prices in the US).

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Table 1: Comparison of lignocellulose feedstock prices to corn (Blaschek et al, 2010)

Feedstock	US $/ton (2010)
Wheat straw	24
Barley staw	26
Corn stover	50
Grass hay	50
Switchgrass	60
Corn	230

Transportation costs of feedstock plays the biggest role in the price of the feedstock that is delivered to the plant. In turn the price of feedstock plays the biggest role on the price of the fuel produced (Ileleji et al., 2010). Thus the logistics must be carefully coordinated to ensure that the feedstock price is low. This limits the size of a biofuels plant as the feedstock can't be transported over too long distances. The limitation on the size of a biofuels plant prevents the building of very large plants to take advantage of the economies of scale.

In this study a second generation ethanol plant that is integrated with a first generation plant is considered, thus the size of the second generation plant depends on the size of the first generation plant. In Brazil the average sugarcane processing plant (autonomous distillery, sugar mill or combination of both) processes approximately 500 metric tonnes of sugarcane per hour (tc/hr). According to a brochure for sugarcane diffusion by De Smet Engineers & Concultants, the largest diffuser they can build can process 20 000 tc/day or 833.33 tc/hr. This is considered as a very large sugarcane processing plant. In sub-Saharan Africa sugar mills tend to be smaller, for example Unfolozi Sugar Mill has a capacity of 300 tc/hr while the Nakambala Factory of Zambia Sugar Co. Ltd has a capacity of 350 tc/day (De Smet Engineers & Contractors, 2010). In this study a plant that processes 493 tc/hr is considered as was done in Dias et al (2009). This plant is larger than the usual sugar mill in sub-Saharan Africa and the reason for this is to improve on the economy of scale, however the building of very large plants to take full advantage of the economy of scale is still prohibited by the increasing transport cost of feedstock with increasing plant size.

Bagasse has considerable potential for use as biofuel since it has been, in effect, collected and delivered to the plant for free since these costs are included in the harvesting/collection and transportation of the sugarcane (Sims, 2002a). By combining a 2nd generation ethanol plant with a 1st generation ethanol plant this will help to overcome the problem of not being able to take

advantage of the economy of scale. The transportation costs of the bagasse are included in the cost of transporting the sugarcane, thus a bigger plant can be constructed (see previous paragraph).

The biggest obstacle in the production of ethanol is to overcome the recalcitrance of the lignocellulosic structure/plant cell wall to degradation (Taherzadeh & Karimi, 2008; Leibbrandt, 2010). Plant cell walls are naturally resistant to degradation by enzymes and microbes. The plant cell wall acts as a barrier between the cell and the outside and it has evolved to be resistant to degradation to protect the plant cell.

Lignocellulose can't be directly fermented due to its recalcitrant structure. First a pre-treatment step and then an enzymatic hydrolysis step is required to break the lignocellulose down into fermentable sugars. These process steps will be explained in sections 2.3.2 and 2.3.3.

The steps of the second generation bio-ethanol production process are outlined in Figure 2 below and explained in the following sections 2.3.2 through 2.3.4. Please note in the figure below that the lignin is not separated from the cellulose by pre-treatment, but they are shown as separate since the lignin is inert in the enzymatic hydrolysis and fermentation steps. Only the hemicellulose is partially separated from the lignin and the cellulose (cellulignin).

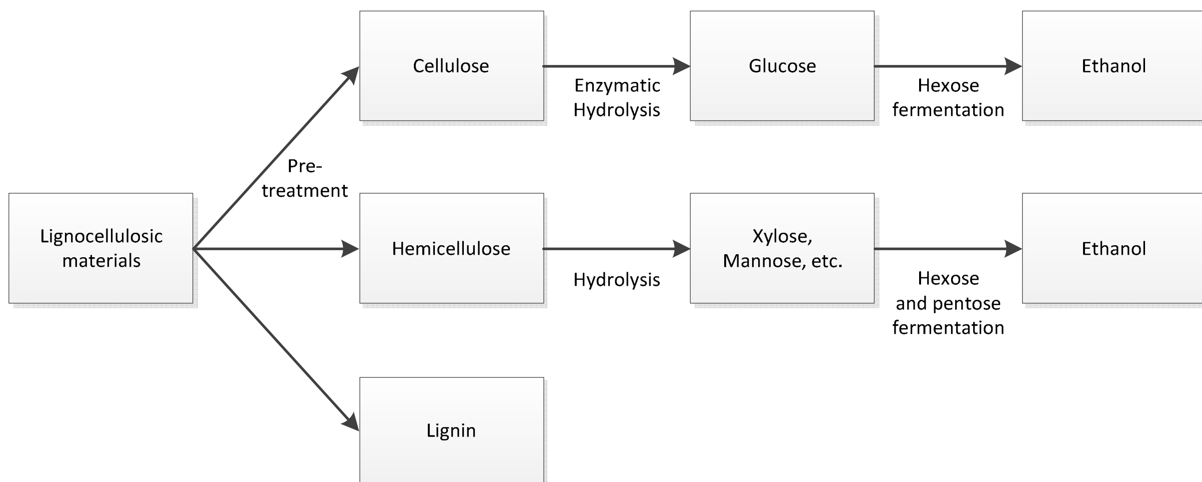

Figure 2: Overall view of ethanol production from lignocellulosic materials (redrawn from Taherzadeh & Karimi, 2008)

Many sources provide a schematic representation of the steps required for cellulosic ethanol production that are similar to the scheme presented here in Figure 3 (Gomez et al., 2008; Leibbrandt, 2010; Girio et al., 2010; Sassner & Zacchi, 2008).

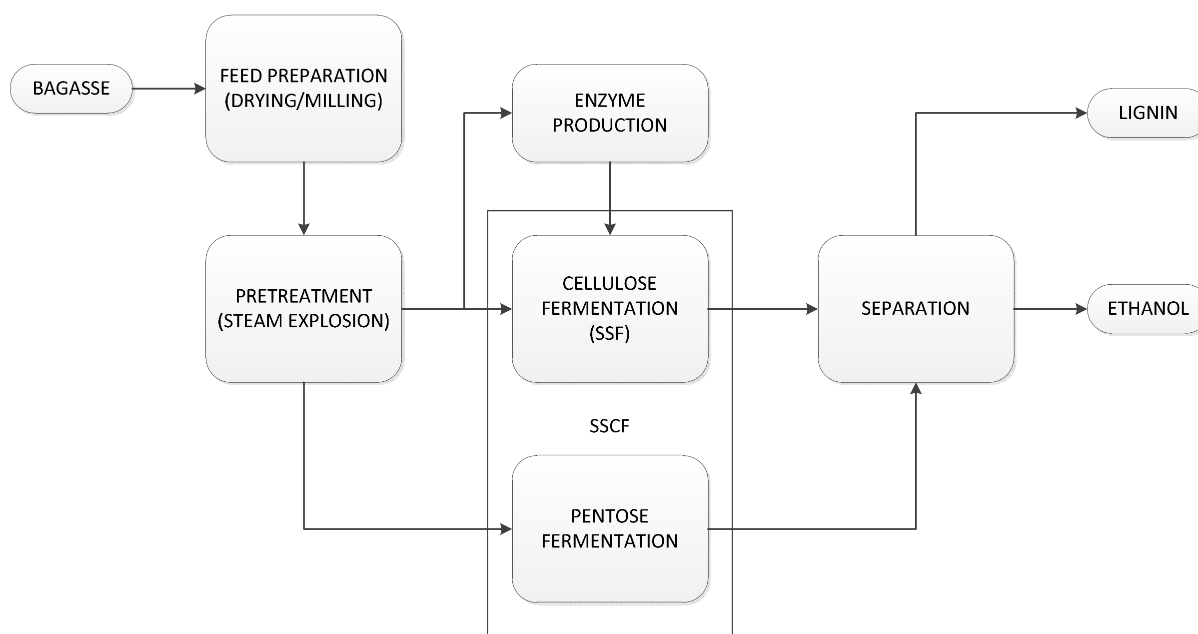

Figure 3: Simplified process steps required for bioethanol production (Leibbrandt, 2010; Girio et al., 2010)

2.3.1 CHARACTERISATION OF LIGNOCELLULOSIC MATERIALS

Lignocellulosic materials consist of a mixture of carbohydrate polymers (cellulose and hemicellulose), lignin, as well as minor components such as extractives and ash (Ezeji & Blaschek, 2010; Gnansounou, 2009; Taherzadeh & Karimi, 2008). The carbohydrate polymers are tightly bound to lignin mainly through hydrogen bonding and some covalent bonding (Taherzadeh & Karimi, 2008).

Cellulose is the main component of most lignocellulosic materials. Cellulose is a long homopolymer of D-glucose linked by β- 1, 4 bonds. Each glucose molecule is rotated 180° relative to the next so that the repeating unit in the polymer is cellobiose, a two-glucose unit (Taherzadeh & Karimi, 2008). Cellulose molecules are arranged into microfibrils. The microfibrils form a highly ordered crystalline structure that makes cellulose recalcitrant to hydrolysis. The crystalline regions are interrupted with less ordered amorphous regions interspersed in the crystalline structure. (Ezeji & Blaschek, 2010; Gnansounou, 2009; Taherzadeh & Karimi, 2008)

Hemicellulose is the second most common component of lignocellulosic biomass. It is a heterogeneous polymer of pentoses (mainly xylose and some arabinose), hexoses (mannose, glucose and galactose) and sugar acids. Hemicellulose is easily hydrolysed to its monomer components. Hemicellulose will consist mostly of either xyloglucans or xylans, depending on the plant. (Ezeji & Blaschek, 2010; Gnansounou, 2009; Taherzadeh & Karimi, 2008)

Lignin is the third most common component of lignocellulosic biomass. Lignin is a heterogeneous polymer that consists of three alcohol monomers: p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. It is an aromatic polymer constructed of phenolic compounds in a three dimensional structure. Lignin is resistant to chemical and enzymatic degradation and contributes significantly to the recalcitrance of lignocellulosic biomass. (Ezeji & Blaschek, 2010; Gnansounou, 2009; Taherzadeh & Karimi, 2008)

The cellulose crystallinity, sheathing of the cellulose by hemicellulose, protection of the cellulose and hemicellulose by lignin all contribute to the recalcitrance of lignocellulosic materials to hydrolysis (Ezeji & Blaschek, 2010).

Typical composition ranges for lignocellulosic materials are presented in Table 2. It can be seen that the composition of lignocellulosic materials vary widely depending on the biomass source. In this study bagasse is considered and Table 3 below gives two literature sources for the typical composition of bagasse.

Table 2: Typical lignocellulosic biomass composition ranges

Lignocellulosic content	Source	
Component	Laxman & Lachke (2009)	Gnansounou (2009)
Cellulose (%DW)	23 - 53	40 - 60
Hemicellulose (%DW)	20 - 35	20 - 40
Lignin (%DW)	10 - 25	10 - 25

Table 3: Typical bagasse compositions

	Source	
Component	Ezeji & Blaschek (2010)	Taherzadeh & Karimi (2008)
Cellulose (%DW)	44.3	40
Hemicellulose (%DW)	26	24
Lignin (%DW)	18.9	25

2.3.2 PRE-TREATMENT

This is the first step in overcoming the recalcitrant structure of lignocellulose. Pre-treatment is required to alter the crystalline structure of the cellulose to a more amorphous structure to render the cellulose more amenable to enzymatic hydrolysis to release glucose (Laxman & Lachke, 2009).

There are many pre-treatment processes that have been developed in laboratories. These include the following:

- Physical pre-treatment – mechanical comminution, irradiation and pyrolysis.
- Physiochemical pre-treatment – steam explosion, ammonia fibre explosion (AFEX), SO_2 explosion and CO_2 explosion.
- Chemical pre-treatment – ozonylisis, dilute-acid hydrolysis, alkaline hydrolysis, organosolvent (Organosolv) process, oxidative delignification and ionic liquid hydrolysis.
- Biological pretreatment
- Combination of pretreatment methods

(Taherzadeh & Karimi, 2008; Blaschek et al., 2010b; Laxman & Lachke, 2009)

Most of the above methods may not be technically or economically feasible. Among the different pre-treatment methods, dilute acid hydrolysis, SO_2 explosion and steam explosion have been successfully developed. These methods show promise for industrial application.

In this particular study steam explosion will be used as the pre-treatment method. The liquid part of the slurry that is formed after pre-treatment is known as the pre-hydrolysate liquor and it contains all the solubles such as xylans, arabinoses and inhibitors (see section 2.3.2.1 for inhibitors). The solid part of the slurry is known as the water insoluble solids (WIS) and contains mainly cellulose and lignin. (Leibbrandt, 2010)

Steam explosion has some inherent limitations: the matrix of lignin and carbohydrates isn't completely broken down and fermentation inhibitors are formed from a fraction of the xylan that is destroyed (Gnansounou, 2009).

The severity of pre-treatment can be characterised by the severity parameter (Alvira et al., 2010; Cantarella et al., 2004) presented below in Equation 4, where T is the pre-treatment temperature in degrees Celsius and t is the pre-treatment residence time in minutes. The severity parameter increases as the pre-treatment becomes more severe. The point of increasing the pre-treatment severity is to make more sugars available for enzymatic hydrolysis and fermentation. However, as the pre-treatment severity is increased to increase the sugar yield there are more fermentation inhibitors that are being formed (see the next subsection 2.3.2.1). This means that the severity factor can't just be maximised to increase the sugar yield but is must be optimised so that the fermentation inhibitors are at acceptably low levels. Optimum sugar yields are reached at values between 3 and 4.5 for $Log(R_0)$ (Tomas-Pejo et al., 2008). Gnansounou (2009) states that lower pre-treatment

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temperatures along with longer pre-treatment residence times will give a better efficiency for the same severity factor. This means that temperature has a larger inhibitor forming effect than time.

$$Log(R_0) = Log\left(t.exp\left[\frac{T - 100°C}{14.75}\right]\right)$$

Equation 4

Sometimes two methods are used to increase the overall efficiency of the pre-treatment (Taherzadeh & Karimi, 2008; Leibbrandt, 2010). For example milling can be used to achieve better steam explosion. This is, in effect, what is done with sugarcane bagasse, because the sugarcane is first shredded to extract the sugar syrup.

2.3.2.1 Inhibitors formed during pre-treatment

During the pre-treatment process some of the sugars released from the biomass degrade to form chemicals that inhibit cell growth, fermentation or both. These degradation products are mainly present in the pre-hydrolysate (Palmqvist & Hahn-Hagerdal, 2000; Cantarella et al., 2004).

Examples of inhibitors include the following:

- Furfural
- Hydroxymethyl furfural (HMF)
- Acids (acetic, ferulic, glucuronic, vanillic, syringic and ρ-coumaric)
- Other (vanillin & syringaldehyde)

Hydrothermal pre-treatments, such as steam explosion, generate acetic acid from thermally labile acetyl groups of hemicellulose (Laxman & Lachke, 2009). Acetic acid is formed at mild pre-treatment conditions and the amount of acetic acid formed doesn't significantly depend on the pre-treatment severity but rather on the raw material itself (amount of thermally labile acetyl groups) (Taherzadeh & Karimi, 2008). However, the pre-treatment severity does determine how much of the acetic acid is released during pre-treatment and how much is released later during enzymatic hydrolysis (see section 2.3.3) (Cantarella et al., 2004).

Figure 4 illustrates the formation of fermentation inhibitors from the different components of lignocellulosic materials.

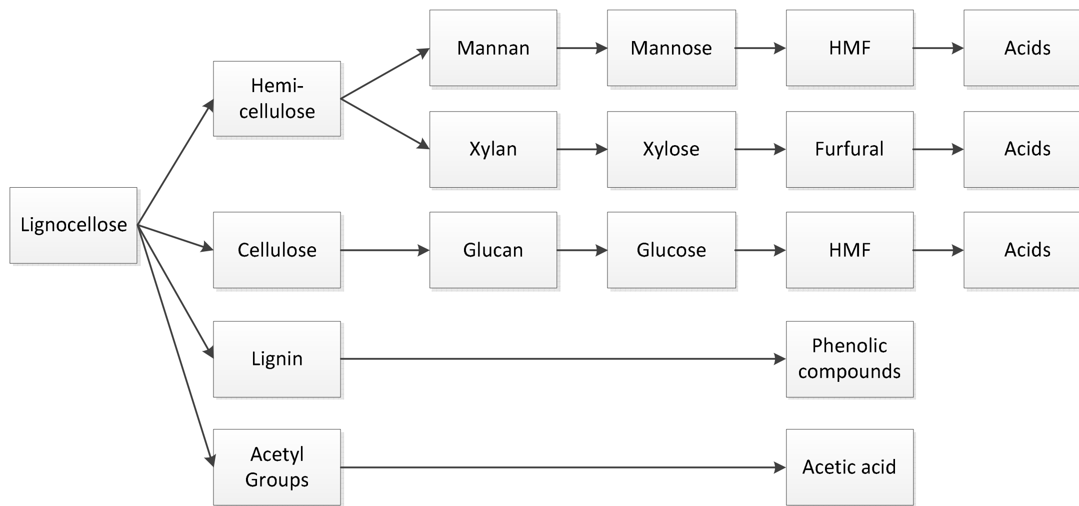

Figure 4: Formation of fermentation inhibitors from lignocellulose during pre-treatment (redrawn from Taherzadeh & Karimi, 2008)

2.3.2.2 Detoxification

The definition of detoxification of lignocellulosic hydrolysates is: "The specific removal of inhibitors prior to fermentation." (Anish & Rao, 2009)

There are many detoxification methods available to remove inhibitors from lignocellulosic hydrolysates after pre-treatment. The detoxification methods may change depending on the biomass source and fermenting organism that is being used because different lignocellulosic hydrolysates differ in their degree of inhibition and different organisms will have different inhibitor tolerances. The detoxifying methods can be divided into different categories. A list of the different categories along with examples of each is presented below (Qureshi et al., 2010; Ezeji & Blaschek, 2010; Anish & Rao, 2009; Wang & Feng, 2010) :

- Biological: the use of enzymes or microbial cultures to detoxify hydrolysates.
- Physical: Evaporation.
- Chemical: Precipitation of toxic compounds, ionization to change the structure of toxic compounds, overliming, adsorption onto activated carbon and ion exchange resins.

In this study evaporation was initially considered to increase the sugar content of the pre-hydrolysate liquor for fermentation. However literature states that evaporation is not effective as a detoxification method since it only decreases the amount of volatile inhibitors such as acetic acid, furfural and vanillin. However, the concentration of non-volatile inhibitors and the overall degree of fermentation inhibition is increased (Wang & Feng, 2010; Anish & Rao, 2009). If evaporation is used to increase the

sugar concentration of a hydrolysate then another detoxification method must be used to remove the non-volatile inhibitors.

An alternative method to detoxification is to adapt the fermenting microorganism so that it can ferment sugars in the presence of inhibitors (Wang & Feng, 2010). This method is not strictly detoxification as the organism will only metabolically convert some of inhibitors but it will be able to tolerate higher concentrations of inhibitors. This is called yeast hardening and it is explained in the fermentation section (section 2.3.4).

2.3.3 ENZYMATIC HYDROLYSIS

Highly specific cellulase and hemicellulase enzymes (glucosyl hydrolases) are used to hydrolyse the cellulose and hemicellulose of pre-treated lignocellulosic material. These enzymes are commonly produced by cultures of Trichoderma reesei or Aspergillus. In this study a cocktail of hemicellulase and cellulase enzymes will be used. The pre-treatment step will hydrolyse most of the hemicellulose. The hemicellulases will hydrolyse the oligomeric pentose sugars and xylan that were not converted to monomeric form by the pre-treatment. This will improve the access to the cellulose so that it can be hydrolysed to glucose by the cellulases. The enzymatic hydrolysis of cellulose is usually accomplished by the synergistic action of three classes of enzymes listed here (Taherzadeh & Karimi, 2008):

- Endo-1,4,-β-glucanases or 1,4-β-D-glucan-4-glucanohydrolases.
- Exo-1,4-β-D glucanases, including both 1,4-β-D-glucan hydrolases and 1,4-β-D-glucan cellobiohydrolases. 1,4-β-D-glucan hydrolases liberate D-glucose and 1,4-β-D-glucan cellobiohydrolases liberate D-cellobiose.
- β-D-glucosidases or β -D-glucoside glucohydrolases, which release D-glucose from cellobiose and soluble cellodextrins, as well as an array of glycosides.

These enzymes work together with a synergistic effect (Taherzadeh & Karimi, 2008). This is because the exo-glucanases attacks the cellulose polymers from the ends while endo-gluconases cleaves the polymers into smaller polymers at any point between the ends, thus creating more endpoints where the exo-glucanases can attack. The synergistic working between endo –and exo-glucanases speeds up the production of cellobiose that is hydrolysed to glucose by the β-glucosidases, thus speeding up the production of glucose. It is a case where the whole is greater than the sum of the parts, i.e. the

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extent and rate of hydrolysis achieved by synergistic action of the two enzymes, is greater than the sum obtained when adding together the performance of the two enzymes individually.

β-glucosidases is usually a limiting agent in the enzyme hydrolysis process of cellulose (Taherzadeh & Karimi, 2008). This limitation is indicated by accumulating cellobiose as β-glucosidases converts cellobiose to glucose (Cantarella et al., 2004). Addition of supplemental β-glucosidases can overcome this limiting step and improve sugar yields and productivities (Taherzadeh & Karimi, 2008; Wyman et al., 1992).

Cellulase activity, substrate properties, substrate concentration and process conditions (temperature and pH) are the variables that affect the enzymatic hydrolysis of cellulose (Taherzadeh & Karimi, 2008). Cellulase activity decreases with time due to the irreversible adsorption of cellulase onto cellulose, and product feedback inhibition caused by glucose and cellobiose (Taherzadeh & Karimi, 2008).

The dosage of enzyme can vary widely. On lab scale enzyme is usually added at a dose of approximately 10 FPU (filter paper units) per gram of WIS (Taherzadeh & Karimi, 2008). This dosage gives reasonable results in a reasonable time of 48 – 72 hours. However, the dosage can vary from 5 – 33 FPU/g WIS depending on the characteristics of the lignocellulosic biomass that is used (Taherzadeh & Karimi, 2008; Gnansounou, 2009).

For the cellulases to be able to have access to the cellulose requires the complete hydrolysis of the hemicellulose. The complete hydrolysis of hemicellulose requires hemicellulose enzymes such as xylanase, β-xylosidase and other complementary enzymes (Ezeji & Blaschek, 2010).

2.3.4 Fermentation

Two factors that influence the production cost more than others are the effective conversion of sugars to ethanol (yield and productivity) and the concentration of ethanol in the fermentation broth (Öhgren et al., 2006).

During fermentation sugars are converted to ethanol by yeast with carbon dioxide as a byproduct. The conversion of hexose sugars to ethanol by yeast has already been shown by Equation 3 (Taherzadeh & Karimi, 2008).

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The theoretical maximum yield of ethanol from hexose sugars is 0.511 g ethanol per gram of consumed sugars. However not all of the consumed sugars are converted to ethanol as the yeast uses some of the carbon source for the cell growth and cell maintenance and this causes the formation of by-products such as glycerol and acetic acid. The ethanol yield usually doesn't exceed 90 – 95 % of the theoretical yield (Taherzadeh & Karimi, 2008).

Conversion of pentose sugars (mainly xylose) to ethanol by means of fermentation takes place anaerobically as presented here in Equation 5 (Taherzadeh & Karimi, 2008):

$$3C_5H_{10}O_5(Pentoses) \rightarrow 5C_2H_5OH(Ethanol) + 5CO_2$$

Equation 5

The theoretical maximum yield of ethanol from pentose sugars is the same as that of hexose sugars, 0.511 g ethanol per gram of consumed sugars. However, the actual yield is usually much lower than the theoretical maximum due to complications in fermenting xylose (Taherzadeh & Karimi, 2008).

In this study the yeast Saccharomyces cerevisiae will be used as the ethanol producing organism. During the last 20 to 30 years new cultures of recombinant S. cerevisiae cultures have been developed, but the overall productivity of these strains are not optimal (Qureshi et al, 2010). The problem with co-fermentation of both hexoses and pentoses in the same vessel is that the hexoses will be fermented before the pentoses are fermented as the yeast has a higher selectivity for hexoses. This results in diauxic growth/fermentation, creating a sequential, two-step fermentation with extended total fermentation time. S. cerevisiae can usually only ferment hexoses. It must be genetically modified to ferment pentoses (Qureshi et al., 2010). P. stipitis is a yeast strain that is able to ferment xylose sugars and it has shown promise in industrial application (Taherzadeh & Karimi, 2008; Qureshi et al, 2010). If the recombinant strain is not successful it may be considered to use more than one type of yeast and ferment the hydrolysate and WIS in separate vessels.

From an economic point of view it is best to take a genetically modified strain and develop a hardened mutant strain from this genetically modified strain via culture adaptation (Qureshi et al, 2010). This genetically modified hardened mutant strain will be able to ferment both pentoses and hexoses in the presence of high inhibitor concentrations. Only recombinant S.cerevisiae strains have been able to produce ethanol from xylose in non-detoxified hydrolysates through culture adaptation (Qureshi et al, 2010).

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A list of criteria for an ideal ethanol producing microorganism is presented below (Taherzadeh & Karimi, 2008; Qureshi et al, 2010):

1. High growth rate and high fermentation rate, but the high growth rate must not be at the expense of a high ethanol yield, by maintaining a low biomass yield under anaerobic conditions.
2. High ethanol tolerance to be able to produce a fermentation broth with a high ethanol content to reduce energy requirements for product separation.
3. Ability to ferment pentoses and hexoses.
4. Osmotolerance allows the use of feedstock with high salt content.
5. Low optimum fermentation pH to prevent contamination.
6. Tolerance to inhibitors released during pre-treatment and other metabolic by-products such as acetic or lactic acid.
7. High optimum temperature to reduce cooling requirements of fermentation and to operate closer to the enzyme's optimum operating temperature.
8. Hardiness under physiological stress to survive handling such as centrifugation.

Fed-batch is used rather than batch to be able to utilise substrates containing fermentation inhibitors and to be able to start the fermentation with less initial lignocellulosic biomass and thus less inhibitors. (Taherzadeh & Karimi, 2008)

There are two main different fermentation schemes employed in the fermentation of hydrolysed sugars from lignocellulosic biomass. They are separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). These two fermentation schemes are explained in the sections that follow.

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2.3.4.1 Separate hydrolysis and fermentation (SHF)

The first step of SHF is the enzymatic hydrolysis of cellulose to glucose. The second step is the fermentation of the glucose to ethanol. The two separate steps take place in two different fermenters in industry due to the continuous nature of an industrial plant. On a laboratory scale an SHF experiment can be performed in the same reactor by first allowing for completion of the enzymatic hydrolysis before commencing with the fermentation.

The advantages and disadvantages of SHF are presented in Table 4.

Table 4: Advantages and disadvantages of SHF (Taherzadeh & Karimi, 2008; Cantarella et al, 2004)

Advantages	Disadvantages
Enzymatic hydrolysis can be performed at the optimum conditions (Trichoderma reesei cellulases: 45°C - 50°C).	Released sugars inhibit enzyme activity (product inhibition).
Fermentation can be performed at the optimum conditions (S. cerevisiae 30°C - 35°C).	Higher capital costs due to more vessels required for separate hydrolysis and fermentation steps.
Yeast can be recycled since the lignin is separated out in the hydrolysis reactor.	

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2.3.4.2 Simultaneous saccharification and (co-) fermentation (SS(C)F)

SSF is the combination of enzymatic hydrolysis of cellulose (with cellulases from Trichoderma reesei) and fermentation into a single step (Taherzadeh & Karimi, 2008; Leibbrandt, 2010). SSF holds many advantages over SHF (Öhgren et al., 2006). The glucose that is produced by the hydrolysing enzymes is immediately consumed by the fermenting microorganism. The advantages and disadvantages of this process configuration are presented in Table 5.

Table 5 Advantages and disadvantages of SSF (Taherzadeh & Karimi, 2008; Cantarella et al., 2004; Wyman et al., 1992)

Advantages	**Disadvantages**
Lower capital costs due to fewer vessels required.	Operation at non optimal conditions for enzymatic hydrolysis or fermentation.
Less contamination during enzymatic hydrolysis as the presence of ethanol reduces the possibility of contamination.	Ethanol inhibition of enzymes. At 30 g/L EtOH enzyme activity is reduced by 25 % (Taherzadeh & Karimi, 2008).
Higher ethanol yield.	Yeast can't be recycled due to difficulties separating lignin and yeast.
Lower enzyme loading requirement due to less enzyme inhibition (the inhibiting sugars are fermented as soon as they are formed).	Ethanol inhibits yeast.
SSF takes less time to complete than SHF because of the elimination of the lengthy hydrolysis step.	Most microorganisms that are used for SSF can't ferment xylose and the ones that can still prefer glucose which may cause a build-up of xylose before it is fermented leading to diauxic growth.

It will be beneficial to use a yeast strain that can ferment both pentoses and hexoses in a process termed simultaneous saccharification and co-fermentation (SSCF). Yeasts that can be used for SSCF include recombinant S.cerevisiae strains and Z. mobilis.

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2.3.4.3 Consolidated bioprocessing (CBP)

Scientists are currently working on organisms that can ferment both hexose and pentose sugars whilst also producing cellulase enzymes, thus enabling the processes of enzymatic hydrolysis and fermentation to take place in the same vessel. This will greatly reduce costs as it would not be necessary to buy costly enzymes. The cost of enzymes is estimated to be as high as US$ 1.47 per gallon of ethanol produced (Klein-Marcuschamer et al., 2012). (Leibbrandt, 2010)

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2.4 ETHANOL RECOVERY

Fermented broth or beer typically contains between 2 % and 12 % ethanol. In this project 4 % is seen as the minimum target for cellulosic ethanol production because at ethanol concentrations of lower than 4 % the ethanol separation costs start to increase rapidly (Öhgren et al., 2006). The broth also contains microbial biomass, fusel oil, volatile components and stillage. Fusel oil is used in solvents for paints and polymers. Stillage represents the non-volatile fraction of materials that are present after distillation. The stillage composition depends on the feedstock. Distillation is the section that uses the most thermal energy in the whole pant (Leal, 2010).

2.4.1 DISTILLATION

The main goal of distilling alcohol is to get a wine with high levels of alcohol with values from 92.6 % to 93.5 % for hydrous ethanol and 99.3 % for anhydrous ethanol (Felipe, 2010).

Distillation is typically used for the separation of ethanol from aldehydes, fusel oil and stillage. The ethanol can be distilled up to a purity of 95.57 wt % where it forms an azeotrope with water. However it is not practical to distil ethanol to the azeotropic purity due to the excessive number of distillation stages that this will require. This form of ethanol is known is hydrous ethanol. Other methods must be employed to further increase purity of the product to the stage of anhydrous ethanol (99.3 wt %) that can be blended with gasoline.

The following parameters must be considered for industrial distillation systems:

- Energy consumption (steam for boiler and cooling water for condensator)
- Ethanol quality (degree of separation of fusel oil and light components)
- How to deal with solids clogging the reboiler of the first column. Special use of a vacuum may be applied for this problem. Using open steam instead of reboiler can prevent clogging, but it will increase the amount of wastewater.
- Simplicity of process control.
- Simplicity of maintenance.
- Capital investment.

There are many multi-column approaches to distil ethanol. In this project a simple two-column approach such as the one shown here in Figure 5 shown is used.

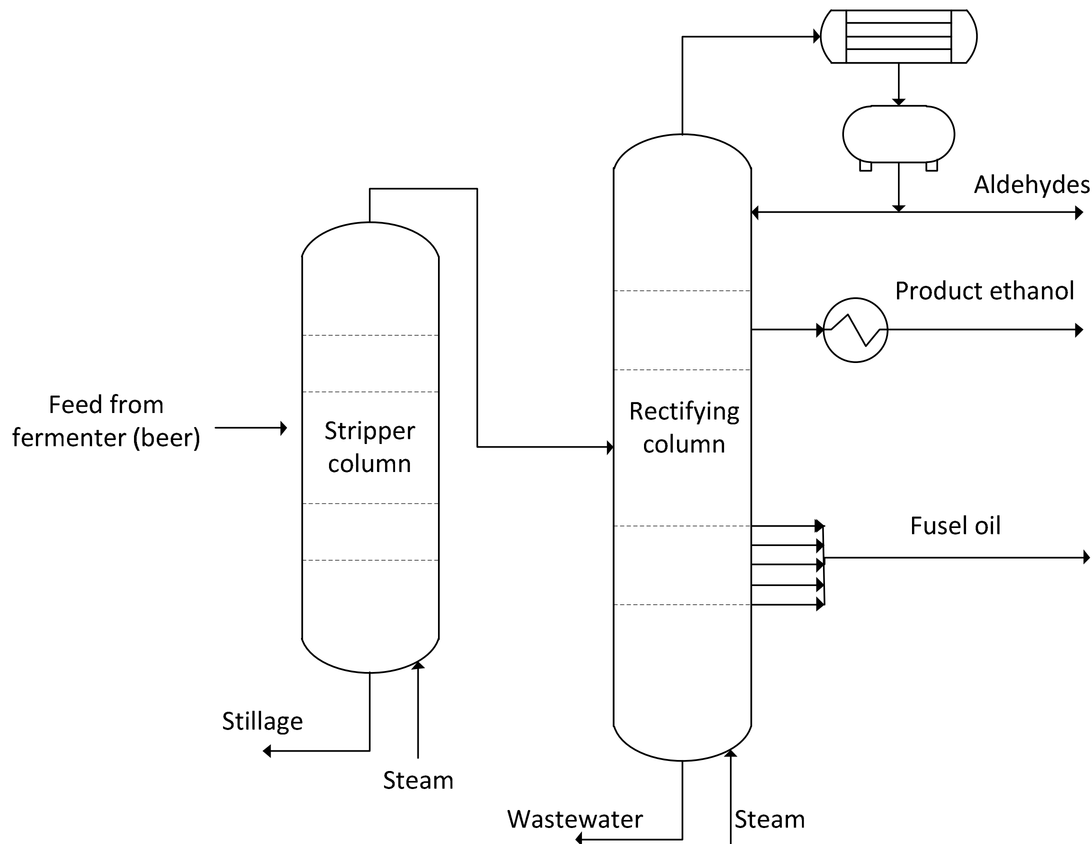

Figure 5: Two-column system for distillation of ethanol (redrawn from Taherzadeh & Karimi, 2008)

In Figure 5 the beer is first pumped into the stripper column where steam is used to heat the mixture to boiling point. The ethanol rich vapour from the stripper column passes to the rectification column. The stillage (mostly lignin in the case of second generation ethanol) is removed from the bottom of the stripper column and concentrated before being sent to the boilers or used as fertiliser in the fields (vinasse from first generation). In the rectification column aldehydes are drawn from the top. Hydrous ethanol is removed near the top. Fusel oil is taken out from several plates.

There has been the introduction of multiple effect distillation (Felipe, 2010) such as double effect distillation (Dias et al., 2011; Dias et al., 2009) to reduce energy consumption. Both atmospheric and double effect distillation has been used in this study.

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2.4.2 Ethanol dehydration

In order to blend ethanol with gasoline, ethanol must contain less than 1 vol % water. This purity of ethanol can't be achieved through distillation due the water ethanol azeotrope mentioned in section 2.4.1. A higher level of water can result in a two phase mixture with gasoline in the one phase and water and ethanol in the other. Removal of water past the last 5 % is called ethanol dehydration. Dehydration methods include azeotropic distillation, membrane technology and molecular sieve adsorption. Molecular sieve adsorption is the chosen method for this project, based on industrial preference.

2.4.2.1 Molecular sieve adsorption

The method of molecular sieve absorption is more energy-efficient than azeotropic distillation. In this method hydrous ethanol is passed through a bed of synthetic zeolite particles that preferentially adsorbs water. Approximately three quarters of adsorbed material is water and one quarter is ethanol. The bed becomes saturated within a few minutes and it must be regenerated by heating or evacuation to remove the adsorbed water. During regeneration a side stream of approximately 50 % water and 50 % ethanol is removed and recycled back to the distillation column(s). (Taherzadeh & Karimi, 2008)

2.5 Co-generation of heat and power

Currently sugar mills around the world are using steam cycles to generate electricity and low pressure steam for process heating (Ensinas et al., 2010). The Biomass Integrated Gasification Combined Cycle (BIG-CC), which utilises the gasification of biomass with both gas and steam turbines, holds the promise of much higher conversion efficiencies than steam only systems, but it is not yet viable for use in sugar mills due to higher capital costs (Ensinas et al., 2010; Sims, 2002a). A steam cycle is therefore considered in this study for heat and power co-generation.

At first electricity generation using bagasse was very inefficient as the goal was only to make the plant energy self-sufficient (Leal, 2010; Ensinas et al, 2010). In the past bagasse was regarded as a

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waste stream with little or no value and was therefore disposed of by burning it in low efficiency boilers to prevent a build-up of bagasse and to provide just enough steam and electricity to make the plant energy self-sufficient (Ensinas et al, 2010). However, due an Energy crisis in Brazil in 2001, the goal has become for sugar mills to sell surplus power to the grid, thus providing incentives to make both the generation and utilisation of steam/electricity much more efficient (Leal, 2010; Ensinas et al, 2010).

Surplus power generation has increased to values above 80 kWh/ton of cane. By including thrash along with bagasse the excess power generation can potentially increase up to 150 kWh/ton of cane. (Leal, 2010).

High pressure steam is used to operate equipment such as roller mills used in sugar cane processing. In this study the mill is replaced with a diffuser, thus all the high pressure steam that is generated will be used for the generation of electricity. Medium pressure steam is used to supply heat to the molecular sieves used for ethanol dehydration (Ensinas et al., 2010). Low pressure steam is used to supply heat to the diffuser and the rest of the process, including evaporation. Boilers of 60 bar and above are needed for the co-generation system, to maximise the efficiency of bagasse utilisation.

Standard boilers in Brazil today are 65 bar/480 °C in the case of backpressure turbines only or 100 bar/520 °C in the case of condensing/extraction or condensing/backpressure turbines (Leal, 2010). State of the art boiler technology used in mills is 100 bar/530 °C with 89 % energy efficiency (Leal, 2010).

An obstacle with exporting electricity is the contractual difficulties associated with only being able to export electricity for 6 – 7 months during the cane crushing season. This can be overcome by burning other waste bio-materials, such as wood chips, during the off season to produce electricity year round and this will also help to improve the process economics (Sims, 2002a).

2.6 PINCH POINT TECHNOLOGY

Pinch technology will be used to perform heat integration. Heat integration will improve the energy efficiency at which the whole facility operates (Ensinas et al., 2010). This will help to reduce the operating costs by reducing the utilities used in the plant, while also increasing the amount of electricity for sale. However the increased energy efficiency will come at the price of increased capital cost due to the installation of heat exchangers and piping. This results in a trade-off between

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reduced operating costs and increased capital costs. Thus an optimum degree of heat integration must be sought to obtain the lowest possible cost of producing ethanol.

The methodology described by Turton et al. (2009) will be used to design a heat exchanger network (HEN). The algorithm that will be followed will ensure a HEN that consumes the minimum amount of utilities and requires the minimum number of exchangers (MUMNE).

The HENSAD (Heat Exchanger Network Simulation and Design) was used to perform the PINCH heat integration (Turton et al., 2009b).

According to Turton et al. (2009) the formalisation of heat integration theory and pinch technology has been attributed to the following researchers: Linhoff & Flower (1978), Hohmann (1971) and Umeda et al. (1978). The approach described in Turton et al (2009) was that given by Douglas (1988).

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2.7 ENGINEERING ECONOMIC ANALYSIS

In this study the economic analyses will be performed according to the method described by (Turton et al., 2009a) for engineering economic analysis for chemical processes. A brief summary and the formulas used for the economic analysis will be provided here.

The first step is to obtain a capital cost estimate. In this study the capital cost will be estimated by using the estimated capital costs determined by (Macrelli et al., 2012) for very similar scenarios. The accuracy of this method is approximately +24 % to -16 %. The capital cost will be scaled to the appropriate flow rate using the six-tenths rule presented below in Equation 6. The effect of inflation will be accounted for by the Chemical Engineering Plant Cost Index (CEPCI). The CEPCI for 2012 will be determined for 2012 by using Equation 7, where f is the inflation rate.

$$\frac{C_a}{C_b} = \left(\frac{A_a}{A_b}\right)^{0.6}$$

Equation 6

$$CEPCI(2012) = (1 + f).CEPCI(2011)$$

Equation 7

In Equation 6 C denotes the capital cost, A denotes the cost attribute (cane flow rate in this case), subscript “a” refers to the required attribute/cost, subscript “b” refers to the base/known attribute/cost and the cost exponent is set equal to 0.6 according to the six-tenths rule.

A module costing approach as described by Turton et al (2009) could not be used in this study since quotes could not be obtained for specialised equipment like cane reception -and extraction equipment. These types of equipment are specialty equipment of the sugar industry and their prices aren’t listed in engineering design texts such as (Sinnott & Towler, 2009) or Turton et al (2009).

The capital investment depreciates over time and this depreciation is considered as a tax deductible expense. In this study linear depreciation over five years is used to account for depreciation. The

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depreciation period is not the same as the plant life. In this study a plant life of 12 years, including two years for construction, is assumed to be conservative.

The next step is to determine the manufacturing cost. This was determined according to Equation 8.

$$COM_d = 0.18.FCI + 2.73C_{OL} + 1.23(C_{UT} + C_{WT} + C_{RM})$$

Equation 8

In Equation 8 COMd refers to the manufacturing cost or cost of manufacture (COM), the subscript d denotes that the depreciation was not considered when calculating the manufacturing cost, FCI is the capital cost or fixed capital investment (FCI), the other subscripts denote the cost of the following: operating labour (OL), utilities (UT), waste treatment (WT) and raw materials (RM).

The cost of operating labour is calculated according to the number of operators required per shift and that is calculated according to Equation 9, where P is the number of solid handling stages and Nnp is the total number of major process equipment, which includes compressors, towers, reactors, heaters and exchangers.

$$N_{OL} = (6.29 + 31.7P^2 + 0.23N_{np})^{0.5}$$

Equation 9

The capital and manufacturing costs along with the revenue, taxation, depreciation, inflation and interest rate will be used to construct a cumulative cash flow diagram (CCFD). All cash flows will be discounted to the present (2012) by using an average interest rate. An average inflation rate will be used to account for future price increases.

The CCFD will be used to assess different economic criteria of the process. These criteria are the discounted payback period (DPBP), the net present value (NPV), the present value ratio (PVR) and the internal rate of return (IRR). The discounted payback period is the time required after start-up to recover the capital costs. A shorter DPBP is obviously better. The NPV is cumulative discounted cash position at the end of the assumed plant life. Since the NPV is greatly influenced by the capital cost it is better to use the PVR. The PVR is the present value of the positive cash flows divided by the present value of all the negative cash flows. The IRR is the interest rate at which all cash flows must be discounted in order to obtain an NPV of zero.

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2.8 Integration of 1st and 2nd generation bioethanol

The integration of first and second generation bioethanol production technologies will be investigated in this project. First and second generation technologies can be integrated into the same facility by sharing certain process sections such as feedstock handling, co-fermentation, ethanol separation, heat and power generation and other infrastructure such as offices to reduce capital expenses. By combining process sections further upstream the capital cost and energy demands can be decreased (Leal, 2010). Heat integration between first and second generation streams in the same facility will improve the plant's efficiency and increase the amount of surplus electricity produced for sale.

It is difficult to obtain high ethanol concentrations in second generation fermentations due to low sugar concentrations. The low sugar concentrations are due to the limitations in the final loading of pre-treated solids that can be achieved, which in turn are caused by the limitations in solids loading due to viscosity, high inhibitor concentrations and the reduced efficiency of hydrolytic enzymes at high solids loadings (requiring an increase in enzyme dosage) (Zhang et al., 2010; Yang et al., 2010; Öhgren et al., 2006). High ethanol concentrations are easily achieved in first generation fermentations due to high sugar concentrations and the absence of fermentation inhibitors that are present in second generation fermentations. The fermented broth from first generation fermentations can easily be distilled, and typically contains 9 to 15% ethanol. However, it is very costly and energy intensive to distil a fermented broth with an ethanol content below 4 % (Öhgren et al., 2006). The concentration of the broth can be increased, to decrease separation costs, by blending the fermented broths from both technologies so that the average ethanol content is higher than 4 % (Leal, 2010).

Furthermore the first and second generation ethanol production technologies can be combined as far upstream as fermentation, using co-fermentation of sugar streams from sugarcane and hydrolysis of fibres. This could mean, for example, that sucrose syrup will be added to the second generation fermentation. In this kind of fermentation ethanol can be removed through gas stripping and glucose can be added in a concentrated stream (Qureshi et al, 2010). In literature only SHF 2nd generation fermentations have been combined with first generation technology in the fermentation step (Dias et al., 2009; Dias et al., 2011b; Macrelli et al., 2012). In this project SSF is combined with first generation fermentation on an experimental scale.

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Building a second generation bioethanol plant holds large risk for investors as second generation bioethanol production is a very new technology that has only recently been proven on an industrial scale. Second generation technology is still in the learning curve phase of a new technology. By combining the new second generation technology with proven first generation technology the risk is reduced whilst the advantages of both technologies are realised making it a more attractive venture for investors (Gnansounou, 2009).

2.9 Technical and Techno-economic studies

There have been many technical and techno-economic studies done on producing ethanol from sugarcane bagasse and/or sugar juice using process simulation software to model the processes and, in some cases, perform economic analyses. This section will show the similarities and differences between the present study and the following other literature sources: Dias et al (2009), Dias et al (2010), Dias et al (2011a), Dias et al (2011b), Modesto et al (2009), Macrelli et al (2012), Ensinas et al (2007), Ensinas et al (2009), Pellegrini & de Oliveira Junior (2011) and Seabra & Macedo (2011). The similarities and differences between the present study and the literature sources mentioned here are highlighted by comparing them in tables (Table 6 to Table 13) and then discussing the reasons for similarities and differences. The reasons for the parameters chosen in this study are also provided, in particular to obtain economic outputs that are relevant to the South African scenario. A very short summary of each of the studies mentioned here will first be given as background information before the similarities and differences are discussed.

Dias et al (2009) evaluated the impact that double effect distillation has on an integrated first and second generation process compared to conventional distillation. It was found that double effect distillation increased the available bagasse for the production of second generation ethanol. However, the additional ethanol production comes at the price of much lower excess electricity production due to the electricity that is required by the compressor in the double effect distillation set-up. Double effect distillation was not considered in this study due to the increase on capital cost that a distillation column operating under vacuum pressure and an extra compressor will have. Another reason for not considering double effect distillation in this study is that the amount of electricity required to run the compressor is too much when considering the marginal gain in ethanol.

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Dias et al (2010) performed analyses of ethanol production costs for different scenarios considering improvements on electricity generation for an autonomous distillery.

Dias et al (2011a) modelled different co-generation, distillation and thermal integration options for ethanol production for an autonomous distillery. A traditional Rankine steam cycle and BIGCC (Biomass Integrated Gasification Combined Cycle) were investigated for the co-generation options.

Dias et al (2011b) compared an integrated first and second generation plant to a stand-alone second generation plant and they found that an integrated plant is better than a stand-alone plant, especially when advanced hydrolysis and pentose fermentation is considered. Dias et al (2011b) considered future (2015) technology for the stand-alone second generation scenario as well as for some of the integrated scenarios. The use of extrapolated future technology precludes the data from the stand-alone second generation scenario to be used in this study since this study only considers current technology. However the data from the current technology, integrated plant from Dias et al (2011b) will be utilised in this study for economic analysis of such a facility.

Modesto et al (2009) evaluated different possibilities for decreasing the thermal energy used in an autonomous distillery. The different possibilities are created by substituting the mills with a diffuser and using pinch heat integration to minimise utility usage.

Macrelli et al (2012) modelled various scenarios where first and second generation technologies were integrated to various degrees to produce ethanol and electricity from sugarcane. The effect of integration on the minimum ethanol selling price of second generation ethanol (MESP-2G) and on the plant energy efficiency was assessed. The process data for ethanol production per ton of sugarcane and the excess electricity production from Macrelli et al (2012) for an integrated plant was used in the economic analysis of this project.

Ensinas et al (2007) analysed the steam demand reduction for an integrated sugar and ethanol production process for different co-generation systems.

Ensinas et al (2009) used exergy analysis to assess an integrated sugar and ethanol production plant to reduce irreversibility generation. Thermal integration was proposed to minimise irreversibility generation.

Pellegrini & de Oliveira Junior (2011) performed exergy analysis on a sugar and ethanol plant and discuss the renewability of such a process according to the results of the exergy analysis.

Seabra & Macedo (2011) compared the technical, economic and environmental performance of second generation ethanol production versus power generation from bagasse considering a plant

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that is adjacent, but not connected to a first generation ethanol plant. The adjacent plant is not connected to the first generation plant to be able to assess power generation versus second generation ethanol production separate from the first generation plant. This is done so that the effect that economy of scale has on second generation ethanol can also be explored in the case of mill clustering with a central stand-alone second generation facility (Seabra et al., 2010). The data from the stand-alone second generation plant will be used in this study to perform an economic analysis for such a facility.

In the present study a first generation process model was built by using parameters from the literature sources that are discussed in this section. The point was to create a process model that is as up to date as possible considering current available technology. The choices for all the parameters are explained in the following tables and paragraphs. The process results from this model were used in to perform an economic analysis for 1st generation ethanol.

Table 6 on the following pages show the differences and similarities of the qualitative process information of the above mentioned literature sources and this current study. The qualitative process information, in this case, includes the type of plant that was studied as well as the technologies used for the following process steps: cane cleaning and juice extraction, pre-treatment, juice concentration, juice sterilisation, fermentation and ethanol recovery.

The first column of Table 6 shows the source from which the information in the table was obtained. The second column shows the type of production plant that was studied (1st or 2nd generation or both). It can be seen that most of the studies are concerned with the production of first generation ethanol or a combination of first generation ethanol and sugar. These studies were included here because they contain information on the production of first generation ethanol, co-generation of heat and electricity, process simulation and thermal integration that were used in building the first generation model that was used in this study. As mentioned earlier the process results from some of the studies were used to perform economic analyses on the integrated 1st and 2nd generation and the stand-alone 2nd generation since these scenarios weren’t modelled in this study.

The third column shows the technology that was used to clean the cane and extract the juice. Most of the studies by Dias (Dias et al., 2009; Dias et al., 2011a; Dias et al., 2011b) use a water saving dry-cleaning system to clean the cane. The same system was adopted in this study also to save water. Most of the literature source use mills for juice extraction. This is because most of these studies were done in Brazil where mills are commonly used for this purpose. However, due to the advantages of diffusers over mills as pointed out by Modesto et al (2009) in section 2.2.1, a diffuser will be used in the present study.

The fourth column shows the pre-treatment method that was used for the production of second generation ethanol for the studies that considered this option. In the present study steam explosion was used for the experimental section. However, since no second generation process modelling was performed in this study, the results from other studies will be used for the economic analysis.

The fifth column shows the method for juice concentration and sterilisation. In this study a five stage multi-effect evaporator (MEE) was used since it is clearly the most common way of concentrating the juice. This study also adopted the sterilisation of the sugarcane juice after concentration at 130 °C (Dias et al., 2009; Dias et al., 2011a; Dias et al., 2011b) to decrease chances of contamination during fermentation.

The sixth column displays the fermentation strategies that were employed. In this study the Melle-Boinot method with yeast recycling is used for the modelling of first generation fermentations. For second generation fermentations in the present study SSF, SHF and hybrid saccharification fermentation (HSF) (see section 3) were used. However, since no second generation modelling is performed in this study, the results from other studies will be used for the economic analysis.

The last column shows the methods used for ethanol recovery. In this study conventional distillation is used. For ethanol dehydration uses molecular sieves since it is the least energy intensive method of all those available (Taherzadeh & Karimi, 2008)

Table 6: Qualitative technical process information

Source	Plant type	Cane cleaning and sucrose extraction [a]	Pre-treatment	Concentration and sterilisation	Fermentation	Ethanol reocvery [b]
Dias et al (2009)	**1G + 2G**	Dry-clean. Mills.	Dilute acid and Organosolv delignification	**5 stage MEE. Juice is sterilised (130 °C).**	**1G + 2G SHF with Yeast recylce.**	**Conv and double effect dist.** Extractive dis.
Dias et al (2010)	1G	Mills	n/a	not given	**Melle-Boinot with yeast recycle**	**Conv dist.** Azeotropic dist and **molecular sieves**
Dias et al (2011a)	1G	Dry-clean. Mills.	n/a	**5 stage MEE. Juice is sterilised (130 °C).**	**Melle-Boinot with yeast recycle**	**Conv and double effect dist.** Extractive dist.
Dias et al (2011b)	**1G + 2G**	Dry-clean. Mills.	**Steam explosion and alkaline delignification**	**5 stage MEE. Juice is sterilised (130 °C).**	**1G + 2G SHF with Yeast recylce.**	**Conv and double effect dist. Molecular sieves**
Modesto et al (2009)	1G	Mills and diffusers	n/a	MEE	not given	**Conv dist.**
Macrelli et al (2012)	**1G + 2G**	Mills	Steam pre-treatment with H_3PO_4.	5 stage MEE.	**1G + 2G SHF with Yeast recylce.**	**Double effect dist.**
Ensinas et al (2007)	1G + sugar	Water wash. Mills.	n/a	5 stage MEE.	**Melle-Boinot with yeast recycle.** Mixed syrup and molasses.	**Conv and double effect dist.** Azeotropic dist and **molecular sieves.**
Ensinas et al (2009)	1G + sugar	Water wash. Mills.	n/a	5 stage MEE.	**Melle-Boinot with yeast recycle.** Mixed syrup and molasses.	**Conv and double effect dist.** Azeotropic dist and **molecular sieves.**

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Table 6: Qualitative technical process information (continued)

Source	Plant type	Cane cleaning and sucrose extraction [a]	Pre-treatment	Concentration and sterilisation	Fermentation	Ethanol reocvery [b]
Pellegrini & de Oliveira Jnr (2011)	1G + sugar	Mills.	n/a	5 stage MEE.	**Melle-Boinot with yeast recycle.** Mixed syrup and molasses.	**Conv and double effect dist.** Azeotropic dist.
Seabra & Macedo (2011)	**1G + 2G**	Mills.	Dilute acid	not given	SSCF	not given
Present study	**1G + 2G**	Dry-clean. Diffuser.	**Steam explosion**	**5 stage MEE. Juice is sterilised (130 °C).**	**1G + 2G SHF with Yeast recylce. Melle-Boinot with yeast recycle (1G). SHF (2G)[c]**	**Conv dist. Molecular sieves**

a: The cane washing method is given first and then the extraction method.

b: The distillation method to obtain hydrous EtOH is given first and then dehydration method.

c: This only refers to the process modelling of this study and not the experiments.

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Table 7 below shows the process simulation information that was used by each study. The first column shows the sources. The second column shows the software that was used. In this study Aspen Plus® was used for the process simulations because of availability and it can simulate the plant as well as the co-generation facilities whereas some of the other software can't simulate the co-generation facilities. Other sources that used Aspen Plus® to perform techno-economic analyses on bioethanol production processes from a wide range of feed stocks include Sassner & Zacchi (2008), Krajnc & Glavic (2009), Seabra et al (2010), Gnansounou & Dauriat, (2010) and Leibbrandt (2010).

The third column shows the equations of state (EOS's) that were used in the simulations. In this study ELEC-NRTL and SRK were used. The fourth column shows the component databases. This study used the NREL component database (Dias et al., 2011b; Macrelli et al., 2012; Seabra & Macedo, 2011; (Leibbrandt, 2010; Petersen, 2011) instead of hypothetical components for more accuracy. The last column shows the heat integration methods. Pinch heat integration was used in this study due to the well-defined steps of this method.

Note: In the process simulation Dias et al (2009) and Dias et al (2011a) uses lithium bromide absorption refrigeration to produce cooling water to keep the fermenters at 28 °C, whereas in the present study only cooling water from a cooling tower was used to keep the fermentation temperature at 32 °C.

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Table 7: Process simulation information

Source	Sotfware	Equations of state (EOS's) [a]	Component database	Heat integration
Dias et al (2009)	Unisim Design	**NRTL (liquid) and SRK (vapour)**	Hypothetical components	**Pinch.** Software by Elsevier.
Dias et al (2010)	Superpro Designer	not given	Hypethetical components	none
Dias et al (2011a)	Unisim Design	**NRTL (liquid) and SRK (vapour)**	Hypothetical components	**Pinch.** Software by Elsevier.
Dias et al (2011b)	**Aspen Plus®**	not given	**NREL biomass databank**	20 % reduction on process steam assumed
Modesto et al (2009)	EES	n/a	n/a	Pinch.
Macrelli et al (2012)	**Aspen Plus®**	not given	**NREL biomass databank**	Basic heat integration
Ensinas et al (2007)	EES	n/a	n/a	Basic heat integration
Ensinas et al (2009)	EES	n/a	n/a	Basic heat integration
Pellegrini & de Oliveira Jnr (2011)	EES	n/a	Only sucrose, ethanol and water.	Basic heat integration
Seabra & Macedo (2011)	**Aspen Plus®**	not given	**NREL biomass databank**	none
Present study	**Aspen Plus®**	**Elec-NRTL (liquid) and SRK (vapour)**	**NREL biomass databank**	**Pinch.**

a: EES (Engineering Equation Solver) doesn't require an EOS.

Table 8 shows the quantitative process information. The first column shows the source. The second column shows the bagasse that is produced per tonne of cane. In this study 120 dry kg/tc bagasse with MC of 50% was used because the process model in this study was based on that of Dias et al (2009) and thus the same cane composition was used. The third column shows the availability of trash. This study used the same amount of trash as Dias et al (2009) because the process model was based on Dias et al (2009).

The fourth and fifth columns show the ethanol production per tonne of cane for first and second generation ethanol, respectively. The first generation ethanol production of this study compares well to the literature values. The value is a bit higher in this study than for Dias et al (2009) for the same cane composition and thus the same sugar content of the cane. This slightly higher value can be attributed to the slightly higher extraction efficiency of a diffuser compared to a mill (99% vs. 97%).

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The second generation ethanol production per tonne of cane for this study (19 L/tc) was adopted from Dias et al (2011b) for a scenario in that is very similar to the one considered in this study. This scenario consists of 1st and 2nd generation integrated plant that uses current enzyme technology, hexose fermentation, pentose bio-digestion and steam co-generation facilities and molecular sieve dehydration. Other studies with higher second generation ethanol production assumed the co-fermentation of pentoses and hexoses(Seabra & Macedo, 2011) and more effective (future) enzyme technology (Dias et al., 2011b; Macrelli et al., 2012) and more effective power generation facilities (see Table 9).

The sixth column shows the size of the production plants by showing the flow rate of cane that is processed. This study used the same cane flow rate, 493 tc/hr, as Dias et al (2009) since the model used in this study was based on Dias et al (2009). See section 2.3 for a discussion on why a very large plant such as the 1000 tc/hr plant of Seabra & Macedo (2011) was not considered.

The seventh column shows the ethanol yield. Again the same value was adopted for this study than that of Dias et al (2009). The value of 90.48% compares well to the other literature values and to the experimental findings of this study.

The last column shows the number of days per year on which the plant is operational. This is also known as the cane crushing season. The highest value of 200 days/y was assumes here to help improve the process economics.

Table 8: Quantitative technical process information

Source	Bagasse (50% MC)	Trash (15% MC)	1G	2G [a]	Cane Flow	EtOH yield	Operating days
Dias et al (2009)	**120 dkg/tc**	**140 dkg/tc**	83.7 L/tc	18.8 - 22 L/tc	**493 tc/hr**	**90.48%**	n/a [b]
Dias et al (2010)	**120 dkg/tc**	**140 dkg/tc**	83.3 L/tc	n/a [c]	500 tc/hr	90%	180
Dias et al (2011a)	**120 dkg/tc**	n/a	85 L/tc	n/a [c]	**493 tc/hr**	not given	n/a [b]
Dias et al (2011b)	**120 dkg/tc**	**140 dkg/tc**	82 L/tc	19 - 35 L/tc or 158 – 181 L/dtb	500 tc/hr	90%	167
Modesto et al (2009)	140 dkg/tc	n/a	85.9 - 87.3 L/tc [ad]	n/a [c]	not given	not given	n/a [b]
Macrelli et al (2012)	not given	not given	85.2 L/tc	13.8 - 46.1 L/tc	540 tc/hr	94%	**200**
Ensinas et al (2007)	140 dkg/tc	125 dkg/tc	n/a [e]	n/a [c]	500 tc/hr	not given	167
Ensinas et al (2009)	140 dkg/tc	n/a	n/a [e]	n/a [c]	500 tc/hr	not given	167
Pellegrini & de Oliveira Jnr (2011)	125 dkg/tc	n/a	n/a [e]	n/a [c]	not given	89%	n/a [b]
Seabra & Macedo (2011)	130 dkg/tc	**140 dkg/tc**	91 L/tc	33 L/tc or **185 L/tb**	1000 tc/hr	not given	167 and 350 [f]
Present study	**120 dkg/tc**	**140 dkg/tc**	**85.5 L/tc**	**185 L/tb**	**493 tc/hr**	**90.48%**	**200**

a: Ranges of values for different scenarios that were considered.

b: Technical reports where the days of operation weren't considered.

c: These studies only consider 1G

d: higher sucrose content in cane was assumed

e: Not applicable due to the inclusion of molasses in the fermentation.

f: 1G plant operates 167 days/yr while adjacent plant (2G and/or power) operates 350 days/yr.

Table 9 shows the information that was used for simulating the co-generation facilities. The first column shows the source. The second column shows the software that was used. Some of the software may differ for the same source between Table 7 and Table 9. This is because, as previously mentioned, not all of the software can simulate both the production plant and the co-generation

facilities and thus extra software had to be used. However, Aspen Plus® that was used in this study is able to simulate both these facilities.

The third column shows the electricity generation cycles that were used in the co-generation facilities. This study used a Rankine steam cycle with a condensing extraction steam turbine (CEST) since it is more effective than a back pressure steam turbine (BPST) (Leal, 2010). Biomass integrated gasification combined cycle (BIGCC) or supercritical steam cycles were not considered since they are not yet commercially viable and they were considered by the other studies mostly for futuristic scenarios.

The fourth and fifth column shows the boiler temperature and pressure and the thermal efficiency of the boilers, respectively. In this study a 90 bar / 520 °C was used since it has a high thermal efficiency (85%) and it would not be as expensive as the higher pressure boilers (Dias et al., 2009; Ensinas et al., 2007). The sixth column shows the generator efficiencies and the value of 98% was chosen for this study since it is the most common value found in literature (Dias et al., 2009; Dias et al., 2011a; Dias et al., 2011b; Modesto et al., 2009).

The seventh column shows the inclusion of trash as extra fuel for electricity generation. This study considers the inclusion of 50% of the available trash as fuel since it is the most common literature value (Dias et al., 2009; Dias et al., 2010; Dias et al., 2011b; Macrelli et al., 2012). By using two values for the inclusion of trash the effect that the inclusion of trash has will be clearly seen.

The eighth and ninth columns show the power demand of the first and second generation facilities. This study uses the value of 28 kWh/tc power demand for first generation ethanol since this is the most common literature value (Dias et al., 2009; Dias et al., 2010; Dias et al., 2011a; Ensinas et al., 2007; Ensinas et al., 2009; Seabra & Macedo, 2011). For the second generation ethanol production the value of 24 kWh per tonne of bagasse (tb) (Dias et al., 2011b) is effectively used since the results from Dias et al (2011b) is used in this study for the economic analysis.

The last column shows the surplus electricity that is produced. There are wide discrepancies in literature between the amounts of surplus electricity produced due to the different electricity generation cycles (Rankine cycles, BIGCC or super critical steam cycles), different boiler pressures and boiler efficiencies, heat integration assumptions and varying assumptions concerning amounts of bagasse and trash contents of cane.

An interesting contrast between Dias et al (2009) and Dias et (2011a) is that in Dias et al (2009) electricity production decreases due to double effect distillation, but in Dias et al (2010) electricity

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production increases due to double effect distillation. This may be due to the fact that Dias et al (2009) considers 1G+2G whereas Dias et al (2010) only considers 1G.

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Table 9: Co-generation simulation information

Source	Software	Cycle	Boiler P & T [a]	Boiler eff	Generator eff	Trash inclusion	1G [b]	2G	Surplus power [b]
Dias et al (2009)	EES	Rankine (BPST)	**90 bar / 520 °C**	**85%**	**98%**	**50%**	**28 kWh/tc**	not given	13.5 - 33 kWh/tc
Dias et al (2010)	Spread sheet	**Rankine (CEST)**	**90 bar / 520 °C**	86%	96%	**50%**	**28** - 34 **kWh/tc**	n/a	68.2 - 154.9 kWh/tc
Dias et al (2011a)	EES	**Rankine (CEST)** and BIGCC	80 bar / 510 °C	**85%**	**98%**	none	**28 kWh/tc**	n/a	6.23 - 144.3 kWh/tc
Dias et al (2011b)	**Aspen Plus®**	**Rankine (CEST)**	**90 bar / 520 °C**	87%	**98%**	**50%**	30 kWh/tc	**24 kWh/tb**	34 - 173 kWh/tc
Modesto et al (2009)	EES	**Rankine (CEST)**	80 bar / 480 °C	80%	**98%**	none	25 - 34.83 kWh/tc	n/a	24.3 - 48.36 kWh/tc
Macrelli et al (2012)	**Apsen Plus®**	**Rankine (CEST)**	65 bar / 500 °C	80%	not given	**50** & 66%	not given	not given	41 - 80 kWh/tc
Ensinas et al (2007)	EES	**Rankine (CEST)** and BIGCC	100 bar/ 540 °C	**85%**	96%	none	**28 kWh/tc**	n/a	65.8 - 172.2 kWh/tc
Ensinas et al (2009)	EES	**Rankine (CEST)**	**90 bar / 520 °C**	**85%**	96%	none	**28 kWh/tc**	n/a	16.4 - 105.1 kWh/tc
Pellegrini & de Oliveira Jnr (2011)	EES	**Rankine (CEST)**, SuSC [c] and BIGCC	120 bar / 540 °C	not given [d]	not given	none	30 kWh/tc	n/a	~30 [e] - 200 kWh/tc
Seabra & Macedo (2011)	Hysys™	**Rankine (CEST)**	65 bar / 480 °C	not given	not given	40%	**28 kWh/tc**	not given	50 - 130 kWh/tc
Present study	**Aspen Plus®**	**Rankine (CEST)**	**90 bar / 520 °C**	**85%**	**98%**	**50%**	**28 kWh/tc**	**24 kWh/tb**	**40.7 – 71.2 kWh/tc**

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a: Rankine cycle values

b: Ranges of values for different scenarios that were considered.

c: Supercritical steam

d: many different boilers were used.

e: This value is approximate as it had to be read off a chart

Table 10 shows the steam consumption of certain unit operations in the process models. These values were used in the present study.

Table 10: Steam consumption of certain unit process operations

Source	Mol Sieves	Diffuser
Dias et al (2011b)	**0.6 kg/L EtOH (6 bar)**	
Modesto et al (2009)		**68 kg/tc (2.5 bar)**
Present study	**0.6 kg/L EtOH (6 bar)**	**68 kg/tc (2.5 bar)**

Table 11 shows the water consumption of certain unit operations in the process models. The imbibition water usage given for a diffuser by Modesto et al (2009) was used in this study. The water usage for the clarifier filter given by Ensinas et al (2009) was used in this study since this value is lower. The water usage for a water washing system for cane cleaning is shown to emphasize the amount of water that can be saved by using a dry-cleaning system to clean cane.

Table 11: Water consumption of certain process unit operations

Source	Imb water	Clarifier Filter	Washing
Dias et al (2009)	28 % of cane flow		
Modesto et al (2009)	235 kg/tc (mills) and **360.8 kg/tc** (diff)		
Ensinas et al (2009)	29.52 % of cane flow	**31.46 kg/tc**	25 % of plant water or 533 % of cane flow
Pellegrini & de Oliveira Jnr (2011)	25 % of cane flow	200 kg/tc	
This study	**360.8 kg/tc**	**31.46 kg/tc**	**dry-cleaning system**

Table 12 shows the assumptions that were used in the economic analyses. This study uses a project lifetime (NPV duration) of 20 years as opposed to the more common 25 years. This is done to be conservative. The construction period is taken as 2 years because it is the most common and conservative option. A linear depreciation over ten years is employed in this study as it is the same method employed by all the other studies cited in Table 12.

Table 12: Assumptions used in economic analyses

Source	Project life	Construction	Depreciation
Dias et al (2010)	25 years	**2 years**	**Linear over 10 years**
Dias et al (2011b)	25 years	**2 years**	**Linear over 10 years**
Macrelli et al (2012)	**20 years**	I year	**Linear over 10 years**
Seabra & Macedo (2011)	not given	not given	**Linear over 10 years**
This study	**20 years**	**2 years**	**Linear over 10 years**

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Table 13 shows some cost information that was used in the economic analyses. The second column shows the methods by which the capital cost estimations were obtained. The capital cost estimations for this study were obtained from literature (Dias et al., 2011b; Macrelli et al., 2012; Seabra et al., 2010). Literature cost data had to be used since vendor quotes or industrial data could not be obtained.

The cost from Dias et al (2011b) was used for an optimised first generation ethanol production plant (218 million US$ (2010)). The cost from Seabra & Macedo (2011) was used for the stand-alone ethanol plant because none of the other sources that where considered provided a cost for a stand-alone second generation plant. The cost from Macrelli et al (2012) was used for an integrated first and second generation plant (311 million US$ (2010)). The cost from Macrelli et al (2012) rather than the cost from Dias et al (2011b) was used for the integrated plant because Dias et al (2011b) considers a future cost whereas Macrelli et al (2011b) considers a current cost that is more conservative. All the costs were scaled to size using the six tenths rule. The costs were scaled to date using the CEPCI. The costs where converted to 2012 South African Rands (ZAR 2012) using an exchange rate of R 8.25/US$ (July – August 2012 average).

The last two columns shows the cane and enzyme prices. The enzyme price used in this study was obtained from (Petersen, 2011). There are large discrepancies between the prices of enzymes in literature. These discrepancies will be cleared up as soon as the production of second generation ethanol is demonstrated on a commercial scale. It can be seen that the cane prices in Brazil are a lot lower than in South Africa. This is because they can produce cane cheaper in Brazil and in South Africa the price of sugarcane is determined by the sugar industry.

The main economic barriers of second generation bioethanol is generally considered to be the cost of producing fermentable sugars (the cost of pre-treatment and enzymatic hydrolysis) (Sims, 2002b; Yang et al., 2010). Other factors that affect the economics is the location of the plant, the by-products produced, the capital costs and the risk associated with new processes (Sims, 2002b; Yang et al., 2010). Case studies have shown that the conclusions depend on technology assumptions and region of application (Seabra & Macedo, 2011).

All of these studies are applicable to the Brazilian context. The only study that has considered the South African context was Leibbrandt (2010). However Leibbrandt (2010) only investigated the stand-alone production of second generation ethanol from bagasse in the South African context whereas this study investigates an integrated plant as well as first and second generation stand-alone plants to determine the effects of integration in the South African and sub-Saharan contexts.

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Table 13: Cost information for economic analyses

Source	Capital Costs					Feedstock costs		Product prices	
	Estimation	conv 1G	opt 1G	2G	1G + 2G	Enzyme price	Cane Price	EtOH price	Electricity price
Dias et al (2010)[a]	Industry info	144 mil US$	205 mil US$	n/a	n/a	n/a	US$ 16.58/tc	US$ 0.40/L	US$ 67.05/MWh
Dias et al (2011b)[b]	vendor quotes, **assumptions and literature**	180 mil US$	**218 mil US$**	75 mil US$ (2010) in 2015 for 268 Mtb/yr [c]	293 million US$	US$ 0.11/L EtOH	US$ 23.25/tc	US$ 0.60/L	US$ 82.88/MWh
Macrelli et al (2012)[b]	Vendor quotes and Aspen.	117 mil US$	not given	not given	**311 million US$**	US$ 0.341/L EtOH	US$ 19.86/tc	US$ 0.53/L	US$ 87/MWh
Seabra & Macedo (2011)[d]	Vendor quotes.	not given	not given	**151 mil US$**	n/a	not given	not given	US$ 0.40/L	US$ 70/MWh
This study	**Literature and assumptions**	**n/a**	**218 mil US$ (2010)**	**151 mil US$ (2007)**	**311 million US$ (2010)**	**0.2 US$/L EtOH (2012)**	**R 331.55/tc or US$ 40.19/tc (2012)**	**R 6.70/L or US$ 0.81/L (2012)**	**R 0.90 – 1.85/kWh or US$ 109.09 – 224.24/MWh (2012)**

a: All amounts based on 2009 US$

b: All amounts based on 2010 US$

c: This is a future price (2015) in US$ (2010) for a plant that is already integrated with the 1G plant.

d: All amounts based on 2007 US$

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2.10 MAIN PROJECT OBJECTIVES

The primary aims of this project were briefly presented under section 1.2 and are elaborated upon here:

- Determine the effect of different fermentation strategies on the ethanol production process from sugarcane juice and bagasse.

1st generation experiments were performed to determine whether a natural hexose fermenting yeast strain can be replaced by a recombinant yeast strain for 1st generation fermentations. This will serve to reduce costs by using only one yeast propagation facility for a future scenario where pentose fermentation also takes place. The other objective of the 1st generation fermentations was to determine ethanol yields, productivities and maximum ethanol concentrations. The data from these experiments served as a baseline for later experiments where 1st and 2nd generation fermentation strategies were combined.

2nd generation fermentations were conducted to determine whether the minimum ethanol concentration of 40 g/L for cellulosic ethanol given by Öhgren et al (2006), to keep distillation costs down, can be reached with the feedstock and yeast strain that were available for this project. The data from these experiments served as a baseline for later experiments where 1st and 2nd generation fermentation strategies were combined.

Different ways of combining first and second generation fermentations were investigated to determine whether a combined fermentation strategy can improve the 2nd generation fermentation. 2nd generation fermentations typically take very long and yield low final ethanol concentrations. 1st and 2nd generation fermentations were combined in two ways. First by feeding sucrose syrup in a fed-batch manner with the bagasse and secondly by performing a pre-hydrolysis step with the pre-treated bagasse at high temperature (50 °C) for 12 hours before inoculation and then adding all the sugar syrup at once before continuing to feed more pre-treated bagasse in a fed-batch manner. These experiments were not performed as an optimisation but rather as a proof of concept. The results from these experiments were compared to pure 1st and 2nd generation fermentation experiments that were performed to determine whether the combination of 1st and 2nd generation fermentations served to improve the problematic 2nd generation fermentation.

- Determine the effect of process integration between 1st and 2nd generation biofuel production technologies on the energy efficiencies and the economic viability of such projects.

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A first generation model in Aspen Plus® was developed. Data from this model was used for the economic analysis of a first generation process. Process data from literature was used to simulate an integrated 1st and 2nd generation process and a stand-alone 2nd generation process. The energy efficiencies for each process were determined and they were all compared. The processes were also compared from an economic point of view in the sub-Saharan African context.

- Determine what financial incentives and market changes are necessary to make biofuels more economically viable and attractive to investors.

Some recommendations were made as to the necessary steps that need to be taken in terms of financial incentives and changes in the market that will have to be legislated to make biofuels in sub-Saharan Africa more economically viable and attractive to investors.

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3. First Generation fermentation experiments

3.1 Introduction

In this section sugar syrup fermentations were performed with two different *S.cerevisiae* yeast strains. The two strains that were used in these experiments were TMB 3400 (Wahlbom et al., 2003) and MH 1000 (Van Zyl et al., 2011).

MH 1000 is an industrial yeast strain that only ferments hexoses. TMB 3400 is a recombinant yeast strain that ferments both pentoses and hexoses (Wahlbom et al., 2003).

The aims of these experiments were to:

- Determine whether a recombinant yeast strain such as TMB 3400 can replace an industrial hexose fermenting yeast strain such as MH 1000, in performing the sugar syrup fermentations. This will serve to reduce costs by using only one yeast propagation facility for a future scenario where pentose fermentation also takes place.
- Determine ethanol yields, productivities and maximum ethanol concentrations.

3.2 Materials and methods

Sugar syrup was fermented using two different yeast strains. The two different yeast strains were TMB 3400 and MH 1000. The sugar syrup was kindly supplied by Umfolozi Sugar Mill. The sugar content of the syrup was determined at the mill to be 67 % (the syrup had a brix value of 67 %). The sugar syrup is a complex medium that contains many unknown nutrients and in the present study no additional nitrogen source was added to be able to determine the ability of the yeast to grow on sugar syrup alone. This will serve to cut costs in an industrial plant by not having to add a nitrogen source.

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3.2.1 Preparation of fermentation medium

The sugar syrup was diluted to approximately 22 % (Dias et al., 2009) and 3.4 g/L of KH_2PO_4 was added as a pH buffer. The amount of water that had to be added to dilute the sugar syrup to 22 %, was calculated by assuming that the concentrated sugar syrup concentration was 67 %. However this value varied somewhat as the sugar concentration throughout the syrup was not uniform and that is why the concentration of the diluted sugar syrup is stated as approximately 22 %. The KH_2PO_4 and sugar syrup were autoclaved separately and added after cooling to prevent a precipitation reaction that occurs when they are autoclaved together. The sugar syrup was autoclaved at 130 °C for 30 mins (Dias et al., 2009). A 68 g/L KH_2PO_4 solution was prepared and autoclaved separately before being added to the sugar syrup to obtain a final concentration of 3.4 g/L KH_2PO_4. The 22 % sugar solution with added KH_2PO_4 will henceforth be referred to as buffered sucrose medium.

3.2.2 Reactor fermentations

3.2.2.1 Preparation of the inoculum culture

The strains were stored at -80 °C in ± 30 % glycerol and plated on YPD plates (see Appendix B: Preparation of YPD plates). The pre-inoculum cultures were prepared in 250 mL Erlenmeyer flasks containing 50 mL of buffered sucrose medium. The pre-inoculum cultures were grown at 30 °C at 150 rpm in an orbital shaker for 18 hours.

The inoculum culture was inoculated with 10 mL of the pre-inoculum culture. The inoculum culture was prepared in a 250 mL Erlenmeyer flask containing 100 mL of buffered sucrose medium. The inoculum cultures were grown at 30 °C at 150 rpm in an orbital shaker for 16 hours. The inoculum cultures where used to inoculate the reactor fermentations at a starting optical density at 600 nm (OD_{600}) of 0.1.

Note: This method was adopted from the method used by NCP Alcohols to produce potable ethanol from molasses. See section 2.2.4.

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3.2.2.2 FERMENTATIONS

The fermentations were performed in 10 L New Brunswick Scientific (NBS) bioreactors with a working volume of 3 L using buffered sucrose medium. The fermentation conditions were the same as that used by NCP Alcohols. The pH was adjusted to 5.5 using 3M KOH. The fermentation was run at 32 °C. A Rushton turbine impeller was used for agitation at 200 rpm.

Sampling was done every three hours for the first 15 hours because, according to OD measurements, that is when the stationary phase was reached. After that a sample was taken at 28 hours and in some of the cases another sample was taken after 56 or 100 hrs. The samples were prepared for HPLC analysis with perchloric acid according to the procedure given in Appendix A: Sample preparation for HPLC analysis.

Biomass characterisation curves were determined for each fermentation run to be able to convert the OD values into cell concentrations (g dry cells/L). The biomass characterisation curves can be observed in Appendix C: Biomass characterisation.

3.3 RESULTS AND DISCUSSION

The fermentation were performed under micro-aerobic that were close to anaerobic conditions. The conditions were classified as micro aerobic due to the oxygen permeable silicone tubing that was used, the headspace was not flushed with nitrogen and the surface was exposed to air. This very closely simulates the micro-aerobic conditions found in industry as these fermentations are exposed to air. The fermentations were not aerated in any way, because aeration would have caused ethanol stripping. It was assumed that the high sugar concentration in the fermentations would were sufficient to induce the Crabtree effect. The Crabtree effect states that at high sugar concentrations (> 5 %) *S.cerevisiae* will ferment sugars to ethanol, even in the presence of oxygen (Bailey & Ollis, 1986; Rudolf et al., 2008). The Crab tree effect was only assumed and could not be verified since CO_2 evolution could not be measured on the fermenters that were used to perform the fermentations.

3.3.1 Growth curves

The growth curves for the two yeasts are presented in Figure 6. It can be seen that the stationary phase is reached at approximately 15 hours. Figure 6 shows good repeatability for TMB 3400 and MH 1000.

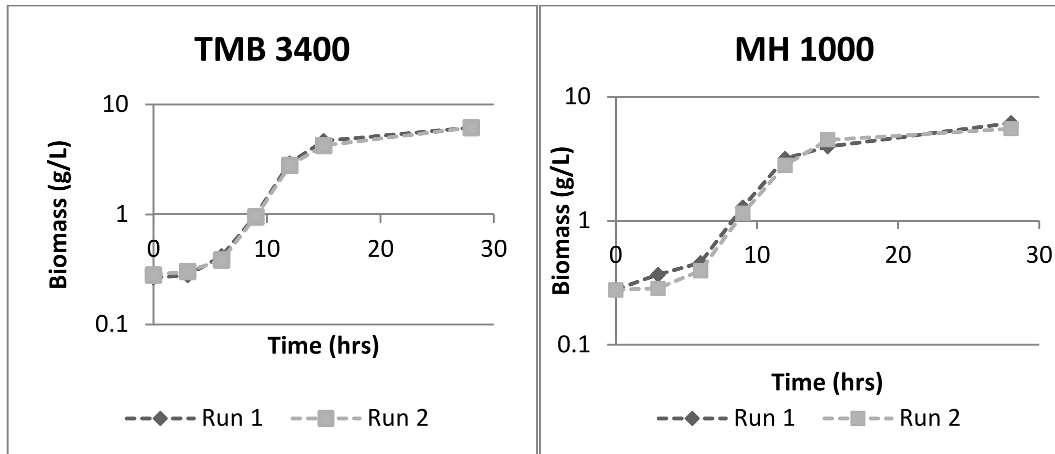

Figure 6: Growth curves for *S. cerevisiae* strains: TMB 3400 and MH 1000

Table 14 shows the maximum specific growth rates (μ_{max}) for both yeast strains that were determined from the growth curves. Both strains have very similar values for μ_{max} indicating that they grow at approximately the same rate. TMB 3400 has an average growth constant of 0.327 which is slightly higher than the average of MH 1000 of 0.325. However this small difference is insignificant. The determination of the maximum specific growth rates can be seen in Appendix D: Determination of maximum specific growth rate

Table 14: Maximum specific growth constants for TMB 3400 and MH 1000 under reactor conditions (as determined from their growth curves)

Strain	Run	μ max (h^{-1})
TMB 3400	1	0.324
	2	0.330
MH 1000	1	0.323
	2	0.326

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After the last samples were taken from the reactors and the agitation was stopped it was observed that the yeast started to flocculate and settle to the bottom of the reactor due to gravity. The yeast flocculates as a survival strategy when it is faced with adverse conditions (Calleja, 1987). Flocculation is a good yeast property since it enhances yeast recovery when the yeast is recycled (Senthilkumar & Gunasekaran, 2009) when the Melle-Boinot fermentation method (Dias et al., 2011a; Leal, 2010) is used.

3.3.2 SUBSTRATES AND PRODUCTS

Figure 7 to Figure 10 shows the substrate and product concentrations of all the fermentations along with the biomass plotted against time. Figure 7 and Figure 9 show the substrate concentrations for TMB 3400 and MH 1000, respectively. Figure 8 and Figure 10 show the product concentrations for TMB 3400 and MH 1000, respectively.

The figures mentioned in the above paragraph are of duplicate fermentation runs. Error bars are included with the data points to show the standard deviation of each data point for the duplicate runs. For the first run with TMB 3400 the last sample was taken at 102.25 hours and for the second run the last sample was taken at 56.25 hours, thus the last two data points of each series on the graphs of TMB 3400 are not duplicate data points. For the first run with MH 1000 the last sample was taken at 56.25 hours and for the second run the last sample was taken at 28 hours, thus the last two points of each series on the graphs of MH 1000 are also not duplicate data points. However the small error bars of all the other duplicate points show good repeatability meaning that these singular points must be accurate.

There were samples taken at a time denoted as t = -1 hours. These were samples taken before the fermentations were inoculated in order to determine the composition of the medium. However the sugar concentrations of these samples differed from the samples taken at time zero (time of inoculation) due to the small amount of invertase that is present in the medium. The small amount of invertase that is present in sugarcane explains the presence of glucose and fructose before the fermentation has started (Del Rosario & Santisopasri, 1977).

It can be seen for all the fermentations that the sucrose must first be converted to glucose and fructose according to Equation 2. This is achieved by the enzyme invertase that is produced by the

yeast. The glucose and fructose is then fermented to ethanol and some glycerol is formed as a by-product (Gnansounou, 2009).

Figure 7 shows that the sucrose decreases as it is converted to glucose and fructose by invertase. The glucose and fructose concentrations are very similar up until 15 hours for TMB 3400. After that the fructose increases to higher levels than the glucose. This shows that the yeast is utilising more glucose than fructose since there are equimolar amounts of fructose and glucose released when the sucrose is broken up by invertase (see Equation 2). This shows that the yeast has a higher selectivity towards glucose than fructose. However, after a long time all of the glucose and fructose have been almost completely consumed.

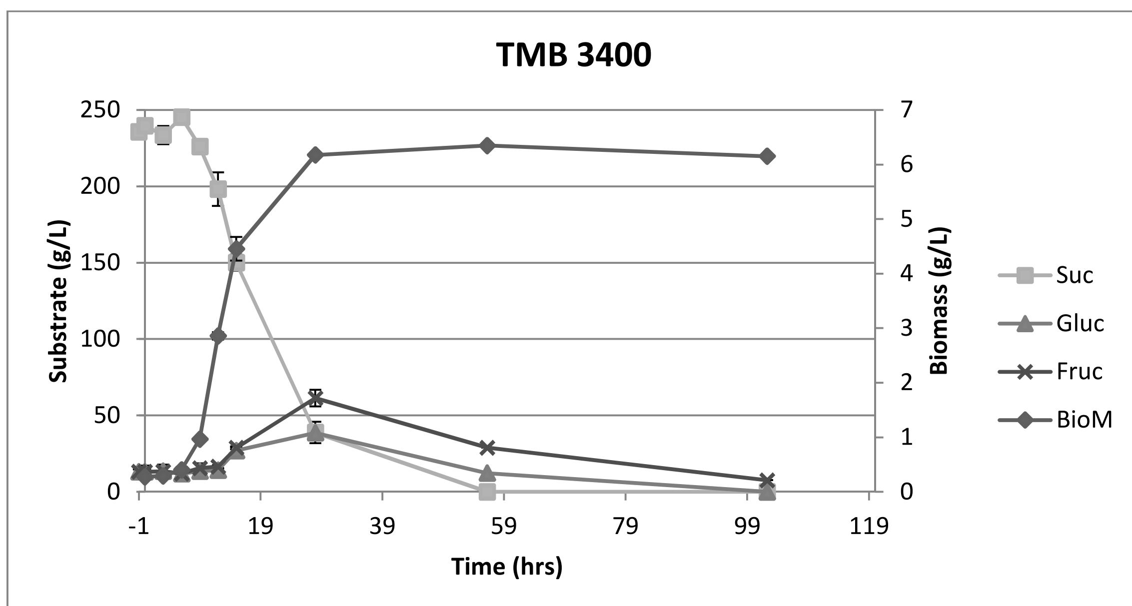

Figure 7: Substrate and biomass concentration for TMB 3400.

It can be seen from Figure 8 that for TMB 3400 a final ethanol concentration of 113.7 g/L was reached after 102.25 hours. This is a bit lower than the literature value given by Leal (2010) of 130 g/L for sugar syrup fermentation. This difference is because Leal (2010) considered an industrial plant with yeast recycle, which means less sugar is required for biomass production and more sugar is available for ethanol production (Dias et al., 2010; Dias et al., 2011a; Ensinas et al., 2007; Ensinas et al., 2009; Pellegrini & de Oliveira Junior, 2011). In this case there was no yeast recycling. However, the final ethanol concentration is higher than the 80 to 90 g/L or 60 to 80 g/L concentrations given by NCP Alcohols (Kitching, 2011) and (Senthilkumar & Gunasekaran, 2009), respectively, for the production of ethanol from molasses (the method used in this study was adapted from a method to

produce ethanol from molasses). This difference in final ethanol concentration can be attributed to the different medium that was used in this study (buffered sucrose medium).

The fermentation time of 102.25 hours is a bit longer than the times of 55 to 60 hours or 80 hours given by NCP Alcohols (Kitching, 2011) and (Senthilkumar & Gunasekaran, 2009), respectively, for the production of ethanol from molasses. However, it should be noted that this time is when the last sample was taken and not necessarily the time when ethanol production was complete (this may have occurred earlier). The longer fermentation time can be attributed to the fact that yeast recycling was not employed and thus the starting yeast culture was small compared to that used in the literature sources (Dias et al., 2010; Dias et al., 2011a; Ensinas et al., 2007; Ensinas et al., 2009; Pellegrini & de Oliveira Junior, 2011). (Taherzadeh & Karimi, 2008) states that yeast recycling will lead to shorter fermentation times.

The ethanol concentration reached 85.61 g/L after 56.25 hours for TMB 3400. The ethanol concentrations after 28 hours were 46.8 g/L and 47.1 g/L for the two runs. This shows good repeatability. The low levels of glycerol production, 12.1 g/L, indicate that the organism was not stressed too much by its environment (Scanes et al., 1998). This shows that there are not high levels of inhibitory compounds present in the sucrose medium.

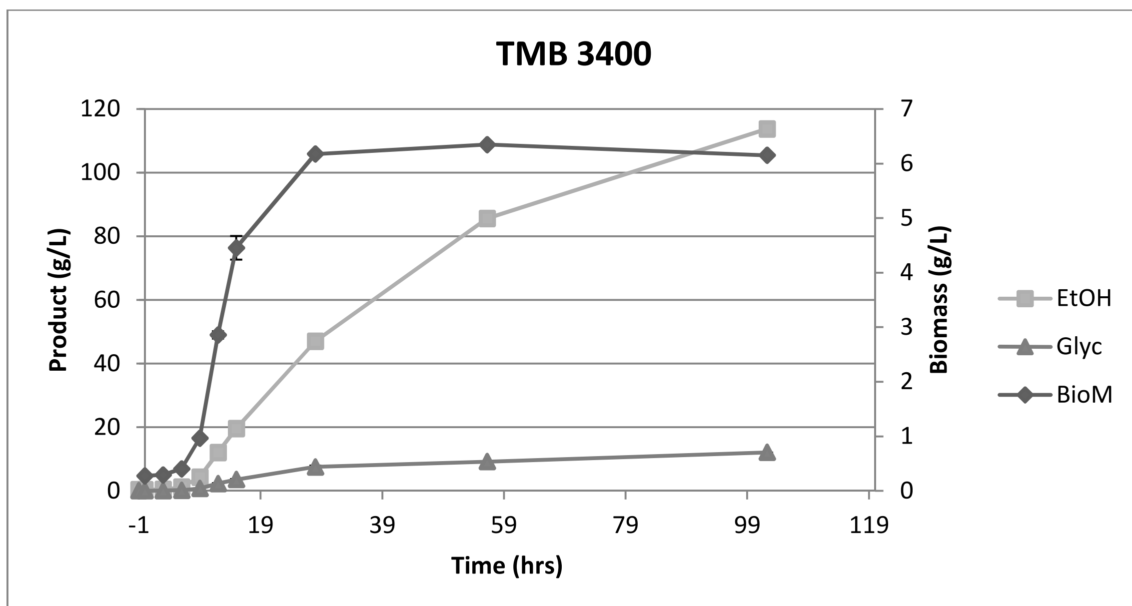

Figure 8: Product and biomass concentration for TMB 3400.

Even though biomass growth has ceased after 15 hours ethanol production was not finished at this point since substrates were still being utilised and products were still being formed after this point. This is good because the object here is not to produce biomass but to produce ethanol. The fact that the population stops growing means that the carbon source is utilised to produce ethanol rather than for biomass production. The fact that biomass growth stops while there are still high concentrations of sugar, shows that the sucrose medium may have some growth nutrient limitation. If the limiting nutrient can be determined it can be added in small amounts to be able to produce larger yeast populations to speed up the fermentations, but this will come at the cost of lower ethanol yield since more of the carbon source will go towards yeast growth.

Figure 9 shows the substrate concentrations for MH 1000. It can be seen that MH 1000 also has a higher selectivity for glucose. This selectivity from glucose can already be noticed after only 9 hours. After 56.25 hours MH 1000 was able to break up all the sucrose and ferment all the glucose, but there was still some fructose left (41.26 g/L) that again shows the yeast's higher selectivity for glucose.

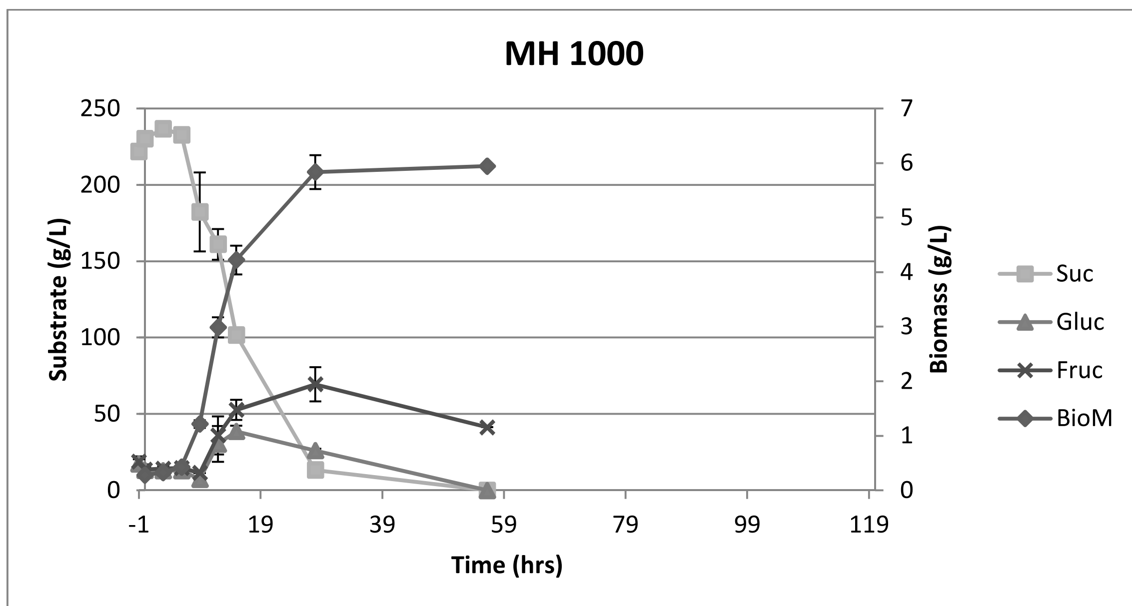

Figure 9: Substrate and biomass concentration for MH 1000.

Figure 10 shows the product formation for MH 1000. A final ethanol concentration of 99.6 g/L was reached after only 56.25 hours. This value is lower than the literature value of 130 g/L for sugar syrup fermentations (Leal, 2010) and higher than the literature values given for ethanol production from molasses (60 to 80 g/L and 80 to 90 g/L) (Kitching, 2011; Senthilkumar & Gunasekaran, 2009). The

final ethanol concentration of MH 1000 may differ from the literature values due to the absence of yeast recycling (Dias et al., 2010; Dias et al., 2011a; Ensinas et al., 2007; Ensinas et al., 2009; Pellegrini & de Oliveira Junior, 2011).

The final ethanol concentration of 99.6 g/L for MH 1000 is lower than the 113.7 g/L that was obtained with TMB 3400. This was because the fermentation time of 56.25 hours for MH 1000 was much shorter than the fermentation time of 102.25 hours for TMB 3400. At 56.25 hours TMB 3400 had a lower ethanol concentration of 85.61 g/L, which shows that MH 1000 has a higher productivity than TMB 3400.

Ethanol concentrations of 57.67 g/L and 55.7 g/L had been reached after only 28 hours for the two runs with MH 1000. This shows that MH 1000 fermented the sucrose faster than TMB 3400 since TMB 3400 had ethanol concentrations of 46.8 g/L and 47.1 g/L for the two runs after 28 hours. This is more evidence that MH 1000 has a higher productivity than TMB 3400.

The last sample was taken at 56.25 hours. This time compares well to the time of 55 to 60 hours given by NCP-Alcohols (Kitching, 2011) and it is much shorter than the time of 80 hours given by (Senthilkumar & Gunasekaran, 2009). However it should be noted that at this point ethanol production was not complete since there was still 41.26 g/L of fructose left. This means that ethanol production would have taken longer than 56.25 hours complete. The long fermentation time may be mainly attributed to the fact that there was no yeast recycle which would have led to shorter fermentation times (Taherzadeh & Karimi, 2008).

For MH 1000 ethanol production also continued after biomass growth had ceased. This again points to a nutrient limitation in the buffered sucrose medium. All of the substrates were not consumed after 56.25 hours (Figure 9), however a high ethanol concentration has been reached (see Figure 10) and the extra time needed to convert the last substrates will have to be justified.

Figure 10: Product and biomass concentration for MH 1000.Figure 10 shows low levels of glycerol production, around 12 g/L, indicating that MH 1000 was not stressed too much by its environment. This shows that the sucrose medium doesn't inhibit fermentation.

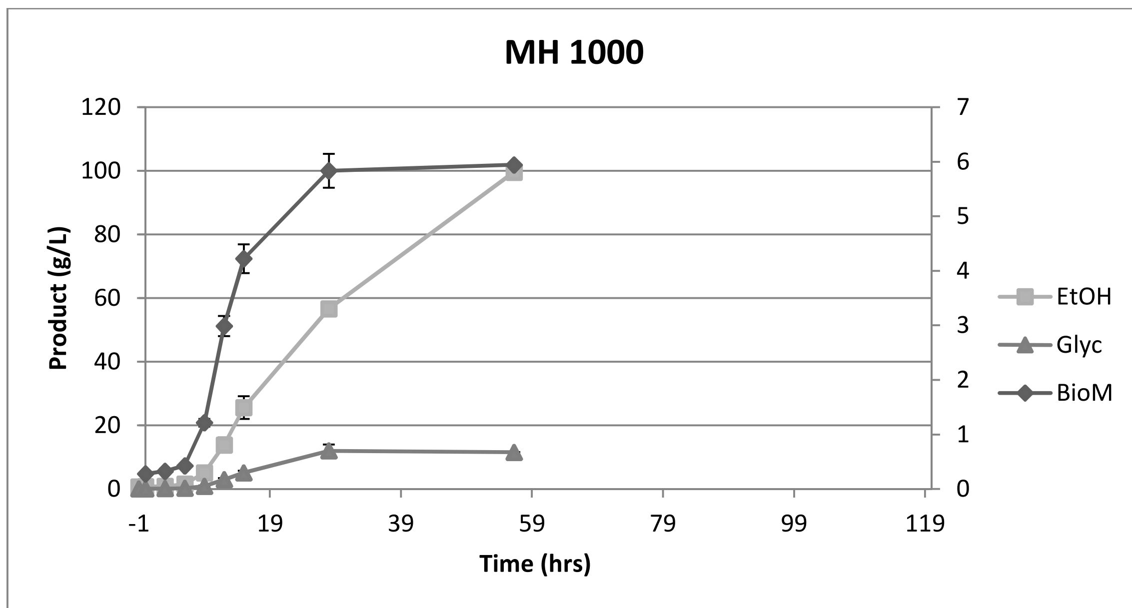

Figure 10: Product and biomass concentration for MH 1000.

3.3.2.1 Fermentation yields

The yields for all the fermentation runs were determined for the time when each of the fermentations was ended. The yield could not be calculated versus time, because the sucrose was consumed by invertase and broken up into its monomers before it was consumed by the yeast to produce ethanol. The lag between sucrose being consumed and ethanol being produced was unknown and thus the yield vs time could not be calculated. It was attempted to calculate the yield against time by using the assumption that the monomers that were consumed was equal to the monomers of the sucrose that has disappeared (1 sucrose molecule gives 2 monomers) minus the monomers that were present in the medium. This method however gave unrealistic results (>100% yield) and thus only end-point yield results were determined.

A summary of all these yields are presented below in Table 15. Some of the yields are quite low for the fermentation runs where ethanol production wasn't complete.

For the two fermentations where ethanol production was complete or almost complete (TMB 3400 run 1 and MH 1000 run 1) the fermentations have shown high yields, close to 85 %. This value will increase to about 90 % when continuous fermentation or the Melle-Boinot method with yeast recirculation is employed because much less biomass will have to be produced (Taherzadeh & Karimi,

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2008). The continuous or Melle-Boinot fermentation methods are inoculated with much larger yeast populations.

Table 15: Fermentation yield summary for first generation fermentation yields.

Strain	Run	Yield % of theo max
TMB 3400	1	85.37
	2	73.06
MH 1000	1	84.18
	2	66.39

3.3.2.2 Mass Balances

Mass balances were performed on all the first generation fermentations. The mass balances were performed by accounting for all the substrates that were consumed by the yeast and all the products formed by the yeast, including biomass. A summary of all the mass balances is presented here in Table 16. The third column in Table 16 shows the mass balance based on the actual mass of all the substrates and products considered and the fourth column is based on an elemental carbon balance. The equations that were used for the mass balances and each individual mass balance can be seen in Appendix E: Mass balance of first generation experiments. The cases where the mass balances are larger than 100 % shows that there are other unmeasured nutrients that was utilised by the yeast or it may be due to analytical or sampling error. The mass balances yielded good results seeing as they are all within the 90 – 105 % range showing that the sampling and analytical errors were minimal. It is thus concluded that the assumed equations (see Appendix E: Mass balance of first generation experiments for equations used for mass balance calculations) that were used in calculating the mass balances are justified.

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Table 16: Mass balance summary for all the 1st generation fermentations.

Mass Balance summary			
Strain	Run	Mass	C-moles
TMB 3400	1	101.87	99.46
	2	92.98	90.72
MH 1000	1	103.38	101.05
	2	92.86	90.75

3.4 CONCLUSIONS AND RECOMMENDATIONS

TMB 3400 performed better than MH 1000 based on final ethanol concentrations. The average final ethanol concentration was 99.56 g/L for MH 1000 and 113.72 g/L for TMB 3400 after 56.25 and 102.25 hours, respectively. The fermentations were thought to be complete after 28 hours due to preliminary experiments that showed that the fermentations had reached the stationary phase after 28 hours. It was not initially anticipated that the ethanol production would continue after biomass growth has ceased. The samples at 56.25 and 102.25 hours were taken as late samples. The higher ethanol concentration of TMB 3400 may be attributed to the longer fermentation time or the fact that TMB 3400 is hardened and can tolerate higher ethanol concentrations. MH 1000 has a higher productivity than TMB 3400 as can be seen from the average ethanol concentrations after 28 hours (56.66 g/L for MH 1000 versus 46.95 g/L for TMB 3400) and after 56.25 hours (99.56 g/L for MH 1000 versus 85.61 g/L for TMB 3400). The lower productivity of TMB 3400 may be attributed to the fact that it was genetically manipulated to withstand higher inhibitor levels and this may have affected its productivity.

The fermentation times were long (102.25 hours for TMB 3400 and 56.25 hours for MH 1000) when compared to fermentation times of 8 hours in Brazilian distilleries where yeast recycling is used to shorten fermentations times and improve yields (Gnansounou, 2009; Leal, 2010). The long fermentation times to produce lower ethanol concentrations than Brazilian distilleries (113.72 g/L for the present study versus 130 g/L for Brazilian sugar mills) mean that the productivity from these experiments are significantly lower than that found in Brazilian distilleries (Gnansounou, 2009; Leal, 2010). The long fermentation times may be attributed to the small starting yeast population and the nutrient limitation in the sucrose medium that caused the yeast to stop growing. This will not be a problem when yeast recirculation is used (Leal, 2010).

It must be determined whether building only one yeast propagation facility for a recombinant yeast strain in an integrated 1st and 2nd generation bioethanol plant will be worth-while despite the lower productivity of the recombinant strain when fermenting sucrose when compared to a hexose fermenting yeast. If a recombinant yeast strain with similar productivity to a hexose fermenting yeast strain can be found then it is recommended that such a recombinant strain be used in an integrated 1st and 2nd generation ethanol production facility on its own.

Both MH 1000 and TMB 3400 have produced very high ethanol concentrations (99.56 g/L and 113.72 g/L, respectively) that will help save on separation costs since these concentrations are high above the limit of 40 g/L (Öhgren et al., 2006). These high ethanol containing broths can be used to increase the ethanol concentration of the broth coming from the second generation fermentation by mixing the two broths before separation (if the 1st and 2nd generation fermentation are separate) (Leal, 2010).

Some of the fermentations have shown high yields for batch fermentations (~85 %) and they will have even higher yields (~90 %) when used in a continuous fermentation or Melle-Boinot fermentation with yeast recycling (Taherzadeh & Karimi, 2008).

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4. WASHING AND PRESSING AS A METHOD OF INHIBITOR REMOVAL

4.1 INTRODUCTION

Washing and pressing experiments were performed to determine how effective it is at the removal of inhibitors present in the pre-hydrolysate liquor (Palmqvist & Hahn-Hagerdal, 2000; Cantarella et al., 2004) from the WIS. This was done to determine the pressure that must be used to press the WIS to its minimum moisture content (to remove as much inhibitor rich pre-hydrolysate liquor as possible) and to determine the amount of washing stages required to lower the inhibitor levels in the WIS to low enough levels that that will help facilitate 2nd generation fermentation (chapters 5 and 6).

The pre-treated material was first pressed to separate the pre-hydrolysate liquor from the WIS. After pressing the WIS was washed and then centrifuged to remove the washing water with the dissolved inhibitors. The washing was repeated three times for each sample to simulate three consecutive cross current washing stages. The pressing and three washing stages that was simulated through these experiments can be seen in Figure 11. The aim of these experiments were to determine how many washing stages will be required (if any) after pressing the pre-treated bagasse to lower the inhibitor concentration of the liquid associated with the WIS to acceptable levels.

The definition of detoxification is the selective removal of inhibitors (Anish & Rao, 2009). Washing and pressing don’t strictly qualify as detoxification according to the definition of detoxification. This is because sugars are also removed during these processes, thus the inhibitor removal is not selective. However it can be used as a method to separate inhibitors from the WIS.

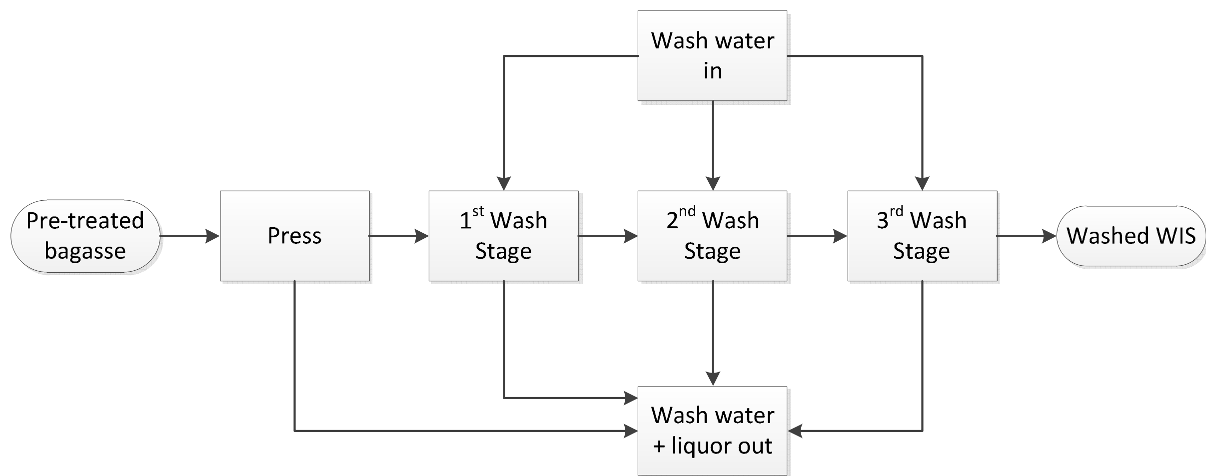

Figure 11: Schematic representation of the pressing and washing set-up simulated by this experiment.

The washing of WIS is usually employed after dilute acid pre-treatment to remove inhibitors (Aden et al., 2002; Stephen et al., 2012; Jackson de Moraes Rocha et al., 2011; Palmqvist & Hahn-Hagerdal, 2000). In these studies the WIS is washed with an abundance of water, however in this study the minimum amount of washing required is determined.

4.2 Materials and Methods

4.2.1 Pressing experiments

Pressing experiments were performed to determine the pressure required to reduce the moisture content of the WIS to approximately 50 %. This value for the moisture content was selected because this is the moisture content of bagasse after it has been through a dewatering mill and it is assumed that pre-treated bagasse and raw bagasse have similar properties when it comes to pressing. This assumption is re-visited in the results section.

A schematic drawing of the piston and cylinder set-up that was used for the pressing can be seen in Figure 12. The cylinder was filled with approximately 50 g of wet sample and then the piston was inserted into the cylinder. The piston and cylinder set-up was then placed on a shop press and the piston of the shop press pressed down on the piston in the cylinder forcing the liquid out through the

holes as the pressure increased. The shop press had a pressure gauge that was calibrated in U.S. tonnes.

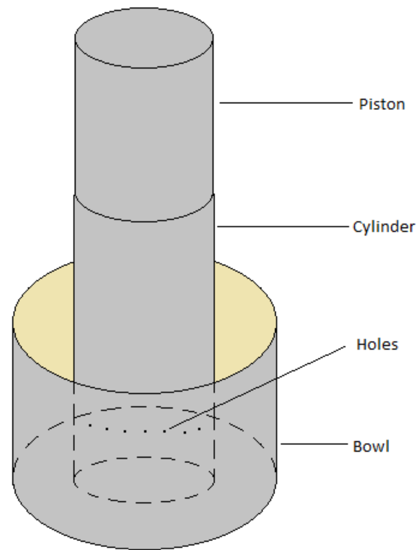

Figure 12: Schematic drawing of the piston and cylinder set-up that was used for the pressing experiments.

These experiments were performed with three samples of WIS that were steam pre-treated at three different pre-treatment conditions. The three pre-treatment conditions along with their severity factors can be seen in Table 17. (Tomas-Pejo et al., 2008) states that the optimum sugar yields are achieved for Log(R_0) of 3 -4.5, which includes conditions 1 and 2.

Table 17: Pre-treatment conditions used for the pressing experiments

Condition	Temp (°C)	Time (min)	Soaked	Log Ro
1	190	5	Yes	3.35
2	200	2	Yes	3.25
3	200	0.5	Yes	2.64

Each sample of pre-treated material was pressed at three different pressures: low (4,77 MPa), medium (9.54 MPa) and high (14.30 MPa). This resulted in nine pressing experiments in total. The pressures that were used can be seen in Table 18. The press force and piston diameter were used to calculate the pressure.

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Table 18: Different pressure levels for the pressing experiments

Pressure level	U.S. tonnes	Cylinder D (mm)	Pressure (MPa)
Low	1	48.75	4.77
Medium	2	48.75	9.54
High	3	48.75	14.30

The moisture contents of the pressed samples were determined by weighing the samples before and after drying them in an oven at 105 °C for at least 24 hours. This was done by first weighing the container in which the sample was placed in the oven. 100 mL glass beakers were used as ovenproof containers. After the containers had been weighed the pressed sample was placed in the container and weighed again. The weight of the sample was determined by subtracting the weight of the container from the combined weight of the sample and the container. The sample was dried in the oven. The sample and the container were weighed again after the sample had been dried. The moisture content was then determined according to Equation 10.

$$Moist\ (wt\ \%) = \frac{pressed\ sample\ (g) - dried\ sample\ (g)}{pressed\ sample\ (g)} \times 100\%$$

Equation 10

Control experiments were performed for each of the three samples. The control experiments were performed by drying samples that had not been pressed to lower the moisture content. The results of the control experiments can be seen in Appendix F: Results for pressing experiments along with the tabulated results for the pressing experiments.

4.2.2 Washing experiments

Approximately 50 g of wet sample was weighed out and then pressed. Approximately 25 g of the pressed sample was weighed out to be used for the washing experiments. The pressed WIS was then placed in a 50 mL Falcon tube and the tube was filled with water. This resulted in water being added on an equal weight basis since approximately 25 mL of water was added. The tubes were then agitated with a vortex for approximately 30 seconds to ensure that the material was thoroughly washed. The wash water was removed by centrifuging the Falcon tubes for 10 min at 10 000 rpm. The supernatant was decanted and the wash water for the next stage was added and the vortexing and centrifugation steps were repeated two more times to simulate two additional washing stages.

For the second and third washing stages there was only about 20 mL of water added because the centrifugation was not as effective as removing moisture from the WIS as pressing.

The pressure that was used for the initial pressing of the washed samples was the minimum pressure that is required to reach a moisture content of 50 %.

The washing experiments were performed in triplicate for each of the three pre-treatment conditions considered. The pre-treatment conditions that were used here are the same ones that were used for the pressing experiments (see Table 17).

The samples were taken from the wash water that was decanted after centrifugation and from the pre-hydrolysate liquor that was pressed out. The samples were prepared for HPLC analysis with perchloric acid according to the procedure given in Appendix A: Sample preparation for HPLC analysis.

4.3 Results and Discussion

4.3.1 Pressing experiments

Figure 13 illustrates how the moisture content of pre-treated bagasse decreased with increasing pressure. The moisture content versus pressure is plotted for all three pre-treatment conditions that were considered. It can be seen that the lowest pressure that was tested, 5 MPa, was enough to reduce to moisture content of all three samples to approximately 50 %. After the pressing the pressed pre-treated material had the appearance of pressed wood.

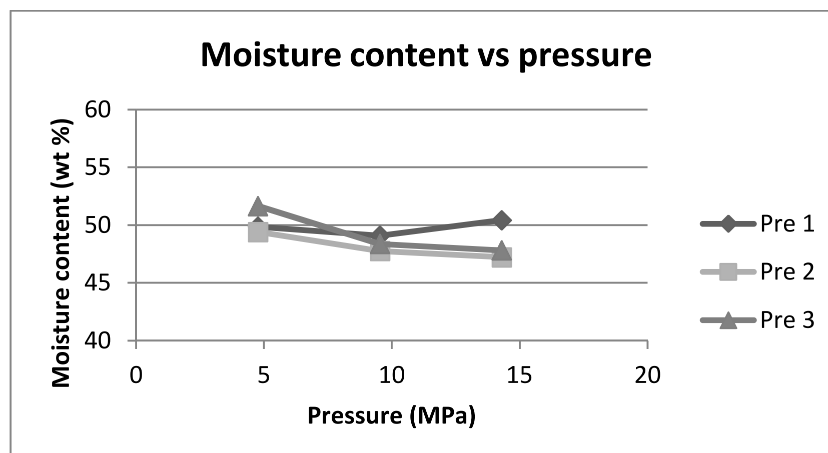

Figure 13: Moisture content versus pressure for pressed, pre-treated bagasse.

The moisture content did not decrease significantly by doubling and tripling the pressure. It is observed that the slope between 5MPa and 10 MPa is steeper than the slope between 10 MPa and 15 MPa. This indicates that the moisture content is not easily decreased below 50 % by pressing. This phenomenon is a limit that is imposed by the structural nature of the material. This observation validates the assumption that raw bagasse and pre-treated bagasse have similar properties when it comes to pressing since many sources cite that raw bagasse has a moisture content of 50 % after it has gone through a dewatering mill, which is essentially a continuous press (Dias et al., 2009; Modesto et al., 2009; Ensinas et al., 2007; Seabra & Macedo, 2011). The material could not be compressed further with the available equipment at that point in time. A larger press was later obtained that was able to press more liquid from the WIS by sustaining a higher pressure for a longer time and with a better design for draining fluid from the WIS (see section 5.2.2).

The data point in Figure 13 for the first pre-treatment condition at 15 MPa is considered as an outlier. This is because the material is not homogeneous and structural differences may affect the ability of the material to retain water. This problem could be solved by increasing the sample size, but a larger press and piston and cylinder set-up were not available at the time that the experiments were conducted.

4.3.2 Washing experiments

Figure 14 to Figure 17 show the concentrations of inhibitors (acids and furans) and sugars (glucose, xylose and arabinose) in the liquid portion of the pre-treated solid during the washing experiments. The concentrations that are given for stage 1 in the afore-mentioned graphs are the concentrations of the specific compounds in the pre-hydrolysate liquor that was pressed from the unwashed pre-treated material. These concentrations are included in the graphs to illustrate how the inhibitor concentrations in the liquid entrapped in the pre-treated solids decreased with an increasing number of washing stages.

The total decrease in concentration of the inhibitors and sugars are presented here:

Formic acid: Condition 1: 0.73 – 0.01 g/L

Condition 2: 0.97 – 0.03 g/L

Condition 3: 0.64 – 0.02 g/L

Acetic acid:	Condition 1:	2.44 – 0.03 g/L
	Condition 2:	2.98 – 0.11 g/L
	Condition 3:	1.73 – 0.04 g/L
HMF:	Condition 1:	0.08 – 0.00 g/L
	Condition 2:	0.12 – 0.01 g/L
	Condition 3:	0.06 – 0.00 g/L
Furfural:	Condition 1:	0.24 – 0.01 g/L
	Condition 2:	0.27 – 0.02 g/L
	Condition 3:	0.11 – 0.01 g/L
Glucose:	Condition 1:	0.34 – 0.00 g/L
	Condition 2:	0.50 – 0.02 g/L
	Condition 3:	0.35 – 0.01 g/L
Xylose:	Condition 1:	3.40 – 0.04 g/L
	Condition 2:	5.08 – 0.16 g/L
	Condition 3:	2.50 – 0.05 g/L
Arabinose	Condition 1:	1.44 – 0.02 g/L
	Condition 2:	1.40 – 0.04 g/L
	Condition 3:	1.59 – 0.03 g/L

Stages 2 to 4 represent the three washing stages. All the experiments were performed in triplicate and the data show very good repeatability, as can be seen from the graphs. There is only one outlier for the glucose concentration of pre-treatment condition number 2 where the value of the outlier is 0.69 g/L compared to the average value of 0.50 g/L for the other two data points (see Figure 16).

Pre-treatment condition 2 will be used as an example to explain the graphs in this section. Pre-treatment condition 2 was chosen for this purpose as it had the highest sugar and inhibitor concentrations which will help to illustrate the effect of washing.

Figure 14 shows the concentrations of formic and acetic acid in the pressed and washed liquor. The concentrations decreased from 0.97 to 0.20 g/L and from 2.98 to 0.65 g/L in the first washing stage for formic- and acetic acid, respectively. The second washing stage decreased the concentration of the acids even further (0.04 g/L for formic- and 0.13 g/L for acetic acid). However, the third stage does very little to decrease the inhibitor concentration further. This is because there are very little inhibitors left to be washed out by the time that the WIS reaches the third washing stage.

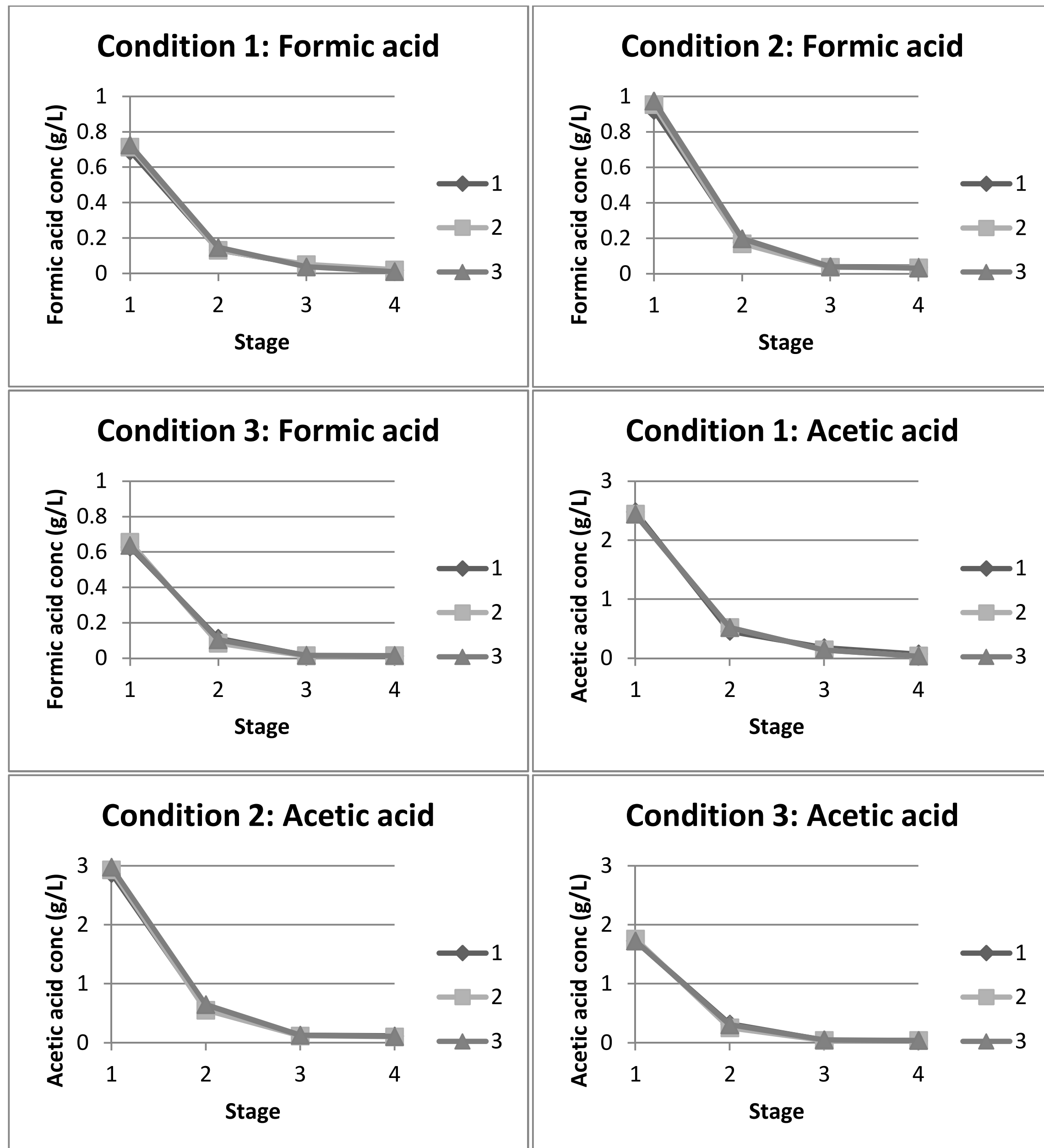

Figure 14: Formic and acetic acid concentrations for washing experiments.

Figure 15 shows the concentrations for HMF and furfural. The same pattern is observed here with the furans as for the acids. Most of the inhibitors are removed by the first washing stage and the second washing stage decreases the inhibitor levels even further. The concentrations decreased from 0.12 to 0.04 g/L and from 0.27 to 0.11 g/L in the first washing stage for HMF and furfural, respectively. The second washing stage decreased the concentration of the furans even further (0.01 g/L for HMF and 0.03 g/L for furfural). The third washing stage, again, does very little to remove any more inhibitors since the WIS entering the third is stage already has a very low inhibitor content in the water associated with the WIS.

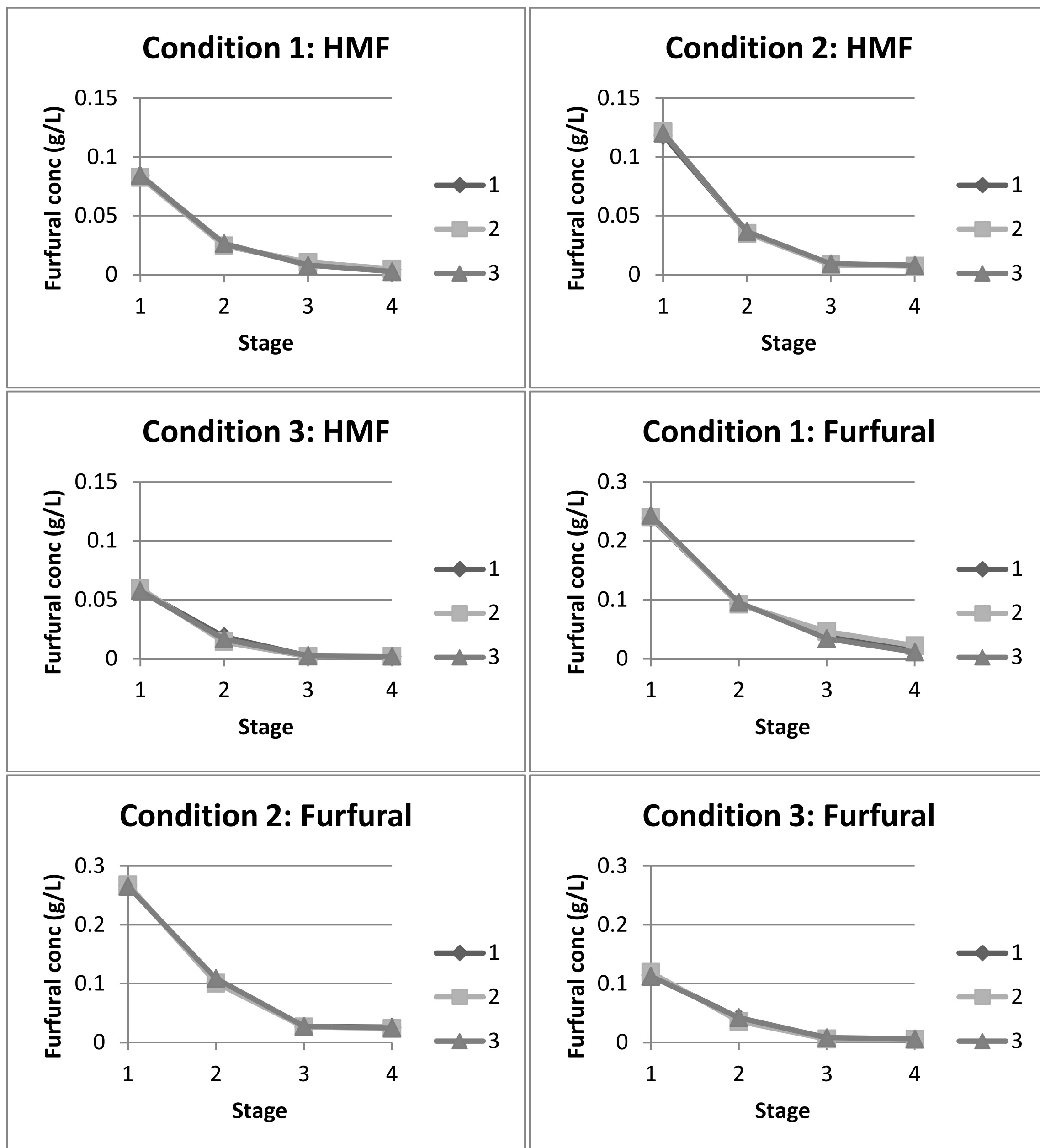

Figure 15: HMF and furfural concentrations for washing experiments.

Figure 16 show the concentration of glucose and xylose. The sugars follow the same trend as the inhibitors (acids and furans). The concentrations decreased from 0.50 to 0.15 g/L and from 5.08 to 1.09 g/L in the first washing stage for glucose and fructose, respectively. The second washing stage decreased the concentration of the sugars even further (0.03 g/L for glucose and 0.20 g/L for fructose). This is the "price" at which inhibitor removal through washing is done – the more inhibitors are removed the more sugars are removed along with the inhibitors. The diluted sugars in the outgoing wash water must be concentrated before it can be fermented or bio-digested. Arabinose follows exactly the same trend as glucose and xylose (see Figure 17 on the next page).

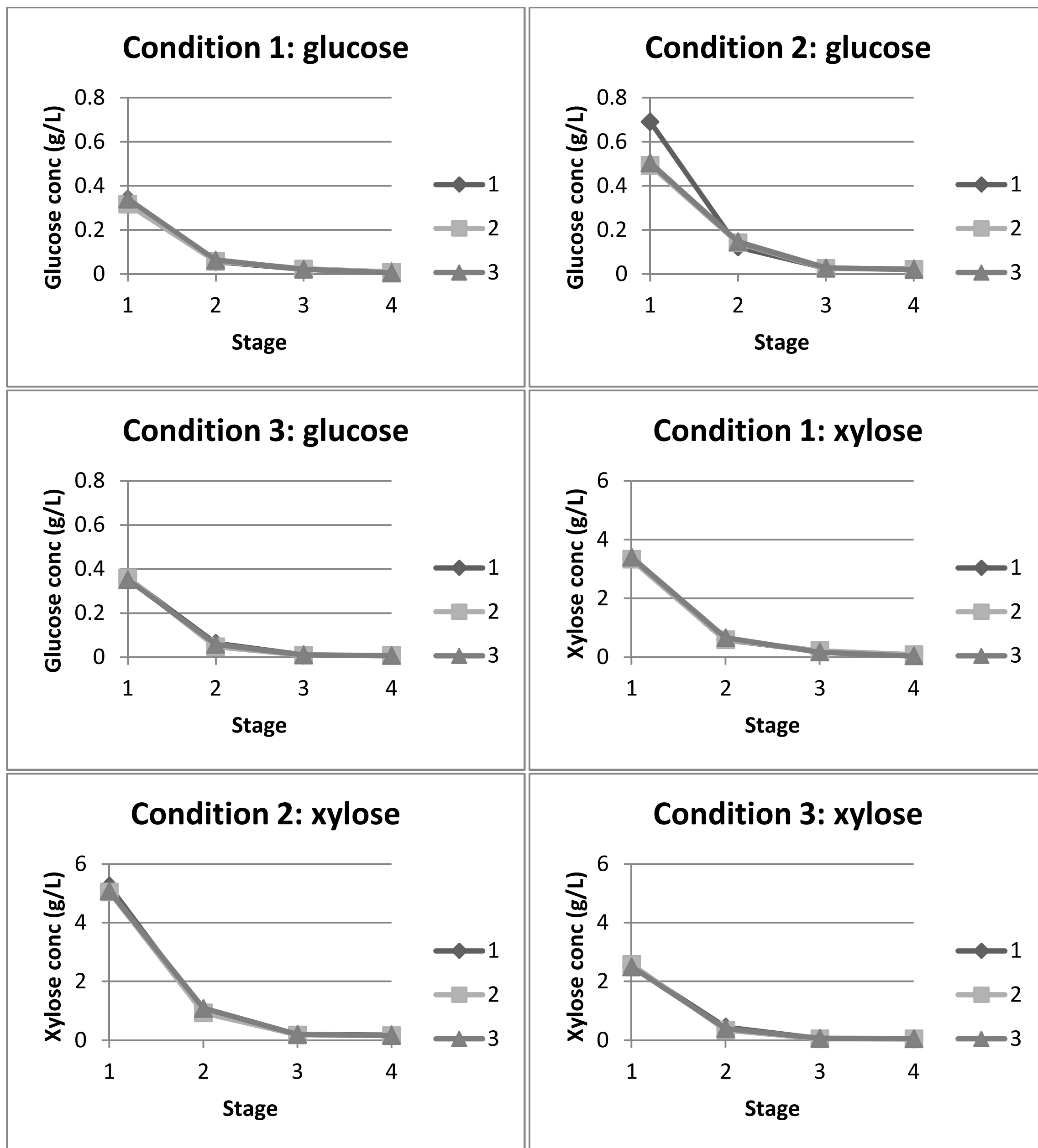

Figure 16: Glucose and xylose concentrations for washing experiments.

The concentration of arabinose decreased from 1.40 to 0.29 g/L in the first washing. The second washing stage decreased the concentration of the arabinose even further to 0.05 g/L.

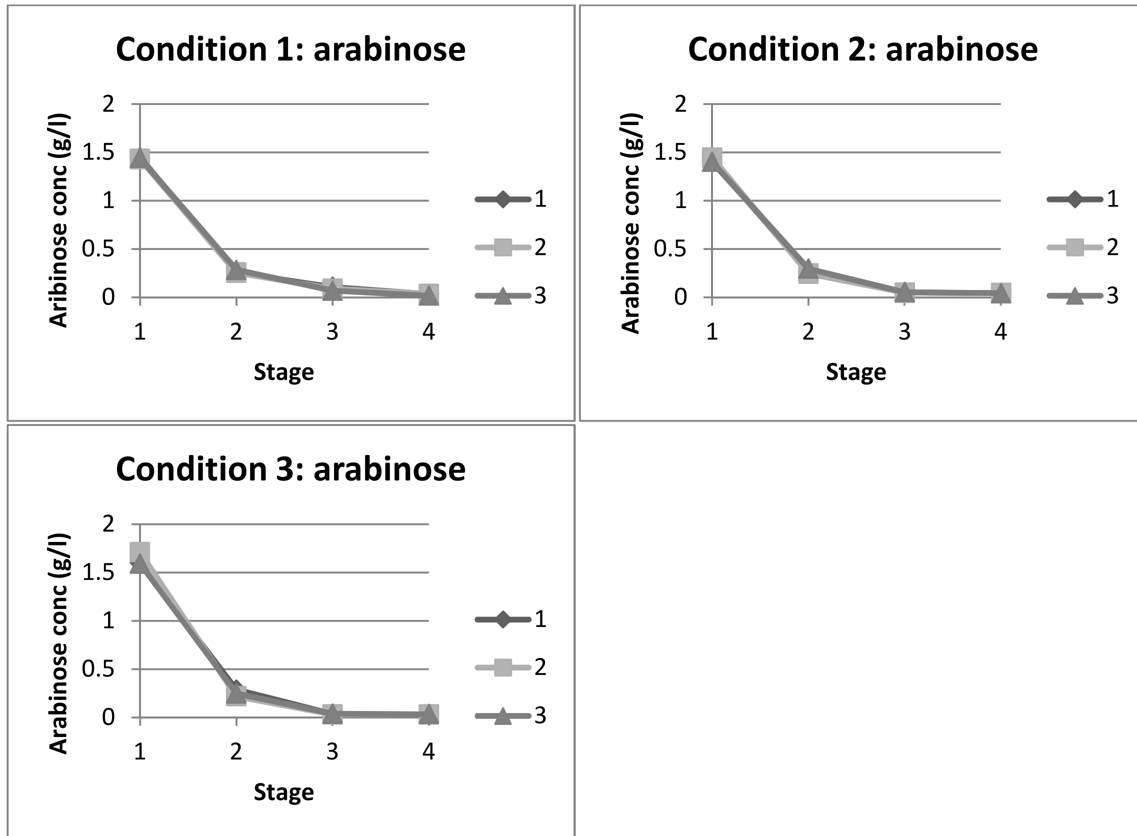

Figure 17: Arabinose concentrations for washing experiments.

Figure 18 and Figure 19 respectively show the total inhibitor and sugar concentrations in the pre-treatment liquor for the three different pre-treatment conditions. It can be observed that high inhibitor concentrations in the liquor correspond to high sugar concentrations in the liquor For example condition 2 has a high total inhibitor concentration of 4.34 g/L and a corresponding high total sugar concentration of 6.98 g/L while condition 3 has a low total inhibitor concentration of 2.54 g/L with a corresponding low total sugar concentration of 4.44 g/L. This is due to the severity of the pre-treatment and sugarcane cultivar. Different cultivars and/or batches of bagasse were used for these experiments. The use of different cultivars may explain why the second pre-treatment condition had higher sugar and inhibitor yields than the first pre-treatment condition even though the first pre-treatment condition was more severe (see Table 17).

Gnansounou (2009) states that pre-treatment temperature has a larger impact than pre-treatment time on inhibitor formation. It is thus better to pre-treat material at a lower temperature for a longer time. This may also explain the lower inhibitor formation of condition 1 when compared to condition 2 since condition 1 was pre-treated at a lower temperature for a longer time than condition 2 (190 °C and 5 mins versus 200 °C and 2 mins).

It should be noted that the total amount of acetic acid that is released (during pre-treatment and enzymatic hydrolysis put together) is a function of the feedstock rather than the pre-treatment severity (Taherzadeh & Karimi, 2008) but the pre-treatment severity does determine how much of the potential acetic acid is released during pre-treatment and how much is released during enzymatic hydrolysis (Cantarella et al., 2004). The fact that the amount of acetic acid that is formed is a function of the feedstock explains why pre-treatment condition 2 has higher a acetic acid level than pre-treatment condition 1 even though the bagasse of condition 1 was pre-treated more severely than that of condition 2 ($Log(R_0)$ of 3.35 versus 3.25). The pre-treatment severity does have an impact on how much acetic acid is initially formed and this explains why the acetic acid level of condition 3 is lower than that of the other two conditions since the pre-treatment severity was much lower for this condition than for conditions 1 and 2 ($Log(R_0)$ of 2.64 for condition 3).

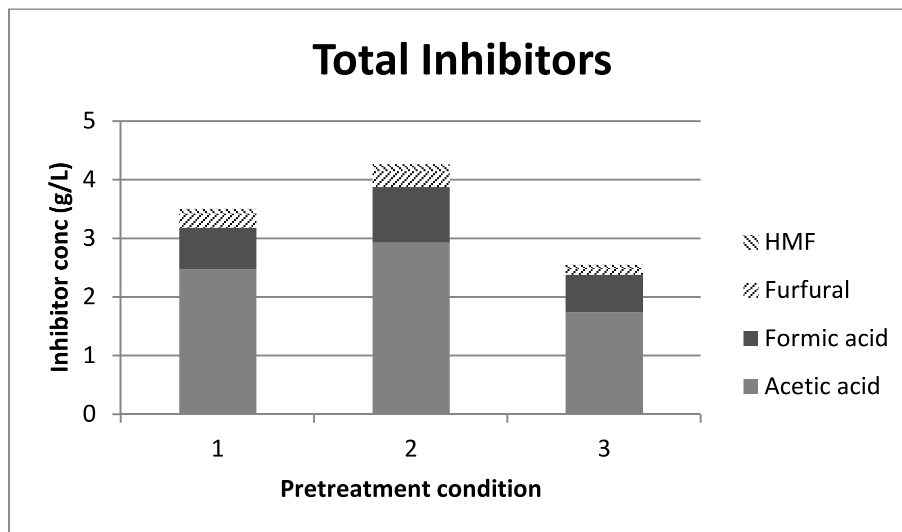

Figure 18: Total inhibitor concentration of the pre-hydrolysate liquor for all three pre-treatment conditions.

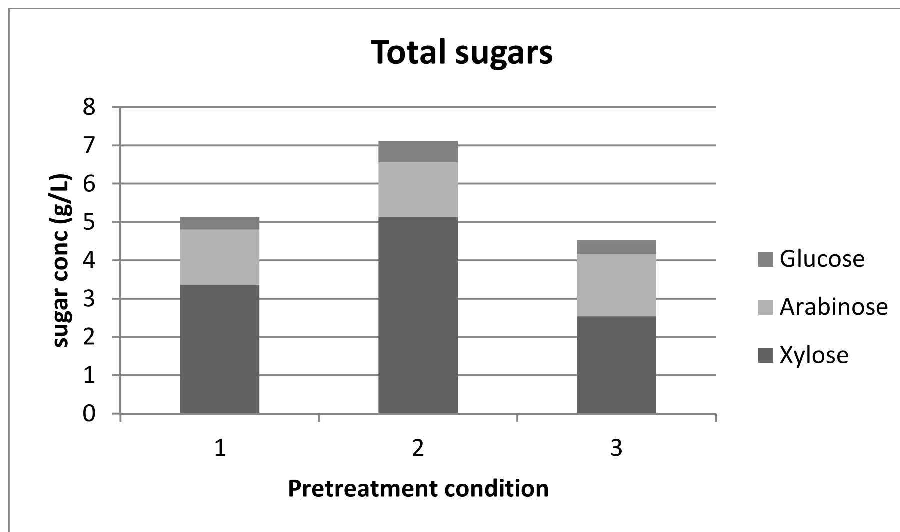

Figure 19: Total sugar concentration of the pre-hydrolysate liquor for all three pre-treatment conditions.

4.4 Conclusions and recommendations

It is concluded that 5 MPa of pressure was enough to reduce the moisture content of pre-treated sugarcane bagasse to 50 %. The pressure to obtain this value of 50 % had to be determined to be able to simulate a dewatering mill (roller mill) that would have been used in a real plant. To reduce the moisture content of the WIS below 50% the pressure must be sustained to give the liquid time to seep out of the WIS (see section 5.2.2) and sustaining the pressure will not be possible when using an industrial continuous dewatering mill. Decreasing the moisture content below 50% on an industrial scale is unfeasible.

It can be seen from all the graphs in this section that pressing removes most of the inhibitors at a pressure of 5 MPa for example the amount of acetic acid for pre-treatment condition 2 was lowered from 2.98 g/L in the pre-treatment liquor to 0.65 g/L in the first washing stage. This is a reduction of 78.2% in the acid concentration.

The second stage on the graphs indicates that the concentrations of inhibitors are very low in the first washing stage (0.65 g/L for the acetic acid of condition 2). It is concluded that only pressing will be necessary since the subsequent washing stages don't contribute much to the removal of inhibitors (0.13 g/L and 0.11 g/L for the second and third washing stages for the acetic acid of condition 2). After pressing the material the water that will be added for the fermentation will dilute the inhibitors to below the levels in the first stage washing water. This is because more water will be added to the

fermentation than will be added to the first washing stage (washing water was added on basis of mass equal to that of the WIS). The final WIS concentration for 2nd generation fermentations used in this study was approximately 17% (w/w) and in the washing stages the WIS concentration is about 25% (w/w) when washing water is added on an equal mass basis to the WIS and the WIS has a moisture content of 50%.

It takes a lot of energy and it is very expensive to concentrate sugars in a stream through evaporation. For this reason it was decided that pressed WIS will be used for the fermentation experiments in this project.

When washing is employed to remove inhibitors from the WIS and the wash water is concentrated through evaporation for fermentation, it is recommended that the concentrated wash water be detoxified by another method since evaporation is not an effective method of detoxification (Anish & Rao, 2009; Wang & Feng, 2010). A hardened yeast strain with high inhibitor tolerance can be used to ferment this stream (Wang & Feng, 2010). This is because evaporation is not an effective detoxifying method and it will concentrate the non-volatile inhibitors along with the sugars.

NOTE:

A larger press was needed to be able to press enough material for the fermentation experiments of this project and for use by other members of the group to press large amounts of pre-treated material. A larger piston and cylinder set-up was designed and built and a 50 ton shop press was brought for this purpose. The design of the larger piston and cylinder set-up and photos of the 50 tonne press and the larger piston and cylinder set-up can be seen in Appendix G: 50 tonne Press.

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5. SECOND GENERATION FERMENTATION EXPERIMENTS

5.1 INTRODUCTION

Second generation fermentation experiments were performed using industrial bagasse and the *S. cerevisiae* yeast strain MH 1000 to assess the possibilities of bioethanol production with the current industrial technology.

The objective of this chapter was to determine to determine whether a fast or a slow WIS feeding strategy (see section 5.2) will work best to assist the yeast in tolerating the inhibitors introduced with the pressed WIS. The data that was generated from the 2nd generation fermentation experiments will serve as a baseline for the combined 1st and 2nd generation fermentation experiments (see chapter 6).

It must be noted that MH 1000 is not a recombinant or hardened yeast strain. Hardened/recombinant strains were not available for these experiments and thus MH 1000 was used instead. *S. cerevisiae* strains used by other authors include a hexose fermenting thermo- and inhibitor tolerant (hardened) strain DQ 1 (Zhang et al., 2010) and the recombinant hardened strain TMB 3400 (Öhgren et al., 2006; Rudolf et al., 2008). A hardened strain will be able to tolerate higher concentration of inhibitors and a recombinant strain will ferment pentoses in addition to hexoses. Both these properties will contribute to better fermentation characteristics and higher ethanol concentrations.

The fermentations were performed using the SSF method due the advantages of SSF given in literature (see section 2.3.4). There are many literature sources that have used SSF to ferment lignocellulosic materials such as sugarcane bagasse (Rudolf et al., 2008) and corn stover (Öhgren et al., 2006; Zhang et al., 2010).

The aims of these experiments are as follows:

- Achieve a minimum ethanol concentration of 40 g/L for cellulosic ethanol as determined by Öhgren et al (2006).
- Determine whether MH 1000 can tolerate the inhibitors that are associated with pressed WIS and stay viable and produce ethanol in the presence of these inhibitors.

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- Determine wheter Cellic Ctec 2 contains enough β-glucosidase since this can be a limiting agent in enzyme cocktails (Taherzadeh & Karimi, 2008; Wyman et al., 1992).

5.2 Materials and methods

Fast (25 % of total WIS per day) and slow (15 % of total WIS per day) feeding strategies were employed to determine the effect that feeding rate has on the ethanol production and inhibitor tolerance of the yeast. For the slow feeding strategy 10 % of the total amount of WIS was fed on the 7th day.

The method for SSF, as it is described here, was adapted from a method that was developed by other members of the research group (Dreyer et al., 2012).

A mix of industrial bagasse was used as the raw material for the SSF experiments. Various industrial bagasse samples from various sugar mills were mixed together using the quarter sampling method to obtain as homogeneous a mixture of bagasse as possible.

5.2.1 Pre-treatment of bagasse

Procedure:

The bagasse was pre-treated using steam explosion. This was done with a high pressure reactor with direct steam injection known as a steam gun. First the boiler that supplies saturated steam at 20 bar to the high pressure reactor must be switched on and allowed to heat up. After the boiler has heated up the reactor must be heated up by steam from the boiler. When the reactor is hot one can insert the bagasse into the reactor and close the valve to the reactor so that steam injection can begin. After the bagasse is inserted into the reactor the time and temperature for the pre-treatment run is entered on the computer console of the steam gun. After the bagasse has been in the reactor for the chosen time at the chosen temperature the pressure inside the reactor is flashed to atmospheric pressure by opening a valve at the bottom of the reactor. This sudden decompression causes the water inside the bagasse to expand rapidly causing the bagasse to “explode”. The pre-treated

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bagasse gets blown into the blow tank where it can be collected and then frozen until it is needed for fermentation.

Pre-treatment conditions:

The bagasse was loaded into the steam gun in 1 kg samples. The bagasse was not soaked in water before pre-treatment. The bagasse was pre-treated for 10 minutes at a temperature of 200 C°. This resulted in a severity parameter of 3.94. This value for the pre-treatment severity is within the range of 3 to 4.5 given by (Tomas-Pejo et al., 2008) for optimum sugar yields. (Zhang et al., 2010) also used steam pre-treatment, but other authors used steam pre-treatment with SO_2 impregnation to lower the amount of inhibitors formed by using less harsh pre-treatment conditions due the extra pre-treatment provided by the SO_2 (Öhgren et al., 2006; Rudolf et al., 2008). All of the pre-treated material was thoroughly mixed using the quarter sampling method to produce a feedstock material that is as homogenous as practically possible to be used in the fermentations.

5.2.2 PRESSING OF THE PRE-TREATMENT MATERIAL

It was assumed from the results of chapter 4 that pressing the material was sufficient to lower the inhibitor level to acceptable levels.

The material was pressed using a 50 tonne hydraulic press that is operated with compressed air. See the note in section 4.4 and Appendix G: 50 tonne Press for more details about the equipment used.

The material was pressed with a force of approximately 20 tonnes that resulted in a pressure of approximately 10 MPa (calculated from a piston diameter of 6 inches). This pressure was sustained until most of the moisture had seeped out of the pre-treated bagasse. The ability of the 50 tonne press to sustain pressure while the liquid seeped out made it possible to reduce the moisture content of the pressed WIS to values below 50 % and the average value for the material used in these experiments was approximately 42 % MC (see Appendix H: Moisture content of pressed WIS used for fermentations for moisture content calculations). The sustained pressure of this press that allows the inhibitor rich pre-treatment liquor to seep out simulates a pneumatic press in industry. The use of a pneumatic press will be more expensive than the traditional roller mills, but it will serve to lower the inhibitors in the fermentation. The moisture content of the material was determined in the same way as described in section 4.2.1.

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It should, however, be noted that in industry roller mills will be used for dewatering purposes. Roller mills don't have the ability to sustain pressure for a long time so that liquid can seep out of the solid. This means that in industry the pressed material will more likely have a moisture content of 50 %. It is unfeasible to reduce the moisture content below 50 % on an industrial scale (see section 4.4).

Other authors also avoided washing the material because they used hardened yeast strains with high inhibitor tolerances (Zhang et al., 2010; Öhgren et al., 2006).

5.2.3 PREPARATION OF THE INOCULUM CULTURE

The strains were stored at -80 °C in ± 30 % glycerol and plated on YPD plates (see Appendix B: Preparation of YPD plates). The inoculum cultivations were done using a mineral medium with glucose as a carbon source (see Appendix J: 2nd generation fermentation inoculation preparation for the preparation of mineral medium).

For each bioreactor three 250 mL Erlenmeyer flasks with cotton plugs were used for the pre-inoculum cultivation. Each flask was filled with 50 mL of mineral medium with added glucose as the carbon source. The pre-inoculum cultures were inoculated from the YPD plates. The pre-inoculum culture was then incubated at 30 °C for 24 hours on a rotary shaker at 150 rpm.

For each bioreactor three 1 L Erlenmeyer flasks with cotton plugs were used for the cultivation of the inoculum cultures. Each flask was filled with 400 mL of mineral medium (glucose included) at 125 % strength and 100 mL of sterilised pre-hydrolysate liquor (the addition of 100 mL of pre-hydrolysate diluted the strength of the mineral medium back to 100 %). The pre-hydrolysate liquor was obtained when the pre-treated bagasse was pressed to lower the inhibitor levels in the WIS for fermentations. The aim of adding pre-hydrolysate liquor to the inoculum cultures was to pre-condition the yeast by introducing the presence of inhibitors before the start of the reactor fermentations (Öhgren et al., 2006). The inoculum cultures were inoculated by adding the whole of the pre-inoculum culture to the inoculum flask. The inoculum culture was incubated at 30 °C for approximately 18 hours on a rotary shaker at 150 rpm.

The yeast cells of the inoculum cultures were harvested using centrifugation (see Appendix J: 2nd generation fermentation inoculation preparation) and all of the harvested cells were used to inoculate the bioreactor fermentations. The size of the inoculation cultures can be seen in Appendix I: Inoculation concentrations for 2nd Gen and 1st and 2nd Gen combination fermentations..

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5.2.4 Simultaneous saccharification and fermentation

The industrial yeast strain MH 1000 was used for these experiments. The pH was controlled at 5.0 (Rudolf et al., 2008) using 3M KOH (Öhgren et al., 2006). The temperature was controlled at 35 °C. Other authors fermented at temperatures as high as 37 °C with thermo-tolerant yeasts to enable the enzymes to operate more efficiently (Zhang et al., 2010); (Rudolf et al., 2008). WIS was added in a discrete fed-batch manner to avoid introducing all the inhibitors formed during pre-treatment at the same time (Öhgren et al., 2006)(Taherzadeh & Karimi, 2008).

Two 10 L New Brunswick Scientific (NBS) bioreactors were used for these experiments. A Rushton turbine impeller was used for agitation. The agitation speed was started at 200 rpm and it was increased to approximately 400 rpm as the volume of the fermentations increased to be able to keep the increasing amount of solids in suspension. Other authors used smaller reactors with agitation speeds of 600 – 750 rpm (Öhgren et al., 2006; Rudolf et al., 2008). The exhaust gases were passed through a condenser that was cooled to approximately 5 °C in order to minimise ethanol evaporation. There was no control over the dissolved oxygen (dO_2) level because the fermentations were not aerated, but rather operated under micro-aerobic conditions.

The Cellic® CTec 2 cellulase enzyme cocktail from Novozymes was used in these experiments. Other authors have used a combination of Celluclast 1.5L and Novozyme 188 (β-glucosidase) (Öhgren et al., 2006; Rudolf et al., 2008) or Accellerase 1000 (Zhang et al., 2010). The enzyme dosage was 15 FPU / g dry WIS (Öhgren et al., 2006). In literature the enzyme dosage can varies from 7 to 30 FPU/g dry WIS (Zhang et al., 2010; Rudolf et al., 2008). The enzymes were added along with some (15 % of the final amount) WIS 90 minutes before the fermentations were inoculated. This was done as a pre-saccharification step to create a sugar rich environment for the yeast.

The same mineral medium, but without the added glucose, that was used for the inoculum cultivations were used here as a buffering medium. The starting volumes of the fermentations were 3L. This volume included the volume of the yeast inoculum, enzyme cocktail, starting WIS and buffering medium.

Samples were taken twice daily. The samples were prepared for HPLC analysis with perchloric acid according to the procedure given in Appendix A: Sample preparation for HPLC analysis. The samples were also used to determine cell concentrations (number of cells per mL) in some cases to gain an

indication of cell growth. The cell counts for determination of cell concentration were performed using a counting chamber (haemocytometer).

The samples were qualitatively tested for glucose with urine strips (Uricheck 10 from Sekunjalo Health Care). This was done as an "on-line" test to be able to quickly determine when glucose accumulation starts. The results for the qualitative glucose tests for the fermentations performed in this chapter and the next can be seen in Appendix K: Qualitative glucose tests for 2nd generation and 1st and 2nd generation combination fermentations.

To determine the total working volume of the fermentation, the volumes of everything that entered and left the reactor had to be determined. This was done by weighing the samples taken from the fermentation, the WIS added to the fermentation and the amount of 3 M KOH added to the fermentation. These weights were recorded twice daily when the samples were taken. It was assumed that everything that was weighed had the same density as water (1000 kg/m^3). This was used to calculate how the fermentation volume changed with time.

The fermentations were performed in duplicate and not triplicate due to limited material and the relatively large starting volumes required for the 10 L NBS bioreactors.

Note: The final WIS concentration was supposed to be 20 % on a dry weight basis. However the, addition of base, the removal of WIS through sampling and a calculation error where the water in the WIS was not considered led to somewhat lower WIS concentrations. There was a fast and a slow feeding strategy. For the slow feeding strategy 3 % WIS was added per day (calculated from the final weight of the fermentation) and 5 % WIS was added per day for the fast feeding strategy. For the 5 % WIS per day, 2 % was added in the morning (09h00) and 3 % in the afternoon (16h00).

5.3 Results and Discussion

The composition of the WIS was determined through chemical composition analysis (Sluiter et al., 2008). The composition of the WIS used in this chapter can be seen below in Table 19.

Table 19: Composition of the WIS used for 2nd Generation fermentations.

Sample	Lignin (%)	Arabinose (%)	Glucose (%)	Xylose (%)	Ash (%)	Total (%)
1	36.37	0.17	50.69	5.32	2.42	95.93
2	36.15	0.15	51.77	5.21	1.16	95.45

3	34.92	0.18	55.02	5.76	1.12	98.84
Avg	35.81	0.17	52.49	5.43	1.57	96.74
St dev	0.78	0.01	2.25	0.29	0.74	1.84

5.3.1 SLOW FEEDING STRATEGY

The fed-batch volume and the actual WIS % of the fermentations can be seen in Figure 20. The actual WIS % is the percentage dry weight of the WIS at a particular point in time, based on the total weight of the fermentation broth at that point in time. The WIS concentration reached a level of almost 17.8 %. This is higher than some other studies that had a maximum WIS concentrations of 7.5 % (Rudolf et al., 2008) and 12 % (Öhgren et al., 2006), but lower than a more recent study that employed a novel helical mixer instead of a Rushton turbine impeller to be able to accommodate higher WIS (pre-treated corn stover) concentrations (up to 30 %) so that the final ethanol concentrations can be increased to 65 g/L (Zhang et al., 2010). Higher solids loadings (as employed in the present study) may lead to higher final ethanol concentrations, which will reduce the energy/economic cost of downstream distillation for ethanol purification.

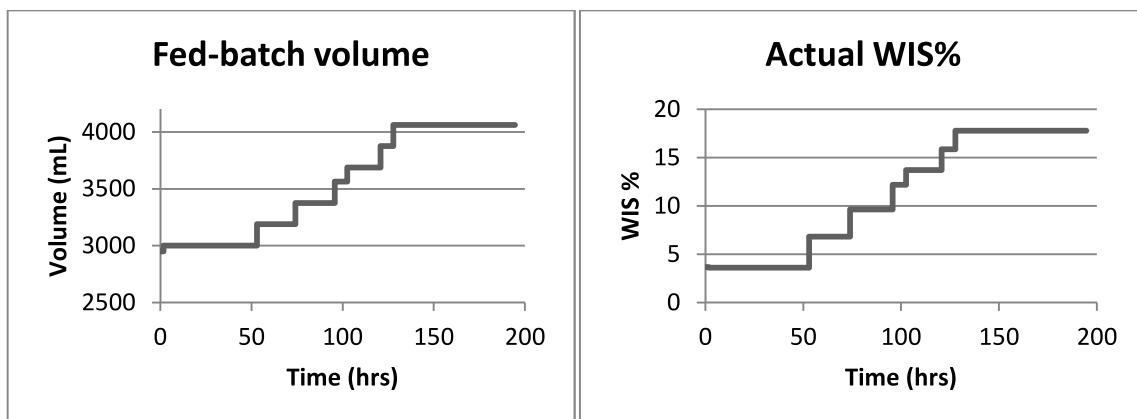

Figure 20: Fed-batch volume and actual WIS concentration of the 15 % of total WIS/day fermentations.

Figure 21 shows the sugar and ethanol concentrations for the 15 % of total WIS per day fermentation. The ethanol concentration decreases after about 100 hours due to the increasing liquid volume that was caused by the WIS being hydrolysed. Reactor 1 showed no glucose accumulation but the maximum ethanol concentration reached was very low (24.72 g/L) when

considering that a minimum of 40 g/L is required. The lack of glucose accumulation and low ethanol production indicate that there was some degree of contamination (as confirmed by a qualitative microscope check). Contamination was always a possibility in these experiments since the reactor had to be opened up (head plate removed) for the feeding of the WIS and this exposed the fermentation broth to the atmosphere and also the WIS that was being added was not sterile. It was assumed that contamination will be minimal due to the presence of inhibitors and ethanol in the broth and the low concentrations of sugars due to the SSF strategy (see section 2.3.4.2 Simultaneous saccharification and (co-) fermentation (SS(C)F). The yeast may also have utilised the sugars for other purposes than ethanol production.

In reactor two there was a problem with the pH-control which caused the control system to pump most of the 3 M KOH into the fermentation causing a pH-shock. This pH-shock prevented the yeast from efficiently fermenting the glucose which probably caused the accumulation of glucose. However this fermentation delivered a similar ethanol concentration than the first fermentation (25.50 g/L). The point at 127 hours for reactor two is seen as an outlier that may have been caused by sampling error.

The maximum ethanol concentration per WIS % was 1.40 g/L/WIS% for the present study which is significantly lower than the literature values of 3.56 g/L/WIS% (Rudolf et al., 2008), 3.07 g/L/WIS% (Öhgren et al., 2006) and 2.15 g/L/WIS% (Zhang et al., 2010). The final ethanol concentrations of 24.7 g/L and 25.50 g/L for the present study are lower than the ethanol concentrations in studies that had much lower WIS concentrations such as 26.7 g/L from 7.5 WIS% (Rudolf et al., 2008) and 36.8 g/L from 12 WIS% (Öhgren et al., 2006). These studies achieved higher ethanol concentrations due to lower inhibitors levels that resulted from lower WIS concentrations, the use of the hardened TMB 3400 yeast strain and faster feeding strategies. A faster feeding strategy was employed in section 5.3.2 (the following section) to show the effect that this has on the fermentation.

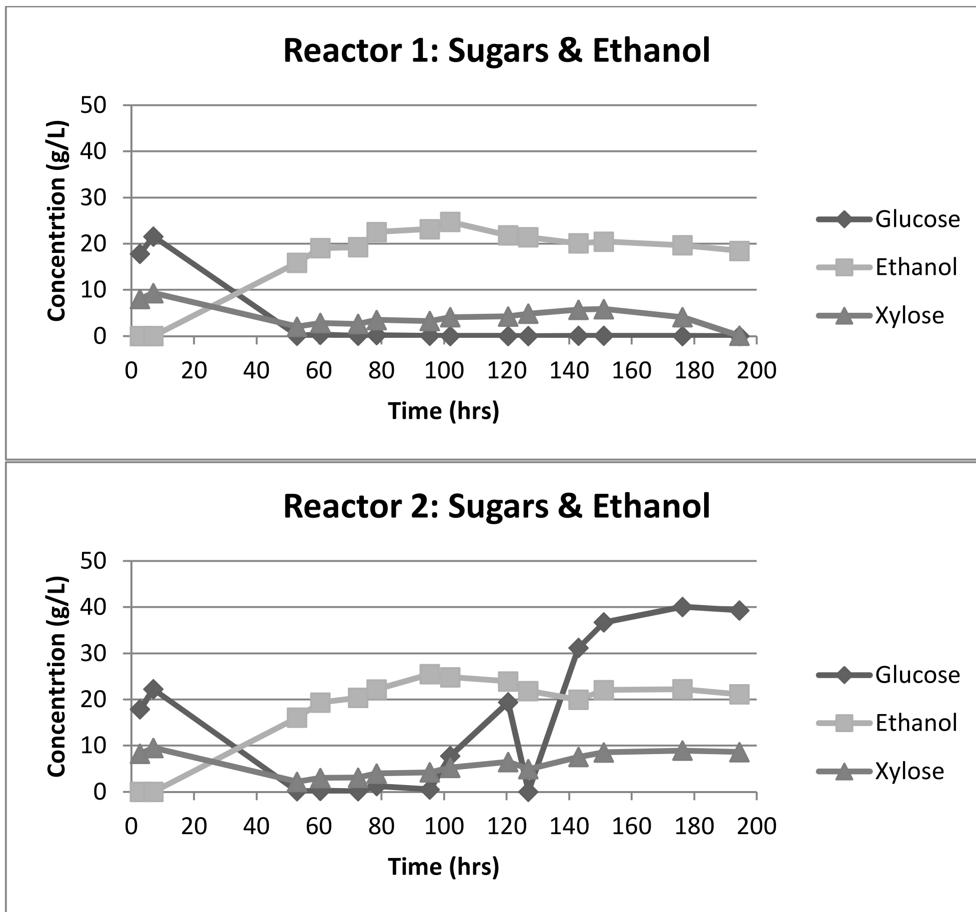

Figure 21: Sugar and ethanol concentrations for 15 % of total WIS/day fermentations.

Figure 22 shows the ethanol and glycerol concentrations for the 15 % per day fermentations. Both fermentations showed glycerol production of below 1 g/L which is low when compared to literature values of 4 g/L (Öhgren et al., 2006) and 4.9 g/L (Rudolf et al., 2008). This is good because the glucose released from the WIS is not used to produce by-products such as glycerol. For both reactors the ethanol concentration was at their highest points around 100 hours. The maximum ethanol concentrations of 24.72 g/L for reactor 1 and 25.50 g/L for reactor 2 were reached after 102 and 95.5 hours, respectively. The decrease in the ethanol concentration may be due to evaporation, utilisation of the ethanol by the yeast or the dilution effect caused by the addition of WIS, the addition of base and the increasing liquid volume caused by the hydrolysis of the cellulose.

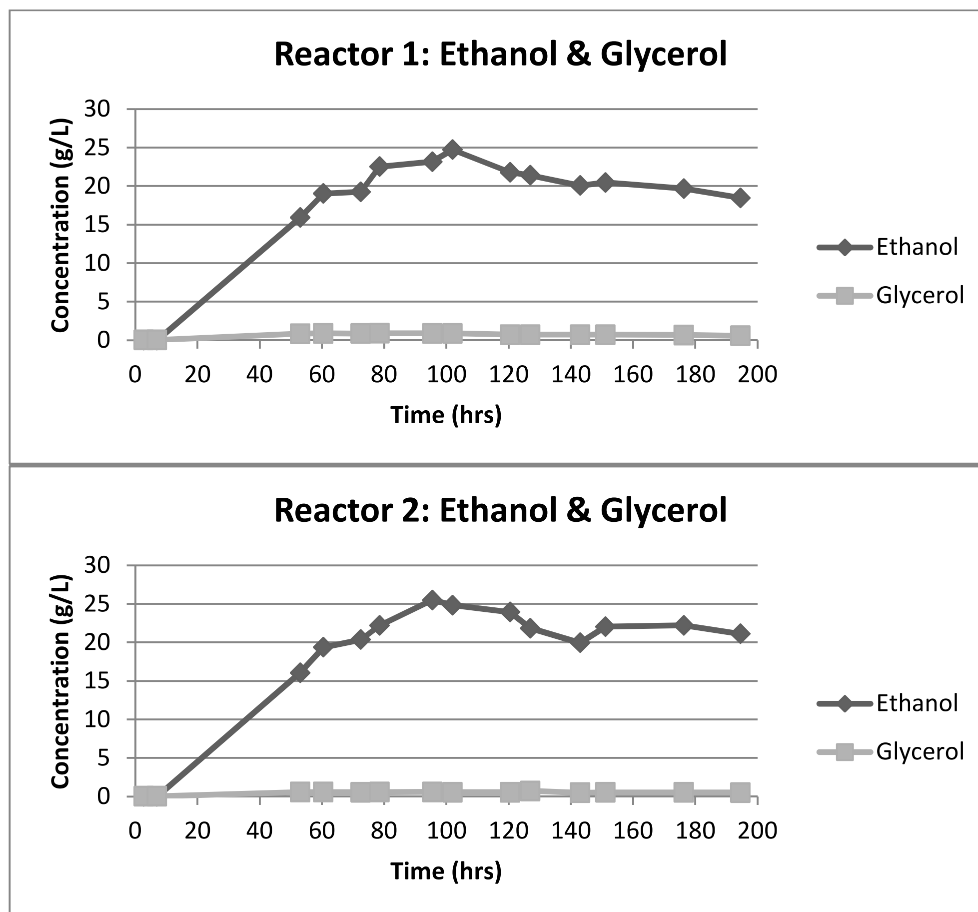

Figure 22: Ethanol and glycerol concentrations for 15 % of total WIS/day fermentations.

Figure 23 shows the HMF and furfural concentrations for the 15 % WIS per day fermentations. Reactor 1 shows that the concentrations for HMF and furfural are initially 0.03 g/L and 0.12 g/L, respectively. After the first 53 hours these concentrations decrease to levels below 0.03 g/L for both inhibitors and they remain at these lower levels for the remainder of the fermentation. This indicates that these inhibitors are metabolised by the yeast when they enter with the WIS.

Reactor 2 shows that the concentrations for HMF and furfural are initially 0.04 g/L and 0.14 g/L, respectively. After the first 53 hours these concentrations decrease to levels below 0.04 g/L for both inhibitors and they remain at these lower levels until about 100 hours after which they start to increase. This is the same point in time when glucose accumulation started, indicating that the yeast is not functioning properly at this point in time. The profile for these inhibitors correspond to the glucose profiles in Figure 21 indicating that the yeast can only metabolise inhibitors when it is busy fermenting glucose. The point at 127 hours for reactor two is seen as an outlier that may have been caused by sampling error. These graphs show that the HMF and Furfural levels are not high enough to inhibit fermentation, since the yeast was able to metabolise these compounds meaning that pressing the WIS was sufficient to lower the HMF and furfural to acceptable levels.

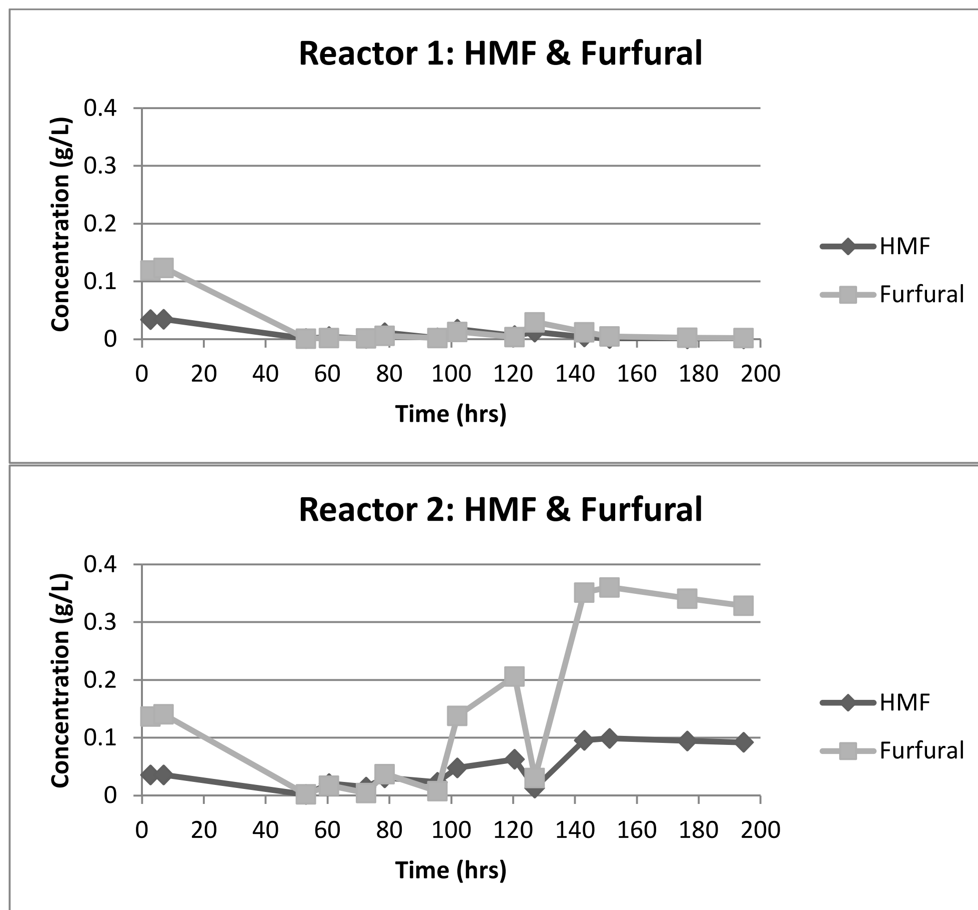

Figure 23: HMF and Furfural concentrations for 15 % of total WIS/day fermentations.

Figure 24 shows the formic and acetic acid concentrations for the 15 % WIS per day fermentations. The formic acid has a stepwise profile in both cases indicating that it enters the fermentation along with the WIS and it is not easily metabolised by the yeast.

The acetic acid has a more linear profile. We know from section 4.3.2 that some acetic acid was released during pre-treatment and is associated with the liquid entrapped in the WIS, but the linear profile indicates that additional acetic acid is released by the enzymes that hydrolyse cellulose. The total amount of acetic acid that is formed during pre-treatment and enzymatic hydrolysis combined is not a function of the pre-treatment severity but a function of the material (Taherzadeh & Karimi, 2008), however the severity of the pre-treatment does determine how much acetic acid is formed during pre-treatment and how much will be formed during enzymatic hydrolysis (Cantarella et al., 2004). This fact that acetic acid is released by enzymatic hydrolysis explains the linear trend of increase in acetic acid. The fact that there is some acetic acid associated with the WIS explains the small jumps in the acetic acid levels that come with feeding. The acetic acid was around 3 g/L when the glucose accumulation started for reactor 2. The high levels for acetic acid are most likely responsible for the inhibition of the yeast.

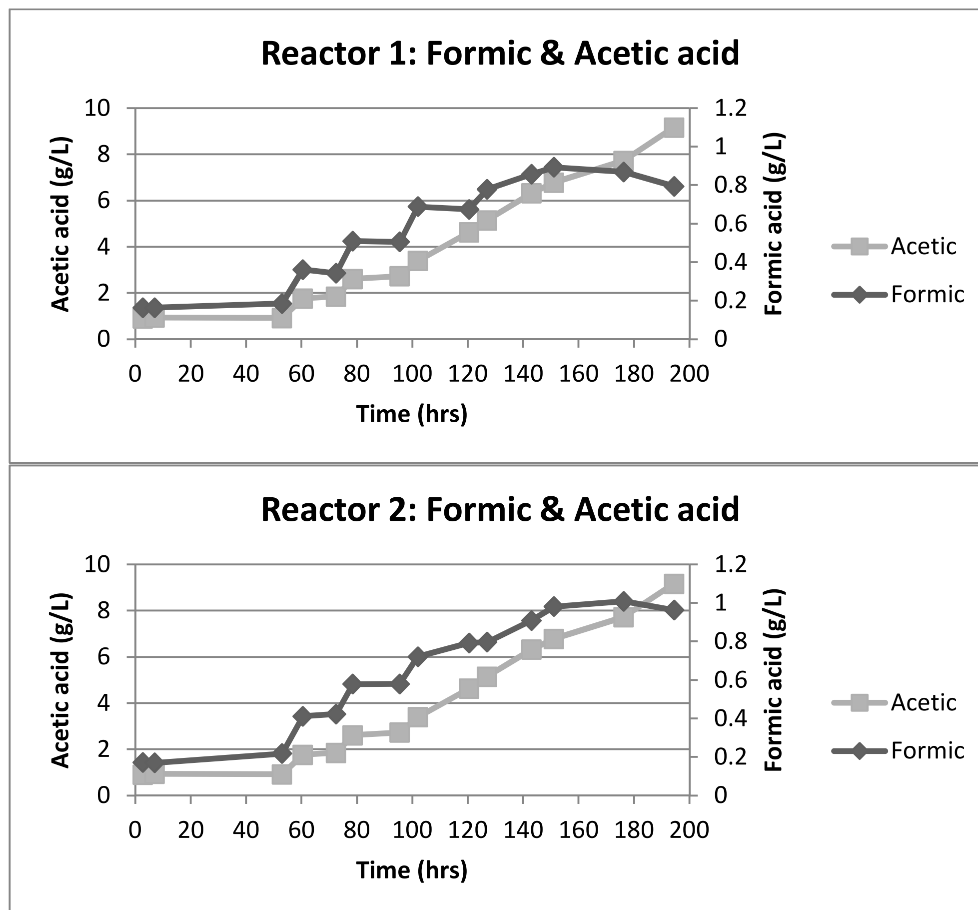

Figure 24: Acetic and formic acid concentrations for 15 % of total WIS/day fermentations.

5.3.2 FAST FEEDING STRATEGY

Figure 25 shows the fed-batch volumes and the actual WIS concentrations for the 25 % of total WIS per day fermentations. The fed-batch volume and actual WIS % was calculated for each reactor since the samples taken from- and WIS and base added to each reactor was measured. The final WIS % was around 17.3 % for both reactors which is higher than usual to increase the final ethanol concentration. This value is higher than the maximum literature WIS concentrations of 7.5 % to 12 % (Rudolf et al., 2008)(Öhgren et al., 2006), but lower than the 30 % of (Zhang et al., 2010) due to inadequate mixing and the low inhibitor tolerance of the yeast that was used.

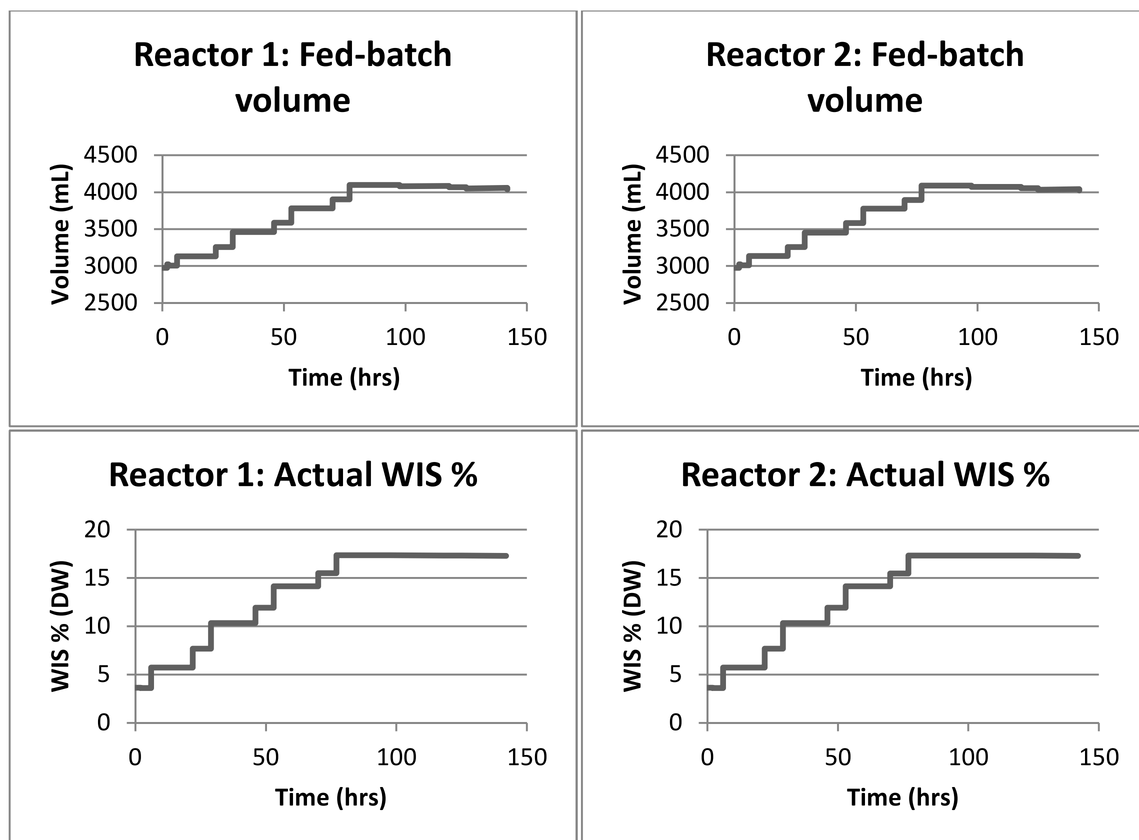

Figure 25: Fed-batch volume of 25 % of total WIS/day fermentations.

Figure 26 shows the cell concentrations for the two reactors. Initially the yeast population increased to 9.25x10^7 cells/mL after 46 hours for reactor 1 and 8.00x10^7 cells/mL after 53 hours for reactor 2. However after the initial increase the population started decreasing and reached values of 2.63 x 10^7 and 4.00x10^7 cells/mL after 97.5 hours for reactors 1 and 2, respectively. This indicates that the yeast's inhibitor tolerance is too low and it is suggested that a yeasts such as TMB 3400 (Öhgren et al., 2006; Rudolf et al., 2008) or DQ 1 (Zhang et al., 2010) with higher inhibitor tolerances be used for the fermentation of lignocellulosic materials. Cell counts could not be taken after 100 hours since there was too much WIS in the samples to see the yeast under a microscope.

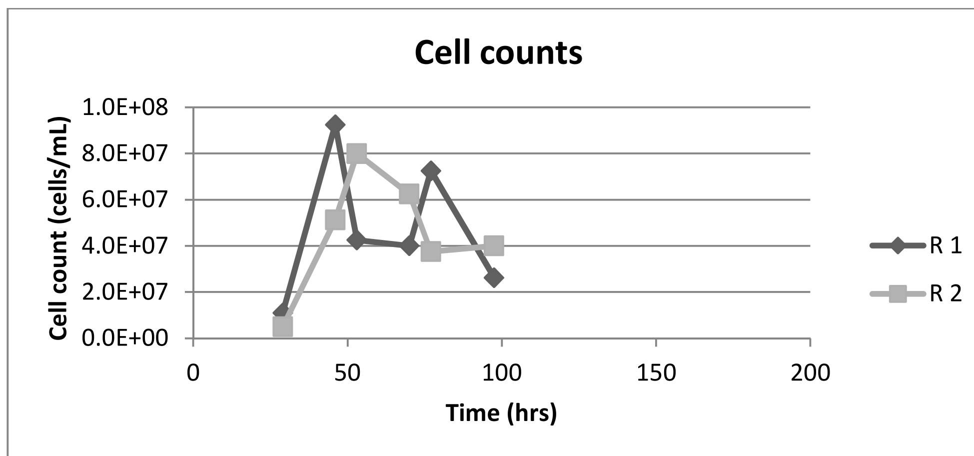

Figure 26: Cell counts for 25 % of total WIS/day fermentations.

Figure 27 shows the sugar and ethanol concentrations for the 25 % of total WIS/day fermentations. These fermentations show higher maximum ethanol concentrations than the 15 % of total WIS/day fermentations (34.67 g/L for reactor 1 and 39.33 g/L for reactor 2 versus 25.11 g/L) indicating that the yeast was able to tolerate the inhibitors better when the WIS feeding rate was increased due to the higher rate at which the yeast received glucose. The maximum ethanol concentration per WIS % was 2.28 g/L/WIS% (calculated from reactor 2) which is significantly higher than the 1.40 g/L/WIS% that was obtained using the slow feeding strategy. It is also higher than the literature value of 2.15 g/L/WIS% (Zhang et al., 2010), but it was lower than the literature values of 3.56 g/L/WIS% (Rudolf et al., 2008) and 3.07 g/L/WIS% (Öhgren et al., 2006). The latter two studies achieved higher ethanol concentrations due to lower inhibitor levels that resulted from lower WIS concentrations and the use of the hardened TMB 3400 yeast strain.

The ethanol concentration in reactor two is almost at the 40 g/L minimum required to be able to achieve acceptable separation costs (Öhgren et al., 2006). It can be seen that the ethanol production has ceased started at 70 hours (reactor 1) and 77 hours (reactor 2). This was apparently due to the low inhibitor tolerance of MH 1000 and it occurred at around the same point in time as when the cell counts started to decrease (see Figure 26). The two graphs show good repeatability.

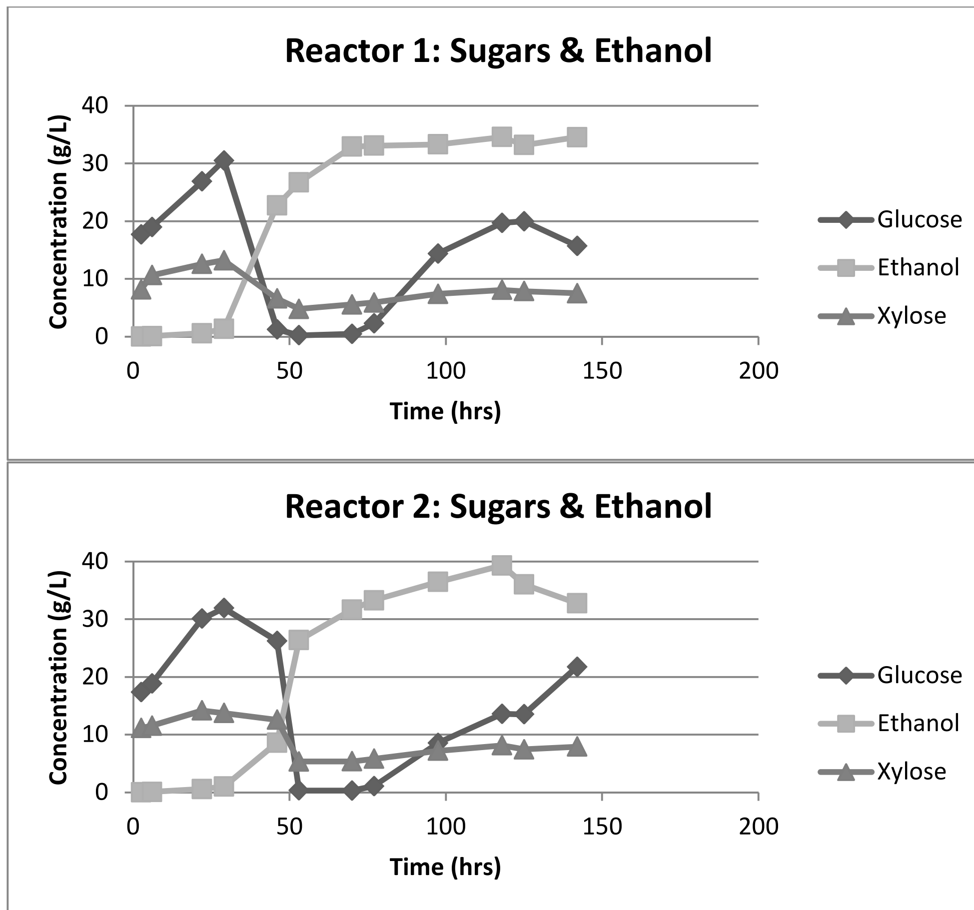

Figure 27: Sugar and ethanol concentrations for 25 % of total WIS/day fermentations.

Figure 28: show the ethanol and glycerol concentration for the 25 %/day fermentations. Both fermentations showed glycerol production of below 2 g/L which is low when compared to literature values of 4 g/L (Öhgren et al., 2006) and 4.9 g/L (Rudolf et al., 2008). The glycerol levels for these experiments are a little higher than for the slow feeding strategy experiments. This may be due to inhibitors that were introduced at a faster rate which caused more stress on the yeast or it may be due to the higher levels of ethanol production.

The low glycerol levels are good because it indicates that the glucose released from the WIS is not used to produce by-products such as glycerol. Both reactors reached their maximum ethanol concentration at 118 hours. The maximum ethanol concentrations were 34.57 g/L for reactor 1 and 39.33 g/L for reactor 2. For the previous experiments the maximum ethanol concentrations were reached after approximately 100 hours, which shows that the faster feeding strategy keeps the yeast producing ethanol for longer, probably because the yeast stays viable for longer. The decrease in the ethanol concentration after 118 hours may be due to evaporation, utilisation of the ethanol by the yeast or the dilution effect caused by the addition of WIS, the addition of base and the increasing liquid volume caused by the hydrolysis of the cellulose.

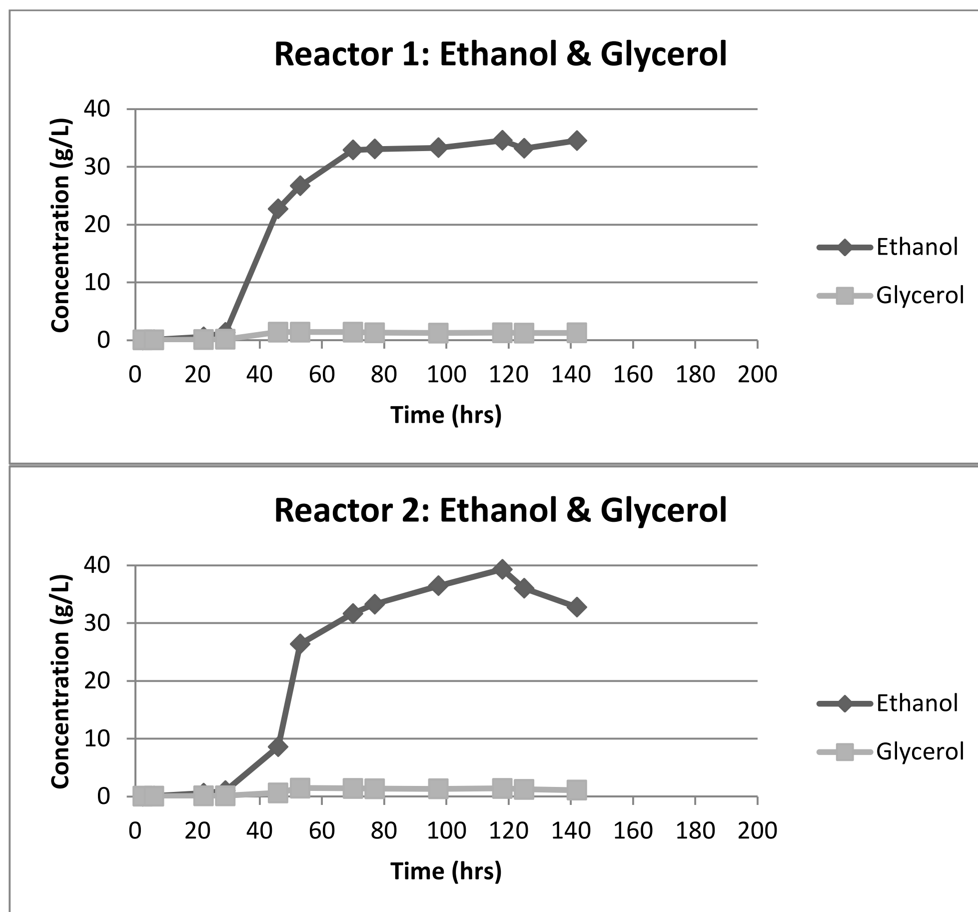

Figure 28: Ethanol and glycerol concentrations for 25 % of total WIS/day fermentations.

Figure 29 shows the HMF and furfural concentrations for the 25 % per day fermentations. The concentrations for HMF reach a maximum of 0.07 g/L for reactor 1 and 0.08 g/L for reactor 2 and the concentrations for furfural reach a maximum of 0.20 g/L for reactor 1 and 0.24 for reactor 2. The maxima for these inhibitor concentrations were reached after 29 hours for both reactors. After 46 hours these concentrations decrease to levels below 0.06 g/L for both inhibitors in both reactors and they remain at these lower levels for the remainder of the fermentation. This indicates that these inhibitors are metabolised by the yeast when they enter with the WIS.

These graphs show that the HMF and Furfural levels are not high enough to inhibit fermentation, since the yeast was able to metabolise these compounds meaning that pressing the WIS was sufficient to lower the HMF and furfural to acceptable levels. The graphs also show good repeatability.

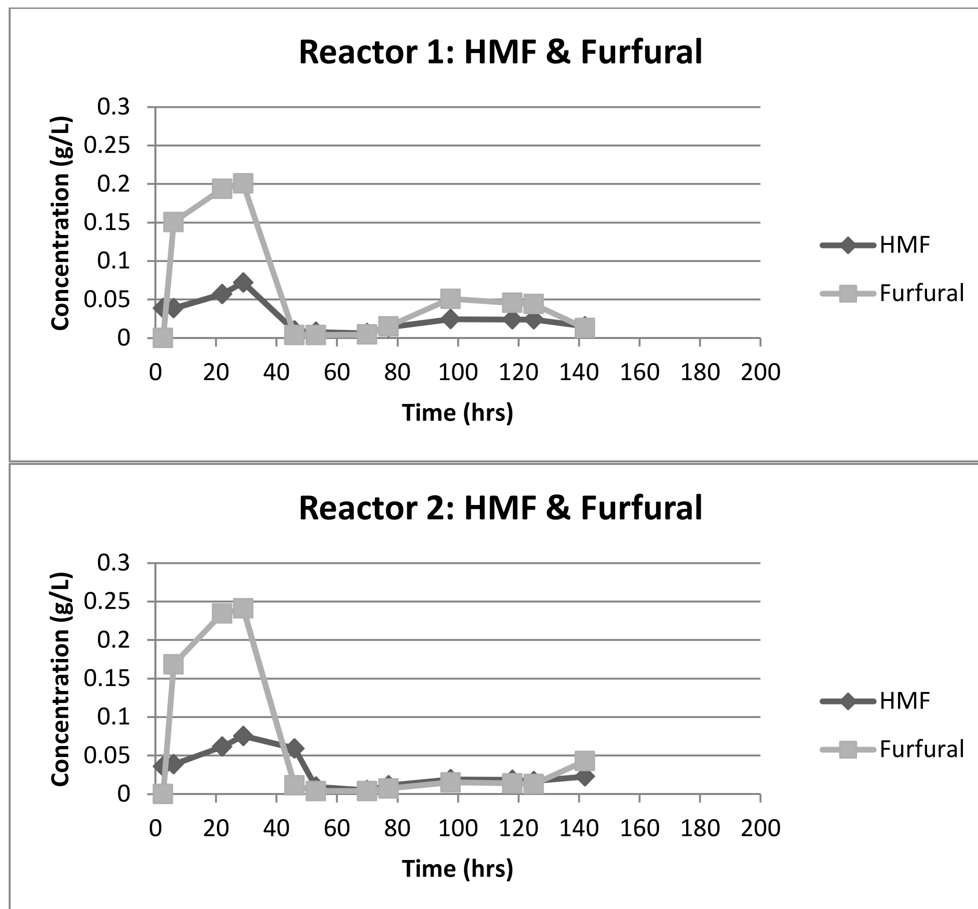

Figure 29: HMF and Furfural concentrations for 25 % of total WIS/day fermentations.

Figure 30 shows the formic and acetic acid concentrations for the 25 % per day fermentations. It can be seen that both acids mostly increase throughout the fermentation indicating that the yeast has trouble metabolising them. The increase in the acids is caused by the acid that enter with the WIS that were formed during pre-treatment and the acid that is released during enzymatic hydrolysis.

Glucose accumulation started when the acetic acid concentration was between 3 and 4 g/L. The high level of acetic acid is most probably the main inhibitor responsible for the inhibition of the yeast. However there were probably other inhibitors present (that were not analysed for) that also contributed to the inhibition of the yeast. The graphs show good repeatability

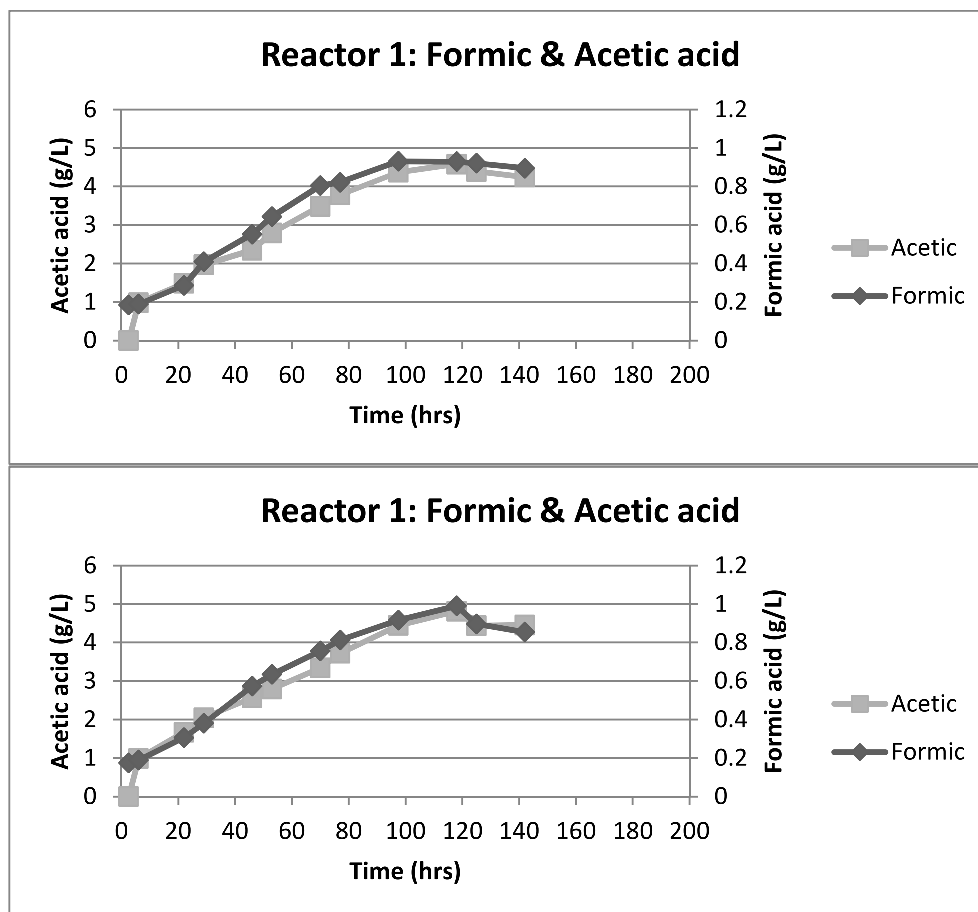

Figure 30: Acetic and formic acid concentrations for 25 % of total WIS/day fermentations.

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There is enough β-glucosidase in the Cellic CTech2 since there was no large accumulation of cellobiose (data shown in Appendix L: Cellobiose concentrations of WIS fermentations), thus backward inhibition is not a problem when the yeast is busy fermenting (Taherzadeh & Karimi, 2008; Wyman et al., 1992). Other authors had to supplemented their enzyme cocktails with additional β-glucosidase (Novozyme 188) to prevent this problem (Öhgren et al., 2006; Rudolf et al., 2008).

5.4 Conclusions And recommendations

Conclusions:

MH 1000's inhibitor tolerance is not high enough for second the generation fermentation of steam pre-treated and pressed industrial bagasse. This was indicated by glucose that started to accumulate at an acetic acid concentration of between 3 – 4 g/L (except for the 15 % WIS per day fermentation in reactor 1, where there was no glucose accumulation). There are many other inhibitors besides acetic acid but acetic acid is used here as an indicator for the inhibitor level, thus acetic acid is not responsible for the glucose accumulation entirely on its own. The low inhibitor tolerance of MH 1000 served to show the improvement that is brought about by different fermentation strategies.

The assumption that pressing the material would lower the inhibitor level to acceptable levels was wrong in this case, but this is due to the low inhibitor tolerance of MH 1000. Other yeast with higher inhibitor tolerances such as TMB 3400 or Q1 might have been able to handle the amounts of inhibitors at the WIS concentrations in these experiments. For example Q1 was used to ferment unwashed steam pre-treated corn stover at 30 WIS% (Zhang et al., 2010).

The faster feeding strategy (25 % WIS per day) performed better than the slower feeding strategy since higher ethanol concentrations were reached and the ethanol concentration per WIS% was higher. The yeast also stayed viable for longer during the fast feeding strategy fermentations showing that the yeast better tolerated the inhibitors. This means that the faster feeding provided higher ethanol yields (based on higher ethanol concentrations for the same amount of WIS added and the same final fermentation volumes) and higher productivities (shorter fermentation times). The ethanol concentrations were too low in all cases to be effectively separated. The faster feeding strategy enabled the yeast to tolerate the inhibitors better due to the higher rate of sugar that it received. The 25 % WIS per day feeding strategy was used as a baseline for the 1st and 2nd generation combination fermentations (see next chapter).

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There is enough β-glucosidase in the Cellic CTech2 since there was no large accumulation of cellobiose, thus backward inhibition is not a problem when the yeast is busy fermenting.

Note: Determining the yield and productivity was not possible because the liquid volume of the fermentation was un-known. The liquid volume was unknown because the degree to which the WIS had been hydrolysed had been unknown. The liquid volume is required to calculate the amount of ethanol produced from the ethanol concentration. The amount of ethanol produced is required to calculate the yield and the productivity.

Recommendations:

It is recommended that a yeast strain with a higher inhibitor tolerance be used for the fermentation. This would be better than to wash the WIS extensively, since adding water to a process in industry causes the separation costs to increase dramatically since all that water will have to be removed again.

It is recommended that a method be sought to determine the liquid volume of the fermentation accurately so that the ethanol yields and productivities can be accurately determined. Such a method must include the analysis of the amount of solids present in every sample to be able to determine how much of the solids have been hydrolysed. This must be done to determine the liquid volume of the fermentation so that the ethanol concentration can be used to determine the exact amount of ethanol that was produced. If the exact amount of ethanol is known it can be used to calculate yields and productivities based on the amount of WIS that is added. It is very important to take large enough samples and to make sure that they are representative of the solid-liquid content of the fermentation. The sample port through which the sample is drawn must be wide enough so that it does not get blocked by solids and the liquid gets sucked through the solids.

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6. COMBINATIONS OF FIRST AND SECOND GENERATION FERMENTATION EXPERIMENTS

6.1 INTRODUCTION

This section is about experimental approaches to combine 1st and 2nd generation fermentations of sugar syrup and pre-treated bagasse. This was done by adding 22 % buffered sucrose syrup to a 2nd generation fermentation of pre-treated bagasse in a continuous fed-batch manner. The aims of adding sucrose to the fermentations were to increase the ethanol concentration and productivity (shortening fermentation times) by increasing the sugar concentrations and diluting the inhibitors by the addition of sucrose syrup. The sucrose was added to SSF-type experiments.

The objective for this work was to assess the possibility of combining 1st and 2nd generation technologies and to determine the advantages that such combinations may hold. Advantages may include higher final ethanol concentrations and higher productivities due to lower inhibition caused by the dilution of inhibitors due to the addition sucrose syrup. In the future when CBP yeasts are used to perform SSF combinations such as this may prove useful.

Other authors have only combined SHF with 1st generation ethanol (Dias et al., 2009; Dias et al., 2011b; Macrelli et al., 2012) where the sucrose is added after the enzymatic hydrolysis step so that the addition of sucrose doesn't inhibit the enzymes. In this study the sucrose is added to SSF, but since the sucrose is added in a fed-batch manner it is assumed that the sucrose will be utilised soon after it is added to the fermentation so that the sugar levels in the fermentation stay low to prevent feedback inhibition of the enzyme. This assumption will be revisited.

The capital cost of fermenters scale up linearly because there is a limit to the maximum size of a fermenter and to increase the total fermentation capacity of a large operation one must increase the amount of fermenters instead of just building one very large fermenter (Aden et al., 2002). This means that one can't take advantage of the economy of scale when it comes to fermenters. This implies that by combining first and second generation fermentations in the same vessel that nothing is gained by integrating unit operations to lower capital costs. The only way that something can be gained from combining these fermentations is by increasing the ethanol concentration to lower

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separation costs, decreasing fermentation times to lower capital costs (fewer fermenters required for lower residence times) and/or increasing the ethanol yield.

6.2 Materials and methods

The same method was followed for performing the pure second generation fermentations (see section 5.2).

In summary: The bagasse was steam pre-treated at 200 °C for 10 min, the pre-treated bagasse was then pressed to remove as much of the pre-hydrolysate liquor as possible to lower inhibitor levels. A fraction of the pre-treated bagasse was then added to the reactor along with the enzymes in a pre-saccharification step (35 °C for 90 min) before the fermentations were inoculated with a yeast culture that had been pre-conditioned using the pre-hydrolysate liquor. The pre-saccharification step for the HSF fermentations took place at a hotter temperature of 50 °C for a longer time of 12 hours.

Sugar syrup was added to the above described fermentations in a continuous fed-batch manner. The sugar syrup was pumped into the fermentations at a constant rate. The sugar syrup flow rates for the SSF + sucrose fermentations (section 6.2.1 and 6.3.1) was 0.325 mL/min and 1.653 mL/min for the one of the HSF experiments (section 6.2.2 and 6.3.2). The reasons for choosing these flow rates can be found in Appendix M: Peristaltic pump calibration. The sugar syrup flow rate was intentionally very low to avoid feed-back inhibition of the enzymes through the accumulation of sugars.

The same amount of WIS was used in these fermentations as in the pure second generation fermentations. The sugar syrup provided additional volume which caused the WIS concentration to decrease. 935 mL and 1095 mL of sugar syrup was added to the SSF + sucrose fermentations and to the one of the HSF experiments, respectively.

The peristaltic pumps that were used to feed the sugar syrup had to be calibrated to be able to determine a relationship between the pump setting (0 – 100 %) and flow rate (mL/min). The pump calibration curves can be seen in Appendix M: Peristaltic pump calibration.

6.2.1 SSF with sucrose fermentations

For these experiments the fast feeding strategy (25 % of total WIS per day) SSF method with pressed pre-treated bagasse was used as a base method, with sucrose syrup being added in a continuous fed-batch manner to improve the fermentation. The sugar syrup feeding did not start when the fermentation was inoculated, but it was started later on when the fermentation had been going for a while (after half of the WIS had already been added). The idea was to start feeding the sugar when the yeast population showed signs of decreasing. The starting time for the sucrose feed was determined from the cell counts in Figure 26 for the 25 % WIS per day SSF experiment (see section 5.3.2). These cell counts show that the yeast populations started decreasing after 46 hours which was selected as the timepoint for the start of sugar syrup feeding. The WIS concentration was approximately 10 % when the sucrose feed was started.

935 mL of buffered (3.4 g/L KH_2PO_4) 22 % sucrose syrup wad added over a time of 48 hours. 48 hours was chosen as the length of feeding so that sugar was continuously fed to the fermentation while the remaining pressed WIS was being added so as to maintain synchronous sugar syrup and WIS feeding, to maximise the dilution effect on inhibitors in the pressed WIS. The idea was that the higher sugar concentrations would help the yeast to metabolise the inhibitors since the higher sugar concentrations will help to speed up the yeast's metabolism and dilute the inhibitors. The calculation of the sugar syrup flow rates can be seen in Appendix M: Peristaltic pump calibration.

This fermentation was performed in duplicate.

6.2.2 Hybrid saccharification and fermentation (HSF)

Hybrid saccharification and fermentation is a hybrid method between SSF and SHF (Bayer et al., 2007). First, half of the total WIS is added along with the enzymes for a pre-saccharification step similar to SHF. Since only half of the WIS was added along with all of the enzymes it means that the enzyme dosage was double during this part of the experiment (30 FPU/g dry WIS). The pre-saccharification step was performed at 50 °C for 12 hours (Zhang et al., 2010). The reactor was then allowed to cool to a fermentation temperature of 35° C. Two hours was allowed for the cooling of the reactor. After the reactor was cooled the fermentation was inoculated with the yeast inoculum that was cultivated in the same way as for the pure SSF cultures (see section 5.2.3). After inoculation the rest of the WIS was added in a fed batch manner, similar to a SSF culture.

In this project sucrose was added to one of the HSF reactor and not to the other one. This was done to be able to determine the effect that sucrose addition will have on an HSF. The experiment without the sucrose addition is seen as the control experiment.

1095 mL of buffered (3.4 g/L KH_2PO_4) 22 % sucrose medium was added to one of the fermentations. The sucrose syrup was again fed in a continuous fed- batch manner. The sugar syrup was added after 36.5 hours, because it was noted that the yeast was not fermenting the glucose. This was realised by doing cell counts on the samples and performing qualitative glucose tests (urine strips) to see whether glucose was still present in the solution (see Appendix K: Qualitative glucose tests for 2nd generation and 1st and 2nd generation combination fermentations). The calculation of the sugar syrup flow rates can be seen in Appendix M: Peristaltic pump calibration.

6.3 Results and Discussion

6.3.1 SSF fermentations with sucrose

Figure 31 shows the fed-batch volumes and the actual WIS % for the SSF + sucrose fermentations. The sucrose causes a linear increase in volume whereas the addition of base, drawing of samples and the addition of WIS causes a stepwise increase in volume since these additions happen in a very short time. The reason that the addition of base is seen as causing a stepwise increase in volume is because it was observed that a lot of base was added directly after the addition of WIS to restore the pH-imbalance that was caused with the acid that enters the fermentation with the WIS. The addition of sucrose syrup causes the WIS % to decrease as can be seen in the figure. The final WIS % is around 14.1 % for both cases.

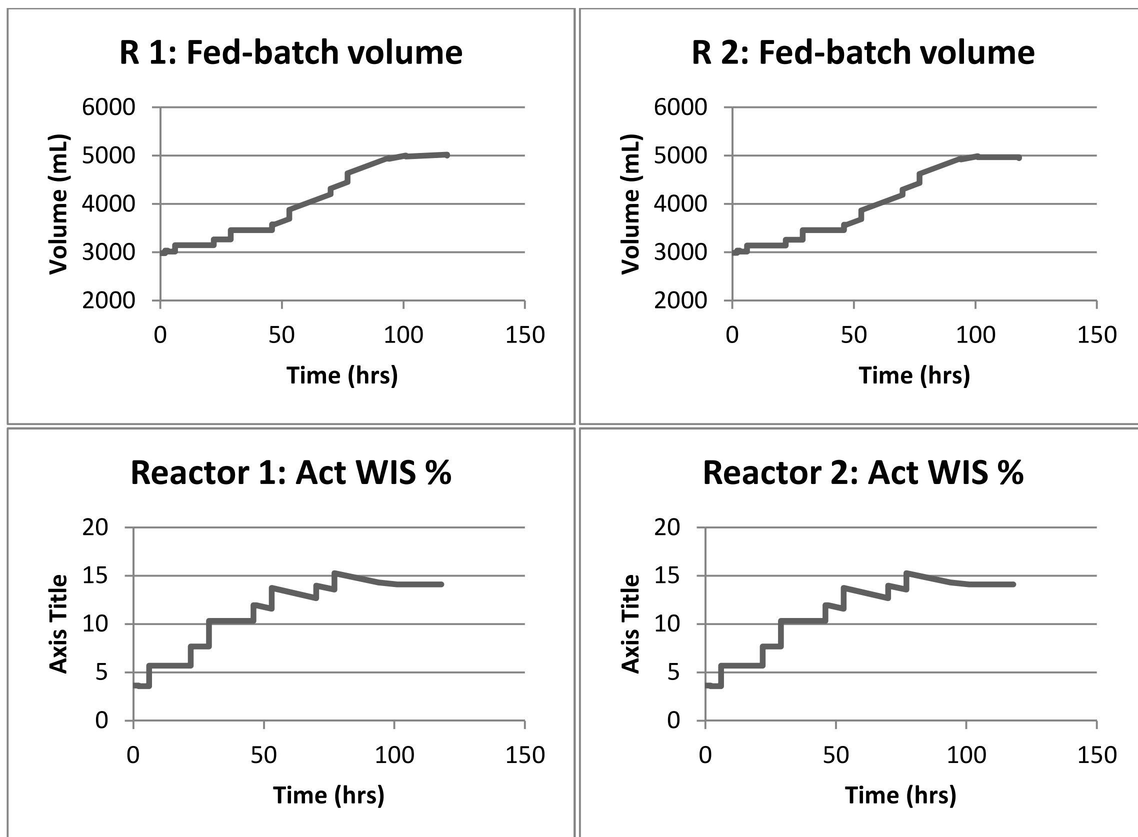

Figure 31: Fed-batch volume and actual WIS percentages of SSF + sucrose fermentations.

This final WIS concentrations of 14.1 % were higher than the maximum literature WIS concentrations of 7.5 % to 12 % (Rudolf et al., 2008)(Öhgren et al., 2006), but lower than the 30 % of (Zhang et al., 2010).

The cell counts for the fermentations can be seen in Figure 32 below. It can be seen that the cell counts initially increased up to 8.375x10^{7} cells/mL after 22 hours for reactor 1 and 7.5x10^{7} cells/mL after 29 hours for reactor 2. After this point the cell counts started to decrease to 1.875x10^{7} cells/mL for reactor 1 and 1.5x10^{7} cells/mL for reactor 2 after 77 hours for both reactors. This indicated that the sucrose syrup was added too late. However when the sugar syrup was added after 46 hours there was a slight increase in the cell count for reactor 1 and the cell count stayed almost constant for reactor 2. Considering that the addition of the sucrose syrup will dilute the yeast cells, it can be deducted that the constant and slightly increasing cell counts indicate positive cell growth due to the addition of sucrose. The decrease in cell counts after 53 hours show that not enough sucrose was added to combat the effect of the inhibitors.

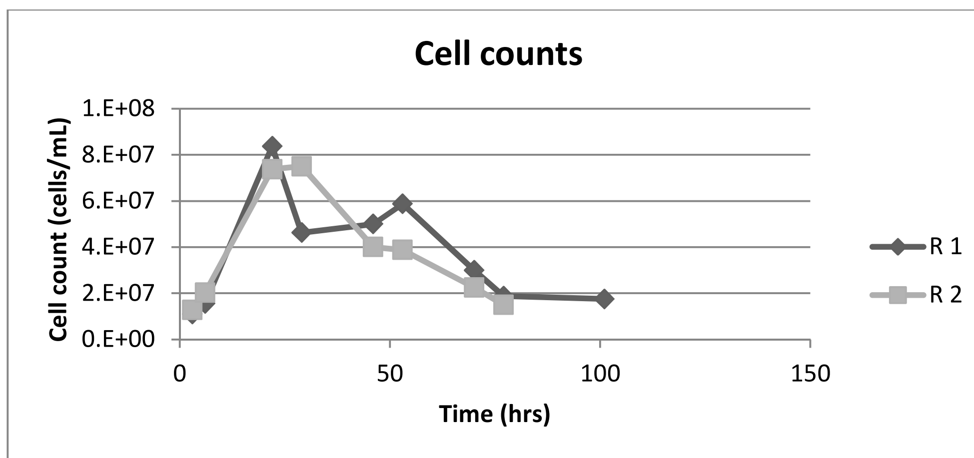

Figure 32: Cell counts for SSF + sucrose fermentations.

Figure 33 shows the concentrations of the sugar and ethanol for the SSF + sucrose fermentations. In both cases sugars start accumulating after 46 hours. This corresponded to the time that the sucrose feeding started. Glucose accumulated faster than fructose, even though the yeast has a higher selectivity for glucose (see previous chapter's results section), apparently due to the glucose released from WIS by the enzymes in addition to glucose released from the sucrose by invertase.

In reactor 1 the sucrose levels remained below 2.5 g/L for the entire fermentation, indicating that the yeast secreted enough invertase to hydrolyse the sucrose. In reactor 2 there was sucrose accumulation up to 33.45 g/L after 77 hours, but the sucrose levels decreased to 7.2 g/L after 118 hours, indicating that the yeast initially did not secrete enough invertase to keep the sucrose levels down. However, later on there was enough invertase secreted to reduce the sucrose levels.

The maximum ethanol concentrations were 31.83 g/L for reactor 1 and 29.06 g/L for reactor 2. These low ethanol levels shows that the addition of sucrose did not help to increase the maximum ethanol concentration as the maximum ethanol concentration for the pure SSF experiments were 34.57 g/L and 39.33 g/L (see Figure 28). The dilution effect of adding sucrose overshadowed any extra production of ethanol and the goal of increasing the final ethanol concentration was not reached.

The maximum ethanol concentrations of these experiments are around 30 g/L. This is lower than the ethanol concentrations that were obtained with the fast feeding strategy SSF experiments (39.33 g/L and 34.57 g/L) that were conducted in this study (section 5.3.2). The low ethanol concentrations, when compared to the experimental results from the fast feeding strategy SSF experiments, in these experiments may be explained by the low inhibitor tolerance of the yeast. It can be seen from Figure 33 that the yeast stopped producing ethanol and sugars started to accumulate. This is due to inhibition of the yeast.

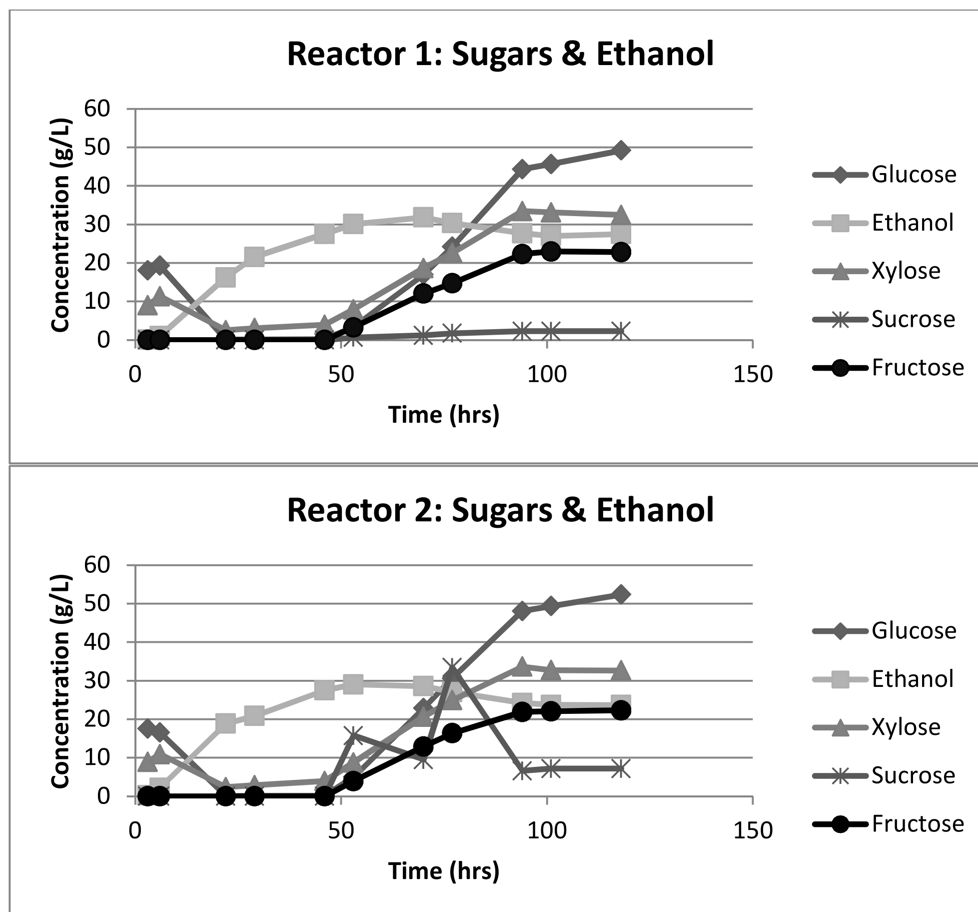

Figure 33: Sugar and ethanol concentrations for SSF + sucrose fermentations.

Figure 34 shows the ethanol and glycerol concentrations for the SSF + sucrose fermentations. Both fermentations showed glycerol production of below 1.5 g/L which is low when compared to literature values of 4 g/L (Öhgren et al., 2006) and 4.9 g/L (Rudolf et al., 2008), which is preferred, indicating that the yeast did not utilise the sugars to produce by-products such as glycerol. The glycerol levels for these experiments are a little lower than for the fast feeding strategy experiments (section 5.3.2). This may be due to the dilution effect that is caused by the addition of sucrose syrup or less glycerol may have been produced due to less stress on the yeast because the sugar syrup diluted the inhibitors.

The maximum ethanol concentrations of 24.72 g/L for reactor 1 and 25.50 g/L for reactor 2 were reached after 70 and 53 hours, respectively. The ethanol production follows the same trend in both cases showing good reproducibility. The decrease in the ethanol concentration after the maxima have been reached may be due to evaporation, utilisation of the ethanol by the yeast or the dilution effect caused by the addition of WIS, the addition of base and the increasing liquid volume caused by the hydrolysis of the cellulose.

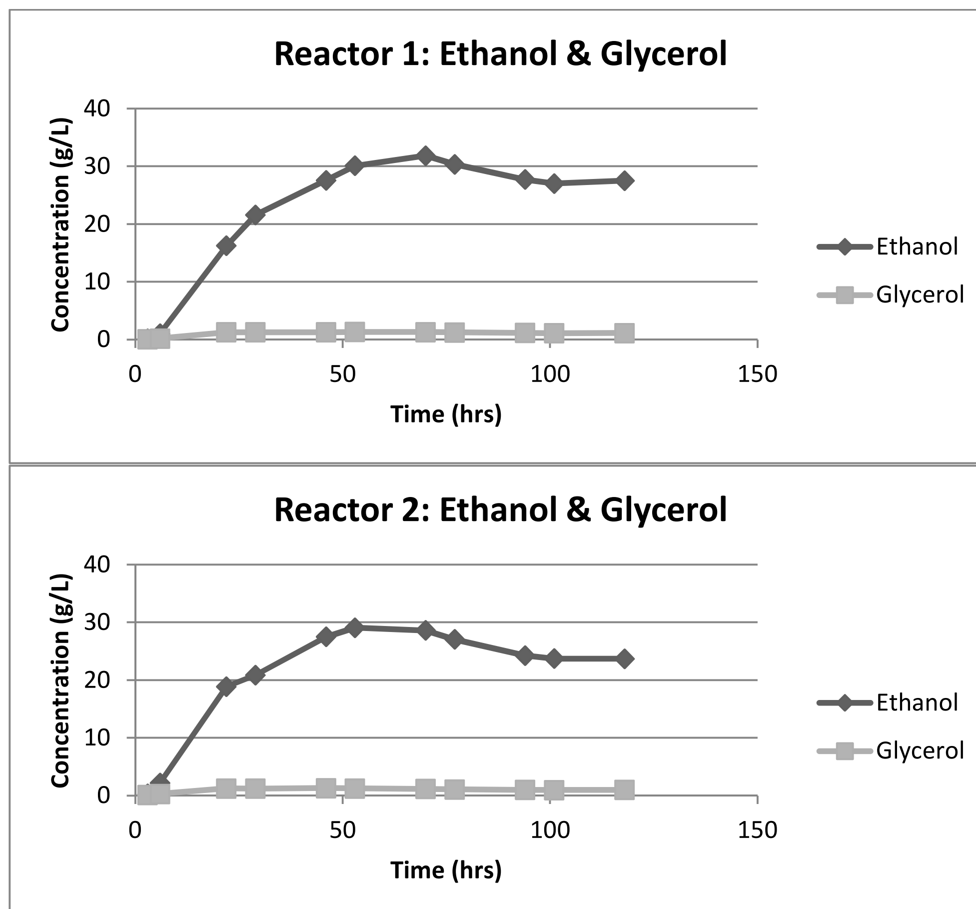

Figure 34: Ethanol and glycerol concentrations for SSF + sucrose fermentations.

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Figure 35 shows the HMF and furfural concentrations for the SSF + sucrose fermentations. The concentrations of both inhibitors were at/near maximum when the fermentations started. The HMF concentrations were 0.03 g/L for both reactors after 3 hours (and still after 6 hours for reactor 1). The furfural concentrations were 0.17 g/L for reactor 1 after 3 hours and 0.16 g/L for reactor 2 after 3 hours. These values are slightly lower than, but very similar to, the maximum values of 0.08 g/L for HMF and 0.22 g/L for furfural that were obtained by the fast feeding SSF experiments (see section 5.3.2) and this is because the fermentations were exactly the same at these timepoints since the addition of sucrose syrup only commenced after 46 hours. The yeast was able to metabolise both inhibitors in both reactors and after 22 hours the concentrations of both inhibitors were reduced to below 0.01 g/L. During the fast feeding strategy SSF experiments the yeast was able to metabolise HMF and furfural until the end of the fermentation, but the yeast was not able to do that in this case, showing that, in this case, the addition of sucrose was not successful in reducing inhibition.

In both cases the HMF started accumulating again at 53 hours and the HMF reached new local maximum concentrations of 0.047 g/L after 101 hours in reactor 1 and 0.058 g/L after 118 hours in reactor 2. The furfural started accumulating again after 70 hours for reactor 1 and after 53 hours for reactor 2 and the furfural reached new local maximum concentrations of 0.11 g/L after 94 hours for reactor 1 and 0.19 g/L after 101 hours for reactor 2.

The accumulation of inhibitors corresponds to the accumulation of sugars (see Figure 33) indicating that the yeast has become inactive. Both graphs follow the same trend, indicating good reproducibility. The fact that the yeast was able to metabolise HMF and furfural indicates that these compounds were not responsible for the inhibition of the yeast because they were at very low levels when the yeast became inactive.

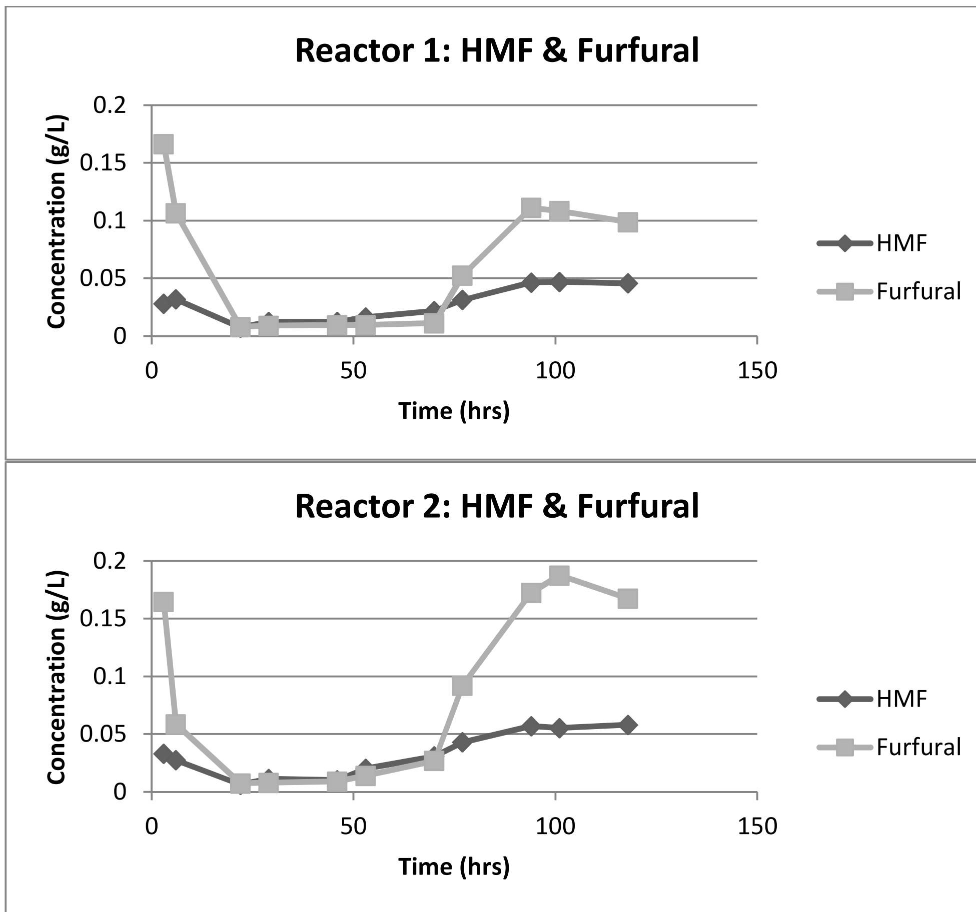

Figure 35: HMF and Furfural concentrations for SSF + sucrose fermentations.

Figure 36 shows the concentrations of formic and acetic acid for the SSF + sucrose fermentations. Both acids increased in a linear fashion in both cases indicating that the yeast can't metabolise these acids fast enough. The final acetic acid concentrations were approximately 3.5 g/L for both reactors after 118 hours. The final formic acid concentrations were approximately 0.75 g/L for both reactors. These values are lower than the values obtained for the fast feeding strategy SSF experiments (see section 5.3.2) due to the dilution effect caused by the addition of the sucrose syrup.

The accumulation of sugars corresponded to a an acetic acid concentration of between 2 – 3 g/L, which is lower than the concentrations at which glucose accumulation started for the pure second generation experiments (3 – 4 g/L). This means that it is not the acetic acid on its own that causes the glucose to start accumulating. The addition of sucrose syrup, although it did help to dilute formic and acetic acid, did not help to reduce inhibition in this case. The graphs show good repeatability

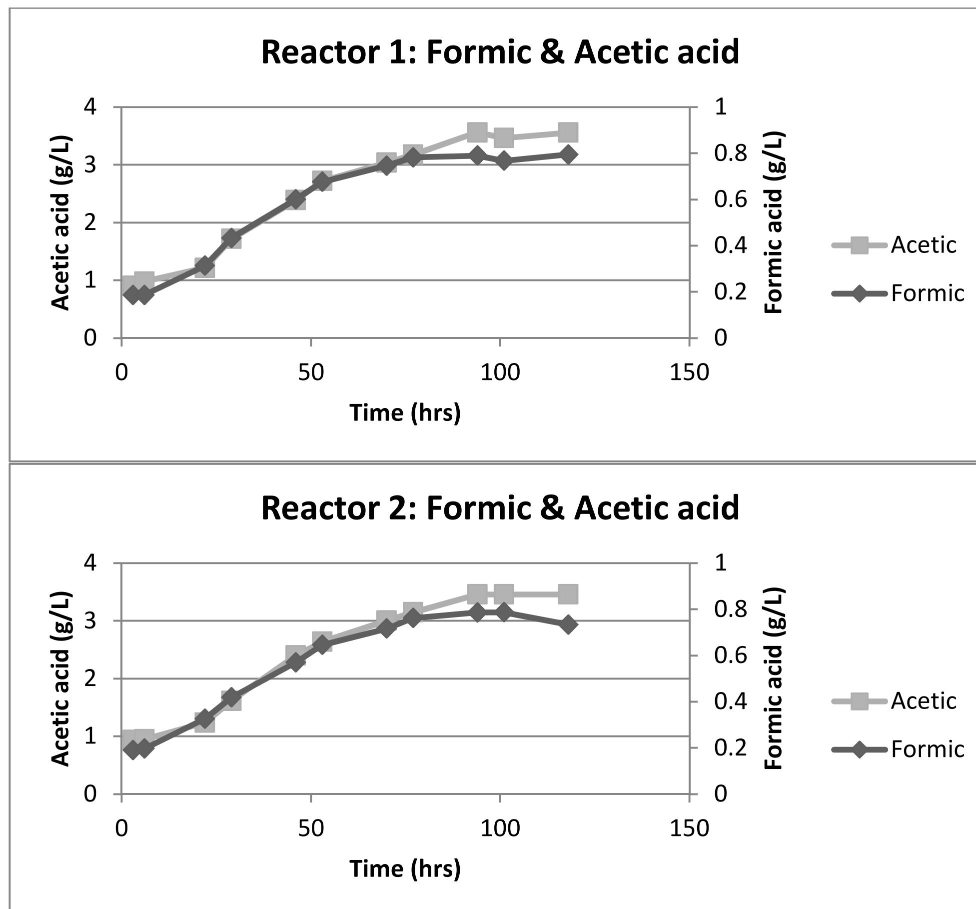

Figure 36: Acetic and formic acid concentrations for SSF + sucrose fermentations.

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6.3.2 HYBRID SACCHARIFICATION AND FERMENTATION (HSF)

In this section two HSF experiments were conducted. In the one experiment sucrose was added to improve the fermentation and in the other fermentation sucrose was not added. The data from the experiment where no sucrose was added served as a baseline for the experiment where sucrose was added. Figure 37 shows the fed-batch volumes and the actual WIS % for the two HSF experiments. The two graphs on the left represent the experiment where sucrose was added. The two graphs on the right represent the control experiment where no sucrose was added.

For the experiment where no sucrose was added the yeast failed to overcome the high inhibitor concentration and the fermentation was stopped. That is why the volume stayed constant and why the WIS % never exceeded 10 %. The graphs for this control experiment are shown along with the graphs of the experiment with added sucrose to emphasize the difference that was made by adding sucrose.

The addition of sucrose can be seen as the linear increase in the volume. Again the addition of sucrose syrup caused the WIS % to decrease (also in a linear fashion).

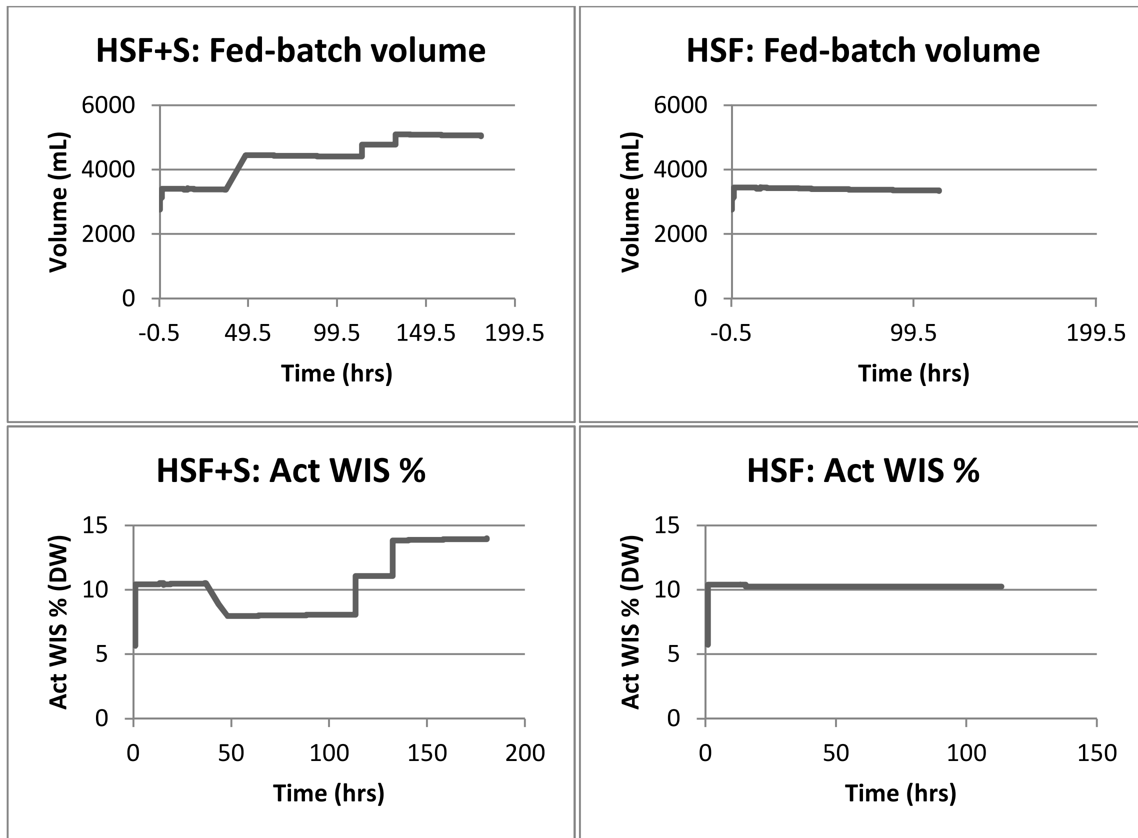

Figure 37: Fed-batch volume of HSF + sucrose (left) and HSF with no sucrose (right) fermentations.

Figure 38 shows the cell count for the HSF experiments. The experiment where sucrose was added is denoted as R1 for reactor 1 and the experiment without sucrose is denoted as R2 for reactor 2 in Figure 38.

For the HSF where no sucrose was added there was no cell growth and the experiment was ended. However the experiment with added sucrose showed significant cell growth after the sucrose was added. The start of the sucrose feed is indicated by the solid vertical line.

Additional sucrose caused cell growth that, in turn, caused the flux of inhibitors that had to be metabolised by each yeast cell to decrease, thus the larger yeast population was able detoxify the broth.

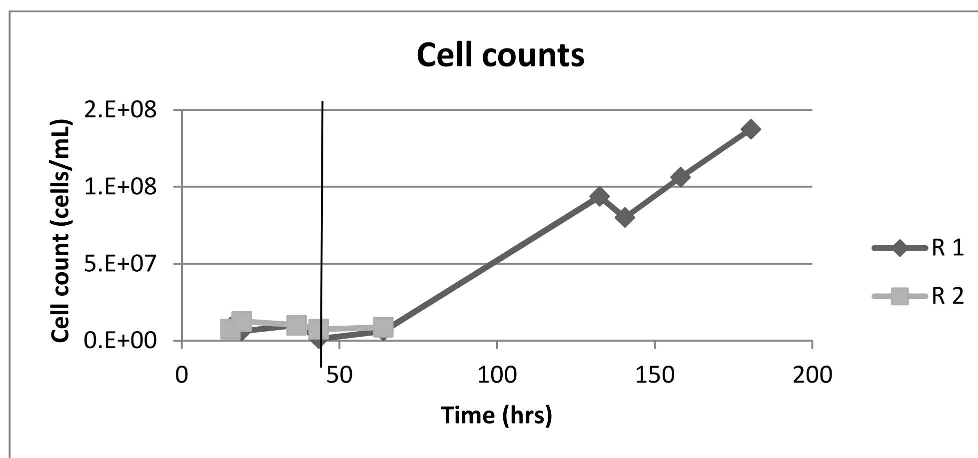

Figure 38: Cell counts for HSF fermentations.

Figure 39 shows the sugar and ethanol concentrations for the HSF experiments. For the HSF experiment with added sucrose the glucose concentration also stayed constant until the start of the sucrose feed, where all the sugar concentrations initially increased and then decreased as they got fermented by the yeast. Before the sucrose was added the yeast was unable to ferment the glucose that was released during the pre-saccharification step. However, after the sucrose was added the yeast became able to ferment the sugars. After the initial increase of the sucrose concentration the sucrose levels quickly dropped indicating that the yeast secreted sufficient invertase to hydrolyse the sucrose.

The control HSF experiment where no sucrose was added showed an almost constant sugar concentration after the initial pre-saccharification step. This indicates that the hydrolysis of the WIS

has completed. If there was feed-back inhibition of the enzymes then the glucose concentration would still have increased, albeit at a slower rate. This means that the pre-saccharification step is long enough for complete hydrolysis of 10 % WIS.

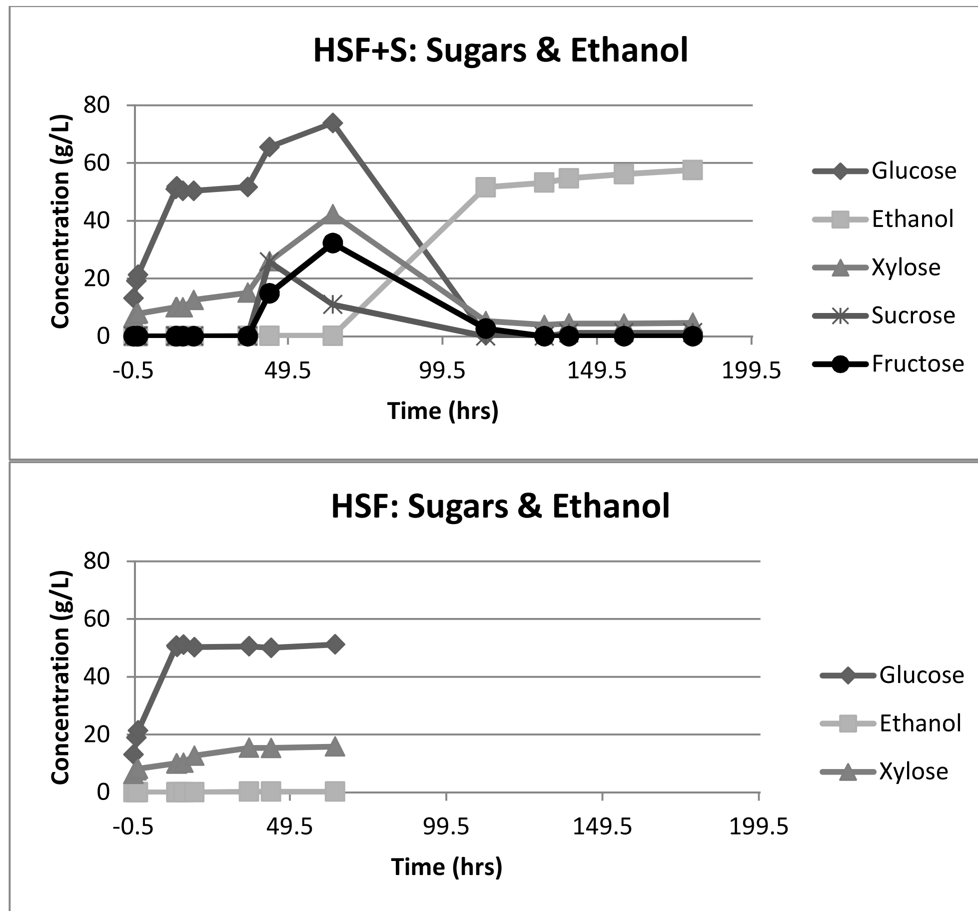

Figure 39: Sugar and ethanol concentrations for the HSF experiments.

The HSF experiment with no added sucrose did not produce any ethanol. However the experiment with added sucrose produced a high final ethanol concentration of 57.59 g/L. This value is much higher than the literature values for second generation fermentations 26.7 g/L and (7.5 WIS %) and 36.8 g/L (12 % WIS) (Rudolf et al., 2008; Öhgren et al., 2006). This value is almost as high as 64.6 g/L (30 WIS %) that was obtained by Zhang et al (2010), but this value was achieved at a much lower WIS %. Furthermore this high value was achieved with a yeast strain that has low inhibitor tolerance. This advantageous because a non-hardened yeast with higher a productivity can be used (In chapter 3 it was shown that the non-hardened MH 1000 had a higher productivity than TMB 3400) .All of this clearly shows the advantages of adding sucrose to a 2nd generation fermentation.

Figure 40 shows the ethanol and glycerol concentrations for the HSF experiments. For the HSF with added sucrose experiment ethanol production started only after the addition of sucrose. The sucrose and sugars released in the pre-saccharification step were quickly fermented and then the ethanol concentration was increased even by the fermentation of the glucose released from the WIS that was added after the sucrose addition (the SSF part of the experiment). The glycerol concentration reached a maximum of 2.7 g/L which is lower than the literature values of 4 g/L (Öhgren et al., 2006) and 4.9 g/L (Rudolf et al., 2008). The glycerol concentrations for this experiment are a little higher than the glycerol concentration reached by the fast feeding SSF experiments (below 2g/L). This is probably due to the higher level of ethanol production.

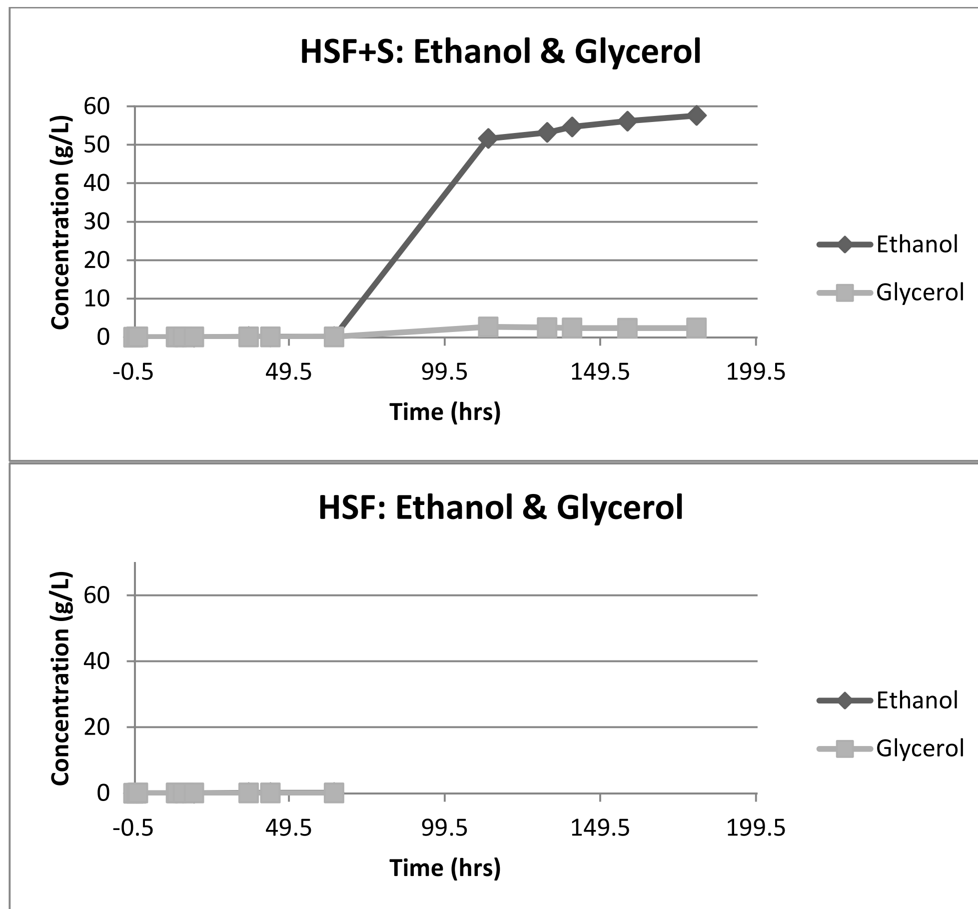

Figure 40: Ethanol and glycerol concentrations for the HSF experiments.

The HSF experiment with no additional sucrose showed no production of ethanol or glycerol.

Figure 41 shows the HMF and furfural concentrations for the HSF experiments. For the HSF experiment with added sucrose the inhibitors are metabolised by the yeast. The rate at which the inhibitors are metabolised increased after the addition of sucrose.

For the HSF experiment where no sucrose was added the HMF concentration stayed constant after the initial increase during the pre-saccharification step. The furfural concentration showed a slight decrease before increasing again.

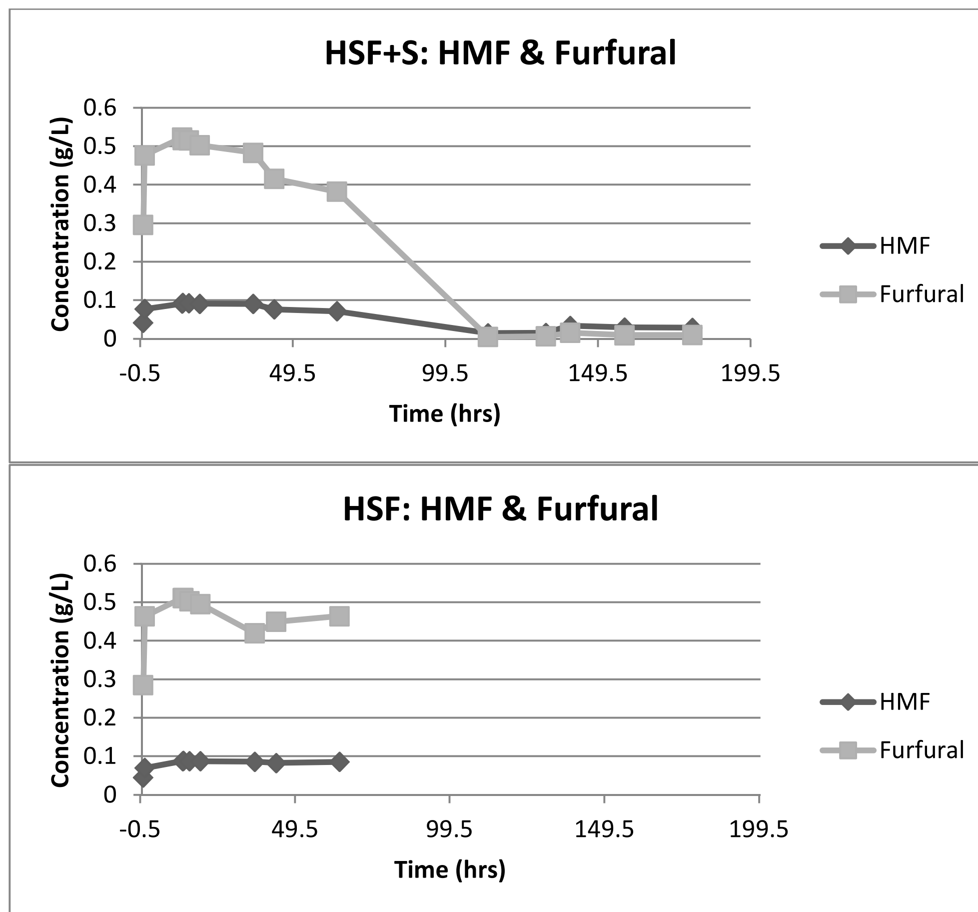

Figure 41: HMF and Furfural concentrations HSF experiments.

Figure 42 shows the formic and acetic acid concentrations for the HSF experiments. For the HSF experiment with added sucrose the acetic acid initially slightly increases after the pre-saccharification step and then the concentration decreases after the addition of sucrose. However the concentration increases again with the addition of the extra WIS during the fermentation. The concentration of formic acid shows the same pattern as that of acetic acid, but without the decrease that acetic has shown due to sucrose addition. This shows that acetic acid is metabolised easier than formic acid by the yeast.

For the HSF without additional sucrose both acid concentrations remain almost constant after the initial pre-saccharification step.

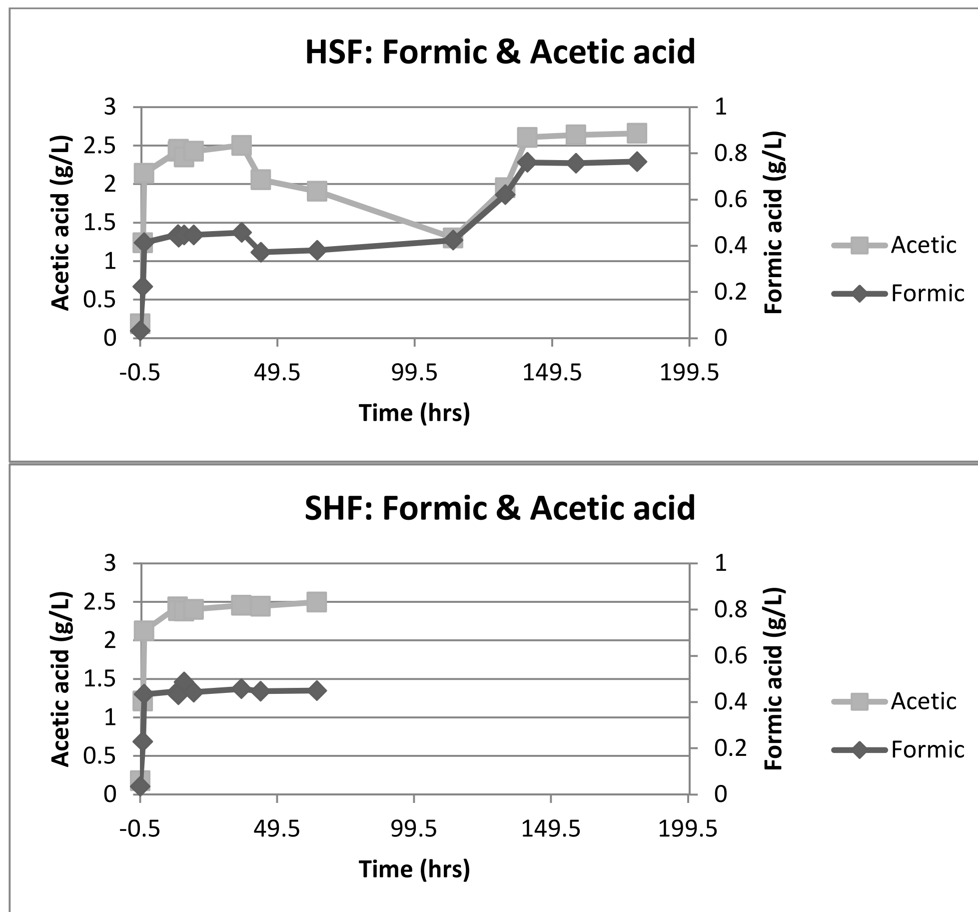

Figure 42: Acetic and formic acid concentrations for HSF (top) and SHF (bottom) fermentations.

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6.4 Conclusions and recommendations

Conclusions:

The addition of sucrose increased the ethanol concentration in the case of HSF with additional sucrose. This increase in ethanol concentration can be attributed to the dilution of inhibitors, increased sugar concentration and the decrease in the flux of inhibitors encountered per yeast cell due to the population growth caused by the sucrose addition. In other words, addition of sucrose provided extra vigour to the yeast's metabolism, enabling it to better perform in the presence of inhibitors.

The fermentation times can be shortened by the addition of sucrose as the inhibitors are diluted meaning that the WIS can be fed faster. In the previous chapter the bottle-neck was the yeast. By providing the yeast with enough sugar and diluting the inhibitors the yeast will be able to ferment the glucose as soon as it is released from the WIS by the enzyme.

Recommendations:

It is recommended that the yeast first be cultivated on the sugar syrup alone (pure 1st Gen) before the WIS and enzyme start to be added in an SSF manner. This will ensure a larger and stronger yeast population that will be able to metabolise the inhibitors. The feeding of the WIS can also be done faster as the stronger yeast population will be able to detoxify the inhibitors as they enter with the WIS.

In future when CBP yeasts are used to perform SSF then the productivity of these yeast strains can be improved by the addition of sucrose. The addition of sucrose will ensure a larger yeast population that will speed up the production of enzymes, the metabolism of inhibitors and the fermentation of sugars. All this will improve the productivity of the fermentation. The addition of sucrose will also help to dilute inhibitors as less WIS will have to be added to obtain the same ethanol concentration.

It is recommended that a method be sought to determine the liquid volume of the fermentation so that the amount of ethanol produced can be calculated from the ethanol concentration to be able to determine accurate fermentations yields. See a description of a proposed method in section 5.4.

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7. TECHNO-ECONOMIC ANALYSIS

7.1 INTRODUCTION

This chapter discusses the process modelling and economic analysis part of the project. Four scenarios were considered in this chapter:

1. A 1st generation process that used all the bagasse that was generated by the process for the co-generation of steam and electricity.

1a. A 1st generation process that sells all the excess bagasse. This was done to determine a bagasse feedstock price for the stand-alone second generation plant as per the method described by Dias et al (2011b)).

2. A 1st and second generation integrated process.
3. A stand-alone 2nd generation process.

In all four scenarios that were considered excess electricity was sold to the grid to provide additional revenue.

The objectives of this chapter are to:

- Determine the effect of process integration between 1st and 2nd generation biofuel production technologies on the energy efficiencies and the economic viability of such projects.
- Determine what financial incentives and market changes are necessary to make biofuels more economically viable and attractive to investors.

For this project a first generation bio-ethanol plant that utilises sugarcane as the feedstock was simulated using the process simulating software Aspen Plus®. This model was created from the most up to date parameters considering current technology from different literature sources (see section 2.8). The point of building a model from scratch was to obtain a model that is as up to date as possible and that can be used by other members of the research group in the future to create an integrated 1st and 2nd generation process model. Literature sources do not publish all the information and parameters that are required to replicate their process models, thus it is necessary to obtain the

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parameters necessary from many different sources. Another problem is that the available parameters aren't always up to date since the sources sometimes simulate older technology that is still in use today. For example some sources simulate typical ethanol plants in Brazil that were constructed a long time ago, but are used in literature as a reference (Macrelli et al., 2012). Thus it is necessary to use many sources to obtain all the parameters required for building a model.

First the 1st generation process was considered on its own. In this case the bagasse that was generated by the process was either used along with 50% of the available trash (Dias et al., 2009; Dias et al., 2010; Dias et al., 2011b) to generate steam and electricity in a co-generation and heating plant (CHP) or sold to a stand-alone second generation plant. Steam and electricity from the CHP was used to meet the steam and electricity demands of the plant. Excess electricity was generated by the CHP for sale to the grid for additional revenue.

Pinch point technology was applied to the 1st generation process model to determine the minimum utility usage so that most of the energy that was generated from the burning of bagasse and leaves could be used to generate extra electricity for additional revenue (Dias et al., 2009; Dias et al., 2011a; Modesto et al., 2009).

Data generated by the 1st generation Aspen Plus® model was used to perform an economic analysis for the 1st generation process, whilst literature data (see next paragraph) was used to perform economic analyses on the integrated 1st and 2nd generation and stand-alone 2nd generation processes. The method for performing the economic analysis was performed according to the method described by Turton et al (2009). The economic analysis was performed under South African economic conditions with capital costs from literature.

A detailed process simulation of the integrated 1st and 2nd generation facilities and stand-alone 2nd generation facilities was not included in this project due to time constraints. The data for the processes producing second generation ethanol for these scenarios were taken from literature. The process data for the stand-alone 2nd generation model was obtained from Seabra & Macedo (2011) and the process data for the integrated facility was obtained from Dias et al (2011b) and Macrelli et al (2012). The process data and economic assumptions that were used in this techno-economic analysis, as well as the reasons for using certain data and assumptions, can be seen in section 2.8.

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7.1.1 Chapter aims

Process modelling

- Build a 1st generation process model in Aspen Plus® that is as up to date as possible by using the most up to date parameters from different literature sources. This model may be used in future to simulate the integration between 1st and 2nd generation technologies by integrating this process model with a 2nd generation model.

Pinch heat integration

- Apply pinch technology to decrease utility usage and increase the surplus bagasse availability in the 1st generation scenarios, for the production of 2nd generation ethanol or the generation of electricity.

Economic analysis

- Determine the price at which sugarcane must be supplied to each process be able to make the processes viable.
- Compare the 1st generation process, modelled in the present study, to a stand-alone 2nd generation process and an integrated 1st and 2nd generation process, from literature, to determine the effect of integration from an economic point of view in the sub-Saharan African context.

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7.2 METHODOLOGY

Four different scenarios were investigated (see Table 20). The model that was used in this study was used to simulate two of the scenarios that consider first generation ethanol only. In scenario 1 all of the bagasse is burned and in scenario 1a the excess bagasse is sold to the second generation stand-alone plant (scenario 3). This latter scenario was considered to be able to compare the stand-alone 1st and 2nd generation scenarios to the integrated scenario 2.

Table 20: Different ethanol production scenarios considered in this study

Scenario	1G	2G	sell surplus bagasse
1[a]	x		
1a[a]	x		x
2[b]	x	x	
3[c]		x	

a: Process data obtained from the Aspen Plus model® developed in this study

b: Process data obtained from Dias et al (2011b) and Macrelli et al (2012)

c: Process data obtained from Seabra & Macedo (2011)

7.2.1 BUILDING THE 1ST GENERATION MODEL

Aspen Plus® software was used to simulate the 1st generation ethanol production process (Dias et al., 2011b; Macrelli et al., 2012; Seabra & Macedo, 2011; Leibbrandt, 2010; Petersen, 2011). The NREL in-house databank was used to simulate the properties of the biological compounds that are not available in Aspen Plus'® own databanks (Dias et al., 2011b; Macrelli et al., 2012; Seabra & Macedo, 2011; Leibbrandt, 2010; Petersen, 2011).

The first generation plant was simulated according to the description is section 2.2.

Figure 43 shows the Aspen Plus® simulation of the 1st generation plant. The plant has been divided into 8 areas using blocks. Each area is described in the following sub-sections:

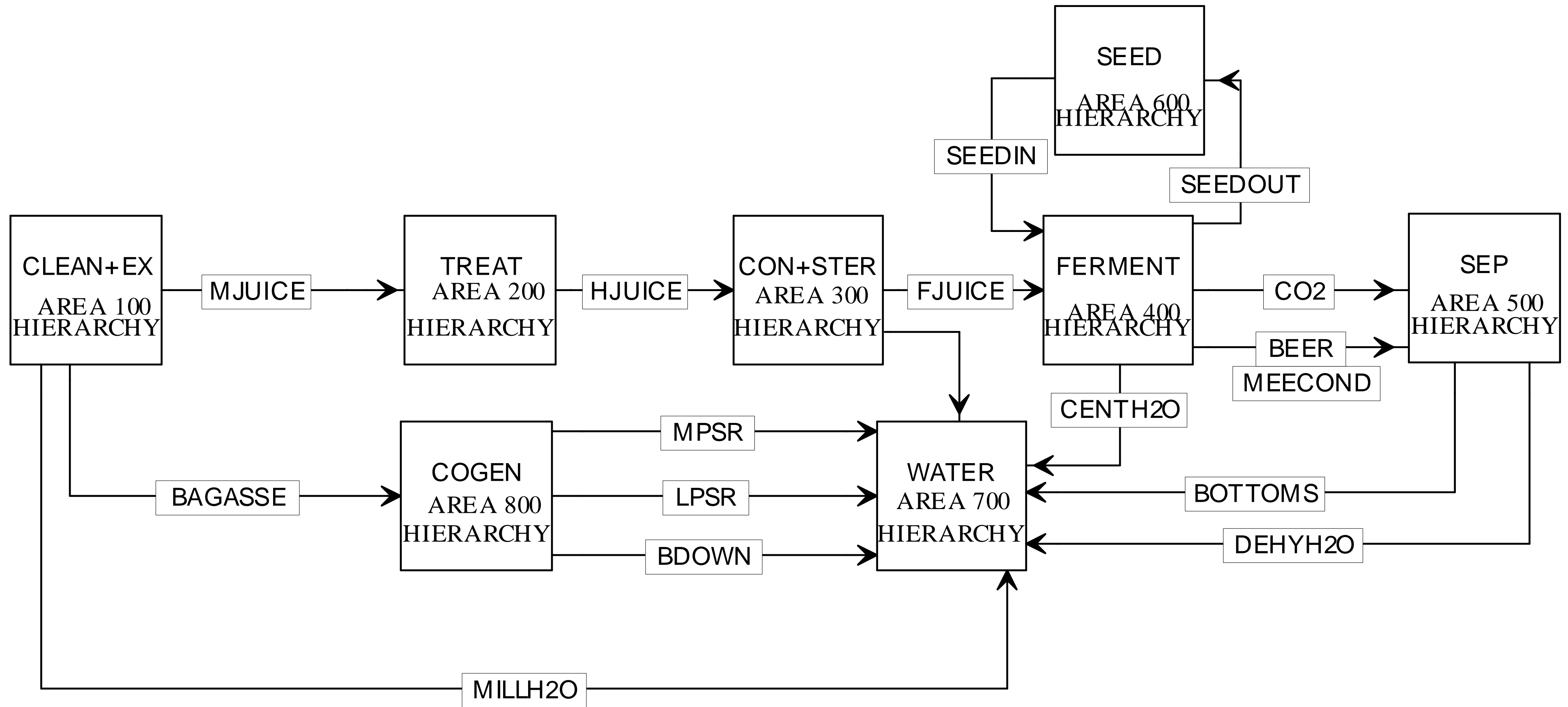

Figure 43: Aspen Plus® simulation of 1st generation ethanol plant.

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7.2.1.1 AREA 100: SUGARCANE RECEPTION, PREPARATION AND JUICE EXTRACTION

The cane flow rate is 493 tons of cane per hour (Dias et al, 2009; Dias et al, 2011a). This flow rate was selected since the model was based on that of Dias et al (2009) and it is a typical flow rate for a Brazilian distillery. The sugarcane composition taken from Dias et al (2009) can be seen in Appendix N: Data for first generation model First the cane is cleaned by a dry cleaning system that removes 70 % of the dirt associated with the cane (Dias et al., 2009; Dias et al., 2011a; Dias et al., 2011b). The cane is then prepared by shredders (hammers) and knives before it goes to the diffuser where 99 % of the sucrose is leached out (this is simulated as one separation unit) (Modesto et al., 2009; Pellegrini & de Oliveira Junior, 2011; Bosch Projects, n.d.). Imbibition water is added to the diffuser at a flow rate of 360.8 kg/tc (Modesto et al., 2009). The bagasse from the diffuser goes through two cane dewatering mills (modelled as one) to lower its moisture content to 50 % (Modesto et al., 2009; Leal, 2010) before it is sent to the boilers in the cogeneration area (Area 800).

7.2.1.2 AREA 200: JUICE TREATMENT AND CLARIFICATION

First the juice passes through screens and hydrocyclones (physical treatment). Phosphoric acid is then added to the juice. The juice is then heated up to 70 °C. After the juice has been heated up to 70 °C, lime is added and the juice is then heated up further to 105 °C. The flow rates of the lime and the phosphoric acid were calculated from Ensinas et al. (2009). The juice passes through a de-aerator before going into the clarifier. The clear juice from the clarifier overflow goes to the concentration and sterilisation section (area 300). The clarifier underflow or mud is filtered to recover sugars. The filtrate from this filter is recycled back to the point where the lime was added. The clarifier filter cleaning water flow rate of 31.46 kg/tc was obtained from Ensinas et al (2009). Process conditions for this area were based on Dias et al (2009), Leal (2010) and Ensinas et al (2009).

7.2.1.3 AREA 300: JUICE CONCENTRATION AND STERILISATION

The clear juice from the clarifier overflow is concentrated in a five effect multi effect evaporator (5 effect MEE). The vapour bleeds from the five effects are used as a heating source and is integrated

with the rest of the process using Pinch heat integration. The steam condensates that results from the vapour bleeds of the five effects are used as imbibition water to reduce the total water usage of the plant (Pellegrini & De Oliveira Junior, 2011; Ensinas et al., 2007; Ensinas et al., 2009).

The pressures and temperatures of the different effects of the MEE were obtained from Dias et al (2009) and can be seen in Appendix N: Data for first generation model. A bypass stream that is not concentrated by the MEE is combined with the concentrate from the MEE to obtain a stream with 22 % sucrose. This 22 % sucrose stream is sterilised by heating it up to 130 °C (Dias et al., 2009) and then cooling it down to fermentation temperature (32 °C) (Kitching, 2011). The 22 % sucrose stream had to be sterilised since the bypass stream does not pass through the MEE and it may carry contaminants. Process conditions for this area were based on Dias et al (2009), Dias et al (2011a), Dias et al (2011b), Macrelli et al (2012), Ensinas et al (2007), Dias et al (2009) and Pellegrini & de Oliveira Junior (2011).

7.2.1.4 Area 400: Fermentation

The 22 % syrup (Kitching, 2011) enters the fermentation vessel along with the recycled yeast inoculum (Dias et al., 2009; Dias et al., 2010; Dias et al., 2011a; Dias et al., 2011b; Ensinas et al., 2007; Ensinas et al., 2009; Macrelli et al., 2012; Pellegrini & de Oliveira Junior, 2011; Leal, 2010). All of the sucrose is converted to glucose and fructose (100 %) (see chapter 0). The ethanol yield on glucose and fructose is 90.5 % and 1.37 % of the glucose and fructose is converted to biomass (Dias et al, 2009). The yeast concentration in the fermentation was determined from the first generation fermentation experiments (see Appendix C: Biomass characterisation). The equations that were used to model the fermentation reactor can be seen in Appendix N: Data for first generation model.

After the fermentation the CO_2 is removed by the vent and sent to the CO_2 scrubber in the separation section to recover the evaporated ethanol (area 500) (Leibbrandt, 2010; Petersen, 2011). The fermented broth is then cooled to 24 °C (Dias et al, 2009). The yeast is removed from the broth by centrifugation by two consecutive centrifuges (Dias et al, 2009).

7.2.1.5 AREA 500: SEPARATION AND PURIFICATION

The CO_2 from the fermenter vent is passed through a small water scrubber to recover the evaporated ethanol in the vent gases. The scrubber has three equilibrium stages and it operates at atmospheric pressures and it recovers 99.9 % of the ethanol. The CO_2 gas goes to the stack. The water and absorbed ethanol stream was combined with the fermented broth coming from the fermenter. The data for the CO_2 scrubber was obtained from Leibbrandt (2010) and Petersen (2011).

The combined scrubber and broth stream is pre-heated to 72 °C (Leibbrandt, 2010; Petersen, 2011). The stream then enters the stripper column. The stripper column has 10 equilibrium stages with the feed stream coming in on stage 1 (Leibbrandt, 2010; Petersen, 2011). The stripper column produces a vapour that contains 55 wt% ethanol (Leibbrandt, 2010; Petersen, 2011). The stream from the bottom of the stripper column is known as vinasse and it can be used as a fertiliser. The ethanol rich vapour stream is fed to the rectification column. Many other authors have also used the similar atmospheric distillation methods (Dias et al., 2009; Dias et al., 2010; Dias et al., 2011a; Dias et al., 2011b; Modesto et al., 2009; Ensinas et al., 2007; Ensinas et al., 2009; Pellegrini & de Oliveira Junior, 2011)

The rectification column has 18 stages with the feed stream coming in on stage 12 (Leibbrandt, 2010; Petersen, 2011). The rectification column produces a 90.9 % pure ethanol vapour (Leibbrandt, 2010; Petersen, 2011). The vapour goes to the molecular sieves. The bottoms from the rectification column is mostly water and it is sent to the water treatment plant (area 700).

The molecular sieves (Ensinas et al., 2007; Ensinas et al., 2009; Dias et al., 2010; Dias et al., 2011b) produce anhydrous ethanol at a purity level of 99.3 %. The water that is separated from the 90.9 % hydrous ethanol is also sent to the water treatment plant (area 700).

7.2.1.6 AREA 600: YEAST PURGE AND RECYCLE

In this section some of the yeast is purged through a bleed stream. This done to prevent the build-up of yeast in the system since there is some growth taking place in the fermenter. The rest of the yeast is recycled back to the fermentation according to the Melle-Boinot fermentation process (Dias et al., 2009; Dias et al., 2010; Dias et al., 2011a; Dias et al., 2011b; Ensinas et al., 2007; Ensinas et al., 2009;

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Macrelli et al., 2012; Pellegrini & de Oliveira Junior, 2011; Leal, 2010). This section was not simulated in detail.

7.2.1.7 AREA 700: WATER TREATMENT PLANT

This section was not simulated in very much detail (Petersen, 2011). It was included to obtain an idea of the flows of waste water. The actual treatment of the water was not simulated. All the water and steam condensate from the system is cooled and recycled.

All the steam that was generated by the boiler is condensed, pressurised and recycled back to the boiler.

7.2.1.8 CO-GENERATION OF HEAT AND ELECTRICITY

The bagasse and the trash enter the combustion chamber of the boiler along with 110 % stoichiometric air (Leibbrandt, 2010; Petersen, 2011). The amount of trash that is included for the co-generation of power and heat is calculated by using the assumption that 50 % (Dias et al., 2009; Dias et al., 2010; Dias et al., 2011b) of the available trash is collected and burnt along with the bagasse. Trash is available for used in the CHP at 140 kg/tc (dry basis) and the moisture content of the trash is 15 % (Dias et al., 2009; Dias et al., 2010; Dias et al., 2011b; Seabra & Macedo, 2011). The heat from combustion is used to heat the boiler that operates at a pressure of 90 bar (Dias et al., 2009; Dias et al., 2010; Dias et al., 2011b; Ensinas et al., 2009) and an 85 % thermal efficiency (Dias et al., 2009; Dias et al., 2011a; Ensinas et al., 2007; Ensinas et al., 2009). The air that enters the combustion chamber is preheated with waste heat from the combustion chamber stack gases (Leibbrandt, 2010; Petersen, 2011).

The 90 bar steam that is generated by the boiler is sent to the first condensing/extraction steam turbine (CEST1). In this turbine the steam pressure is reduced to 21 bar (Dias et al., 2009). The mechanical energy from the turbine is used to generate electricity with a generator that is 98 % efficient (Dias et al., 2009; Dias et al., 2011a; Dias et al., 2011b). The electricity requirement of the plant is taken from this electricity stream. The electricity demand of the plant is determined to be 22 kWh/tc, because most sources (Dias et al., 2009; Dias et al., 2010; Dias et al., 2011a; Ensinas et al.,

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2007; Ensinas et al., 2009; Seabra & Macedo, 2011) cite that a plant using a mill requires 28 kWh/tc and it is also known that a diffuser uses 6 kWh/tc less energy than a mill (Bosch Projects, n.d.). The remaining electricity is exported to the grid for additional revenue.

The steam at 21 bar goes through the second turbine (CEST2) (Dias et al., 2009). In this turbine the steam pressure is reduced to 6 bar (Dias et al., 2009). Steam is extracted at 6 bar to provide energy to the molecular sieves (Dias et al, 2009). The remaining 6 bar steam is used to generate more electricity, but since all the electricity needs of the plant have been met by the previous turbine (CEST1) all the electricity from this turbine can be exported to the grid. The same amount of steam passes through both CEST1 and CEST2 but they are modelled as two different turbines due to different isentropic efficiencies for each (see Appendix N: Data for first generation model).

The flow rate of 6 bar steam required for the molecular sieves was 0.6 kg per litre anhydrous ethanol produced (Dias et al., 2011b; Ensinas et al., 2009). The rest of the 6 bar is steam is sent to CEST3 to generate more electricity for sale. The steam exits CEST3 at 2.5 bar.

The 2.5 bar steam is used for plant utilities such as heating the imbibition water for the diffuser (Modesto et al., 2009), supplying heat to the MEE and to supply heat to the rest of the heating utilities. The 2.5 bar steam that is not used for the above mentioned utilities is expanded in a final turbine (CEST4) to generate more electricity for sale (Dias et al., 2010; Dias et al., 2011a; Dias et al., 2011b; Modesto et al., 2009; Macrelli et al., 2012; Ensinas et al., 2007; Ensinas et al., 2009; Pellegrini & de Oliveira Junior, 2011; Seabra & Macedo, 2011). The diffuser requires steam at a flow rate of 68 kg/tc at a pressure of 2.5 bar (Modesto et al., 2009). The steam required for the utilities was determined from the PINCH analysis that was performed on the first generation model (see section 7.2.3).

All the steam condensates are sent to the water treatment plant (area 700) for recycling as boiler feed water.

The isentropic efficiencies of the turbines and other parameters for the modelling of the co-generation system was obtained from Dias et al (2009) and can be found in Appendix N: Data for first generation model. It should be noted that the generators all have the same mechanical energy efficiency, but the turbines have different isentropic energy efficiencies (see Appendix N: Data for first generation model)

7.2.2 PROCESS ENERGY EFFICIENCIES

The energy efficiencies of different scenarios were determined according to the definitions for energy efficiencies given by Leibbrandt (2010). The first energy efficiency is the liquid fuel efficiency and the second is the overall energy efficiency and they are presented in Equation 11 and Equation 12, respectively. The liquid fuel efficiency is to determine how effective each scenario is to produce ethanol. The overall efficiency combines energy products in the form of ethanol and electricity to provide an indication of the overall effectiveness of the whole process.

$$\eta_{liquid\ fuel} = \frac{E_{th}fuel}{E_{th}biomass - (E_{Elec}/\eta_{Elec})}$$

Equation 11

$$\eta_{overall} = \frac{E_{th}fuel + E_{Elec}}{E_{th}biomass}$$

Equation 12

In the above equations $E_{th}fuel$ is the thermal energy of the ethanol produced, $E_{th}biomass$ is the thermal energy of the biomass that goes into the process, E_{Elec} is the thermal energy of the electricity and $\eta_{Elec}$is the efficiency at which electricity is generated from the biomass. A value of 35 % efficiency is assumed for the electricity generation since this is a typical value for the efficiency of a Rankine steam cycle for electricity generation (Çengel & Boles, 2006).

7.2.3 PINCH HEAT INTEGRATION APPLIED TO 1ST GENERATION PROCESS MODEL

7.2.2.1 PINCH ALGORITHM

The general algorithm that was used to perform the Pinch analysis was obtained from Turton et al (2009) and a short version of this algorithm is presented below. This algorithm provides a minimum utilities and minimum number of heat exchangers (MUMNE) solution to a heat integration problem. The algorithm consists of the following five steps:

1. Decide what the minimum approach temperature (ΔT_{min}) should be.

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This is the minimum temperature that two streams leaving or entering a heat exchanger can have. Typical values range from 5 to 20 °C. As the minimum approach temperature decreases the capital cost increases while the utility requirements decrease. In Appendix Q: HENSAD printouts there is a graph that shows the hot and cold utilities and the heat exchanger area as a function of ΔT_{min}. That graph shows that the heat exchanger area tends to become very large as ΔT_{min} becomes very small. The value chosen for this project is 10 °C since this is the same value used by Dias et al (2009).

2. Draw a temperature interval diagram.

In this diagram all the streams are represented by vertical lines. Streams that need to be cooled (hot streams) are on the left-hand side while streams that need heating up (cold streams) are on the right. The direction of temperature change is indicated by an arrow head at the end of the vertical lines. The left- and right-hand axes of the diagram are shifted by the value of ΔT_{min}. The left-hand axis for the hot stream temperatures is shifted down by the value of ΔT_{min}. The diagram is divided into intervals by drawing horizontal lines through the ends of all the lines. The net energy available for all the streams in an interval is shown in the right-hand column. An excess of energy is shown by a (+) and an energy shortage in an interval is denoted by a (-). Since the axes have been shifted all the energy from the hot streams in an interval is available to the cold streams without violating the second law of thermodynamics.

3. Draw a cascade diagram to determine the pinch temperatures and the minimum utility requirements.

A cascade diagram shows the net amount of energy available in each temperature interval. If there is excess energy in an interval this energy can be cascaded down to the next energy interval since energy can always be transferred to lower temperatures. The excess energy is continuously cascaded down until a point is reached when no more energy is available to be cascaded down. At this point energy will most likely be needed to be transferred to the process from the hot utility. Below this point the cascading down of excess energy is continued until a point where the excess energy must be rejected to the cold utility. The point where no more energy can be cascaded downwards and energy must be supplied from the hot utility is known as the pinch temperature.

4. Calculate the minimum number of heat exchangers above and below the pinch.

From this point onwards the heat transfer problem must be split into two parts: above the pinch and below the pinch. The minimum number of heat exchangers above and below the pinch can be calculated suing Equation 13:

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$$Min\ \#\ HE's = \#\ hot\ streams + \#\ cold\ streams + \#\ of\ utilities - 1$$

Equation 13

5. Design the HEN

Design above the pinch:

A temperature interval diagram is drawn for all the streams above the pinch. Hot streams and cold streams are matched at the pinch and then they are matched moving away from the pinch. Matching two streams means that those streams will exchange heat in a heat exchanger. At the pinch the streams are matched using the criterion presented in Equation 14 below. The criterion in Equation 14 ensures that ΔT_{min} is not violated.

$$\dot{m}_{hot} c_{P,hot} \leq \dot{m}_{cold} c_{P,cold}$$

Equation 14

The above mentioned criterion is only valid for streams at the pinch. At temperatures away from the pinch the remaining streams are matched while ensuring that neither ΔT_{min} nor the second law of thermodynamics is violated.

Design below the pinch:

Again a temperature interval diagram is drawn for the streams below the pinch. At the pinch the streams are matched using the criterion presented below in Equation 15. The criterion in Equation 15 ensures that ΔT_{min} is not violated.

$$\dot{m}_{hot} c_{P,hot} \geq \dot{m}_{cold} c_{P,cold}$$

Equation 15

The above mentioned criterion is only valid for streams at the pinch. Away from the pinch the remaining streams are matched while ensuring that neither ΔT_{min} nor the second law of thermodynamics is violated.

Note: This last step of the algorithm is included here for the sake of completeness, but this step was not performed in this study since the minimum utility requirements were already determined by the previous step.

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7.2.2.2 Composite temperature enthalpy diagram

This diagram is an aid to graphically represent the system on which the pinch heat integration is to be performed. This diagram is created by respectively plotting the sum of the enthalpies of all the hot and cold streams as a function of temperature.

The point where the vertical distance between the two curves is at a minimum is the pinch point. This graph also graphically portrays the minimum utility requirements. The distance between the ends of the curves on the left-hand side represents the minimum cold utility requirements while the distance between the ends of the curves on the right-hand side represents the minimum hot utility requirements. The distance between the start of the cold curve and the end of the hot curve is the maximum amount of energy that can be saved by heat integration.

7.2.4 Economic Analysis

An engineering economic analysis was performed according to the methods of by Turton et al (2009) as described in section **Error! Reference source not found.**.

The sugarcane price in South Africa is determined by the South African Sugar Association (SASA). It was found that this sugarcane price is too high to make the process economically viable. The sugarcane price in South Africa was R 331.55 per tonne of cane (tc) in 2011 (SASA, 2012). It was then decided to determine the price at which sugarcane must be supplied to achieve different internal rates of return (IRR). Thus the IRR was varied and the sugarcane price was determined for the different IRR's.

The idea of determining the sugarcane supply price stems from the work of (Watson, 2011) that states that there is 6 million hectares of suitable land available in sub-Saharan Africa for the production of sugarcane for biofuels. Furthermore, the production of sugarcane specifically for ethanol production, with no co-production of sugar, would not be subjected to the prices set by SASA mediated agreements. Finally, sugarcane production for ethanol production will have different optimisation targets, such as maximum ethanol production, rather than maximum sucrose content, which may result in significant variations in cultivation/harvesting of sugarcane. Thus a different price regime may be expected for new first generation ethanol plants built in South Africa, outside of the SASA-determined sugarcane prices for sugar production.

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The determination of the inputs for the different parameters of the economic models and the list of assumptions that were used are shown in the next two subsections.

7.2.4.1 Economic model inputs

The capital costs were determined from literature values that were adapted to the appropriate scale, currency and year (inflation) by using certain assumptions (see section 7.2.4.2). The capital cost for scenario 1 was adapted from Dias et al (2011b) and it amounted to 1881.786 million R for a plant that processes 493 tc/hr. The capital cost for scenario 1a was determined by scaling down the cogeneration facilities of scenario 1 using the six tenths rule since the surplus bagasse (57.5 % or 68.034 tb/hr) was sold to scenario 3. The capital costs for co-generation facilities were obtained from Dias et al (2011b). This resulted in a capital cost of 1674.694 million R. The capital cost for scenario 2 was taken from Macrelli et al (2012) and it amounted to 2455.836 million R for a plant that processes 493 tc/hr. The extra capital cost of scenario 2 is due to the facilities that are required for the production of second generation ethanol. The capital cost for scenario 3 was obtained from Seabra & Macedo 1140.581 million R for a plant that processes 68.034 tb/hr.

The minimum ethanol selling price was determined according to the South African Industrial Biofuels Strategy that was published by the South African Department of Energy and Minerals (DME, 2007; Petersen, 2011). The determination of the MESP can be seen in Equation 16 below. In Equation 16 BFP is the basic fuel price and Tax is the tax exemption that applies to bioethanol. GGE is the gas gallon equivalent and it represents the gallons of a certain fuel that has the same energy content as one gallon of gasoline. The GGE is 1.5 for ethanol. In October 2012 the basic fuel price was 708.590 c/L and the applicable tax exemption was 197.5 c/L (www.dme.gov.za).

$$MESP = BFP/GGE + Tax$$

Equation 16

Sensitivity analyses were performed by varying key parameters by 25 and 50 % above and below their base values to determine the effect that these parameters have on the minimum ethanol selling price (MESP). The MESP for the base cases of the sensitivity analysis was determined according to Equation 16.

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The hourly operator wage is R52/hr for a plant operator with two years' experience (www.mywage.co.za). This experience level was chosen as it is the average that was given by www.mywage.co.za.

The base cost for enzymes were taken from Petersen (2011) to be 0.17 US$ per gallon of ethanol produced (2007 US$).

The effect of the enzyme price on ethanol produced by an integrated first and second generation plant and a stand-alone second generation plant was also investigated. The cost of enzymes was varied according to a wide range of enzyme prices (US$ 0.10/gal EtOH to US$ 1.5/gal EtOH) that have been published in literature (Klein-Marcuschamer et al., 2012).

The economic analysis was performed using South African economic parameters to represent the African context.

An exchange rate of R 8.25/US$ was used to convert costs to 2012 South African Rand (ZAR 2012). This exchange rate is based on an average that was calculated during July and August 2012.

Electricity prices were obtained from Co-generation feed in tariff (COFIT) at R1.835/kWh for electricity generated from sugarcane bagasse (NERSA, 2011).

7.2.4.2 LIST OF ASSUMPTIONS:

- Capital costs used in this analysis were based on capital costs from Dias et al (2011b) for the first generation scenarios, Macrelli et al (2012) for the integrated scenario and Seabra & Macedo (2011) for the second generation plant.
- The Chemical Engineering Plant Cost Index (CEPCI) was used to account for inflation of capital costs.
- Capital costs from literature scaled to capacity using the six-tenths rule.
- 2 year plant construction period (60 % of capital cost in 1st year and 40 % in the 2nd year).
- Assumed plant operating life of 20 years (see Table 12).
- Linear depreciation over 10 years (see Table 12).
- Tax rate of 35%.
- The plant operates for 200 days of the year during the cane crushing season (reference).
- The working capital is 10 % of the fixed capital.

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- Green fields project.

All the assumptions were made conservatively according to the engineering economic analysis method presented by Turton et al (2009) except for the plant life (see Table 12).

7.3 Results and Discussion

7.3.1 Process yields and energy efficiencies

Table 21 below shows the comparison of ethanol produced, surplus electricity produced and process energy efficiencies for all the scenarios that were considered in this chapter. Dias et al (2011b) used the assumption that including heat integration will reduce the total steam usage by 20 %. Seabra and Macedo (2011) did not use heat integration in their stand-alone second generation plant. It was determined that a 43 % reduction in steam usage resulted from applying Pinch heat integration to the 1st generation process model developed in this study (see section 7.3.2). In both the afore-mentioned literature processes and in the 1st generation process model developed in here, trash is included as a fuel for the co-generation of heat and power.

Dias et al (2011b) included 50 % of the available trash (same as this study) whereas Seabra & Macedo (2011) only included 40 % of the available trash. The amount of available trash was 140 kg/tc for the two literature sources cited here and it is also the value that was adopted in this study. The fact that Seabra and Macedo (2011) only includes 40 % of the available trash doesn't affect the results of the second generation stand-alone plant (which is the only scenario from this source that was used in this study), because the inclusion of trash only increases the bagasse surplus (69.6 % for this source) that goes to the second generation plant. The bagasse surplus for the first generation model in this study was 57.5 % or 68.034 tb/hr. The difference in the excess amounts of bagasse between this study and Seabra & Macedo (2011) is due to the assumption by Macedo that more bagasse is produced per tonne of cane being crushed than in this study (260 kg/tc versus 240 kg/tc).

It can be seen from Table 21 that scenario 1 and 1a both produces 85.54 litres of anhydrous ethanol per tonne of cane (L EtOH/tc). This compares well to literature values that range from 82 L/tc (Dias et al., 2011b) and 91 L/tc (Seabra & Macedo, 2011) for 1st generation ethanol. Macrelli et al (2012) produced 85.2 L/tc 1st generation which is very close to the value obtained in this study. The slightly

higher ethanol yield in this study is due to the higher extraction efficiency of the diffuser (99%) compared to that of the mill (max 97 %) (Modesto et al., 2009). Scenarios 1 and 1a produce 70.2 and 40.7 kWh/tc of excess electricity, respectively. These values fall well within the large range of electricity production (24.3 kWh/tc to 154.9 kWh/tc) found in literature for 1st generation processes using Rankine steam cycles for power generation (Dias et al., 2010; Modesto et al., 2009). Scenario 2 produces 128 L EtOH/tc and 50 kWh/tc of excess electricity (Macrelli et al., 2012). Scenario 3 produces 185 litres of anhydrous ethanol per tonne of bagasse at 50% moisture (L/tb) or 25.53 L/tc and 103.6 kWh of excess electricity per tonne of bagasse (kWh/tb) or 14.3 kWh/tc.

Scenario 1 produces less ethanol than scenario 2, because scenario 2 utilises the excess bagasse for the production of ethanol and not for the generation of electricity. Scenario 1a produces less ethanol and less electricity than scenario 2 because the excess bagasse is sold to scenario 3. In scenario 2 the lignin and the xylan, from the excess bagasse that is used for 2G ethanol, is used to generate extra electricity by using these streams to produce biogas (Macrelli et al., 2012). If the ethanol and electricity production from scenarios 1a and 3 are added together to compare stand-alone plants to an integrated process, the integrated process produces more ethanol but less electricity than the stand-alone processes (111.7 L EtOH/tc versus 129 L EtOH/tc and 55 kWh/tc versus 50 kWh/tc).

The integrated plant produces little electricity due to the assumption that heat integration will only reduce steam consumption by 20 % (Dias et al., 2011b), whereas it was found in this study that by using Pinch heat integration the steam demand can be reduced by almost 45 % for the 1st generation scenarios (see section 7.4.2).

There is very little difference between the liquid fuel efficiency and the overall process efficiency for all the scenarios except for scenario 3, where the liquid fuel efficiency is 4.1 % higher than the overall efficiency (see Table 21). This is because more ethanol is created in this case which is a more energy efficient process than steam cycle-based electricity production (Macrelli et al., 2012). The first generation plant that burns all the bagasse has the lowest efficiencies (η_{liq} = 40.20 % and η = 39.51 %). This is because more electricity is produced in the first generation process and the efficiency of electricity generation is low (35 %) (Çengel & Boles, 2006) and this decreases the process energy efficiencies. Scenario 1a has much higher energy efficiencies than scenario 1 (η_{liq} = 47.56 % and η = 46.37 %) because the bagasse is sold and not used to produce electricity at a low efficiency. The energy efficiencies of scenario 2 (η_{liq} = 57.18 % and η = 55.09 %) is between that of scenario 1a and scenario 3 and much higher than the efficiencies of scenario 1. This shows that the production of ethanol from bagasse is much more energy efficient than the generation of electricity with a steam based cycle. Scenario 3 is the most efficient process (η_{liq} = 63.92 % and η = 59.82 %) showing again

that the production of 2G ethanol is more efficient than electricity generation and also more energy efficient than 1G ethanol (compare scenario 3 to scenario 1a).

Table 21: Comparison of ethanol- and electricity production and process efficiencies between different processes

Scenario	L EtOH/tc (L EtOH/tb)	Elec kWh/tc (kWh/tb)	Process efficiencies (%)	
			liquid fuel	overall
1	85.54	70.2	40.20	39.51
1a	85.54	40.7	47.56	46.37
2	102	50	57.18	55.09
3	25.53 (185)	14.30 (103.6)	63.92	59.82

All the stream tables for the first generation model can be found in Appendix O: Aspen Plus® stream results. All the unit operation summaries can be found in Appendix P: Aspen Plus® unit operation summaries

7.3.2 PINCH HEAT INTEGRATION

Pinch heat integration was performed on the 1st generation scenarios. The objective of this section was to minimise utility usage to increase the amount of surplus bagasse available for the generation of excess electricity that can be sold to the grid for additional income. The streams were identified for heat integration according to Dias et al (2009). Dias et al (2009) suggests that only streams with a heat flow of larger than 1000 kW must be used in the pinch analysis. Table 22 shows the hot streams that were identified for heat integration. However there may be streams that have high heat flows (more than 1000 kW) but the energy is of low quality (low temperature) that have been excluded from the analysis.

Table 22: Hot streams identified for pinch heat integration.

Hot streams	Data from Aspen Plus®						
Stream Name	Str # in	Str # out	Block code	flow (kg/hr)	Supply T (°C)	Target T (°C)	Heat Duty (kW)
1. 2nd eff cond	309	312	E301	3281.35	108.8	108.34	-2048
2. 3rd eff cond	315	318	E302	3103.08	100.57	99.91	-1955
3. 4th eff cond	321	324	E303	2997.38	83.73	82.73	-1923
4. 5th eff vapour	326	327	E308	28371.59	62.68	60.2	-18650
5. Sterilised juice	329	330	E310	284180.75	130	32	-27277
6. Fermented Wine	404	406	E401	258220.68	32	24	-2333
7. Stripper cond	507	508	T502	453557.60	105.9	102.1	-19888
8. Vinasse	509	510	E502	273674.73	115	35	-25255
9. Rectifier bottoms	512	514	E506	23726.83	114.3	35	-2194
10. Rectifier condenser	508	511	T503	133474.20	86.6	85.97	-25648
11. Anhydr EtOH cool	515	517	E505	33272.48	116	35	-9771

Table 23 show the cold streams that were identified for heat integration.

Table 23: Cold streams identified for pinch heat integration.

Cold streams	Data from Aspen Plus®						
Stream Name	Str # in	Str # out	Block code	flow (kg/hr)	Supply T (°C)	Target T (°C)	Heat Duty (kW)
12. Mixed juice	205	206	E201	422093.03	30	70	17414
13. Limed juice	209	210	E202	440571.50	70	105	16059
14. Sterilisation	328	329	E309	284180.75	93.72	130	10223
15. Stripper feed	506	507	E501	333769.43	25	72	17744
16. Stripper reboiler	507	509	T502	365827.17	114.76	115.07	57531
17. Rectifier reboiler	508	512	T503	42110.88	133.78	144.31	11487

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The data in Table 22 and Table 23 was entered into the HENSAD software to design the HEN. The minimum approach temperature was selected to be 10 °C. The pinch summary report that was generated by the HENSAD Elsevier software can be seen Appendix Q: HENSAD printouts.

The summary report in Appendix Q: HENSAD printouts shows the data for the temperature interval diagram. The temperature interval diagram required 27 temperature intervals (see Figure 44 for the cascade diagram where the temperature intervals are indicated by the lettered boxes).

It was calculated that a total of 136941.4 kW of energy was available from the hot streams and that -130459.3 kW was required by the cold streams. The hot pinch temperature was 116 °C which means that the cold pinch temperature was 10 °C lower at 106 °C. The minimum hot utility requirement was 71885 kW (44.90 % reduction) and the minimum cold utility requirement was -78367 kW (42.77 % reduction). These values represent the utility requirements after the process has been thermally integrated using the pinch method. It can be seen that these utility values are significantly lower than the utility values that would be required if no heat integration is performed.

The minimum number of heat exchangers required above and below the pinch is 4 and 14, respectively, to ensure that the minimum utility targets are reached.

Figure 44 shows the cascade diagram that was generated by the HENSAD software. It can be seen that the pinch point occurs between the temperature intervals G and H. The values that are cascaded between interval and the utility requirements can also be seen from this figure.

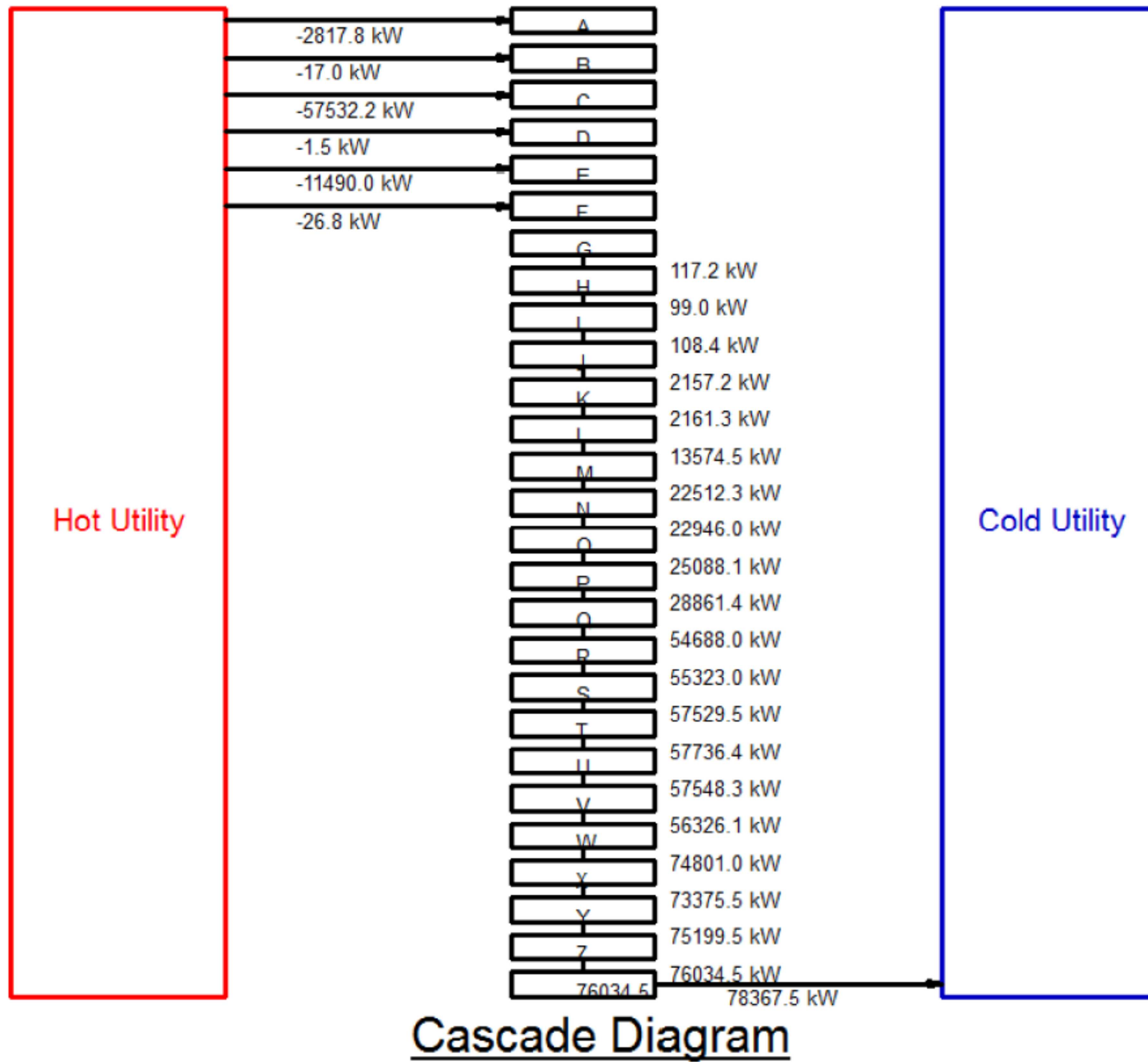

Figure 44: Cascade diagram generated by HENSAD software for 1st generation scenarios

Figure 45 shows the composite temperature enthalpy diagram that was generated by the HENSAD software. It can be seen that the minimum vertical distance between the hot and cold curves are around approximately 115 °C. This corresponds well to the calculated values for the pinch temperatures. It can also be seen from the figure that the hot (QH) and cold (QC) utility requirements are roughly equal.

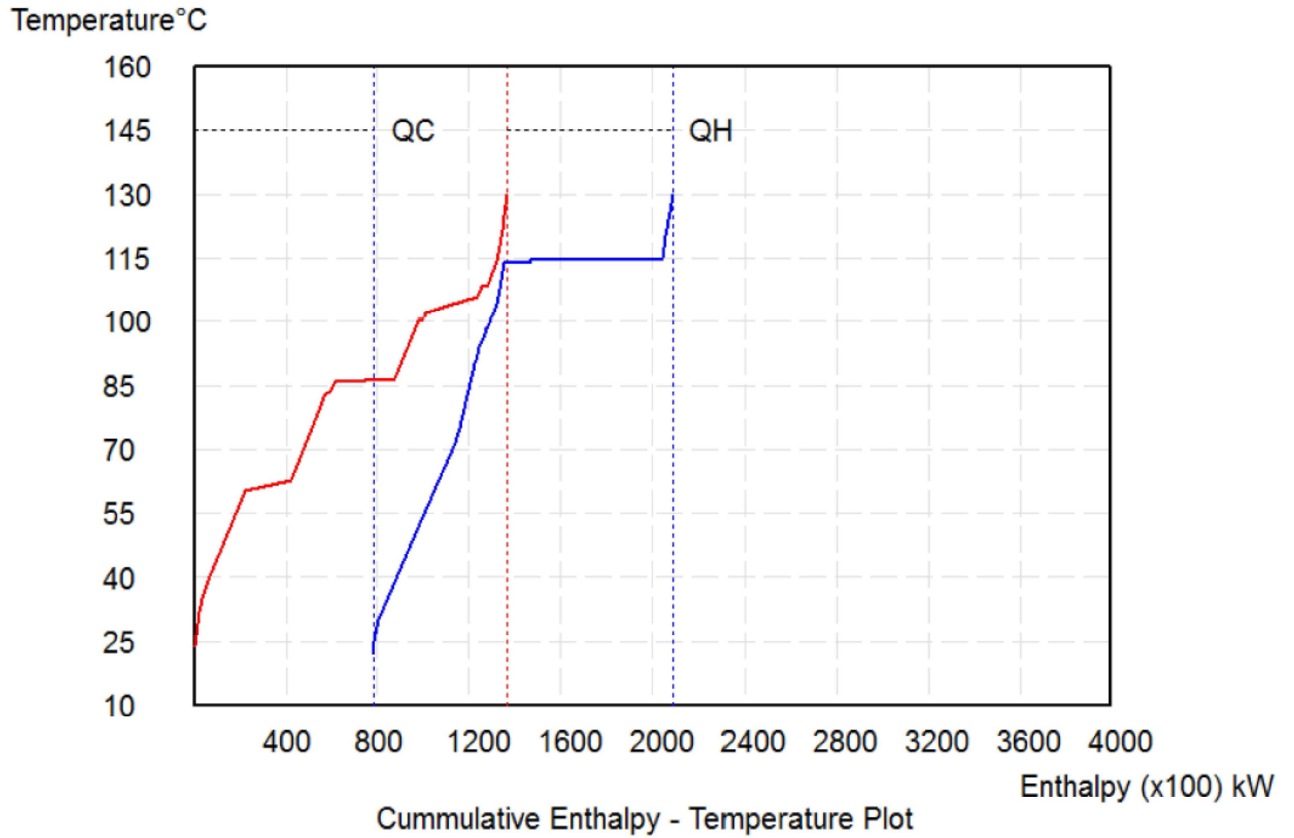

Figure 45: Composite temperature-enthalpy diagram for 1st generation scenarios

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7.3.3 Economic analysis

In Scenario 1a the surplus bagasse was sold to the stand-alone 2nd generation plant (scenario 3). The bagasse price was determined to be R 188.697/tb (50% moisture), because this price provides the same IRR as that of scenario 1 where all the bagasse is burned. This price is directly related to the electricity price because the income from selling the bagasse replaces the income that would have been obtained if the surplus bagasse was burned to generate surplus electricity for sale. This same method of determining a price for bagasse was used by Dias et al (2011b). The purpose of doing this is to be able to compare the stand-alone 1st and 2nd generation plants to the integrated plant to be able to ascertain the effects of integration.

Figure 46 shows the effect that different IRR's have on the sugarcane supply price. The IRR is varied from 15 % to 30 % for scenarios 1, 1a and 2 (scenario 3 is not considered here since it does not use sugarcane as a feedstock). The sugarcane supply price that can be absorbed becomes lower with an increasing IRR. The ranges of sugarcane supply prices that can be absorbed are R 213.15/tc to R 21.03/tc, R 226.18/tc to R 55.20 and R 281.81/tc to R 31.08/tc for scenarios 1, 1a and 2, respectively. The integrated scenario 2 (see Table 20) can absorb a higher sugarcane price than the first generation scenarios 1 and 1a. At an IRR of 15 % scenario 1 and 1a can only absorb sugarcane prices of R 213.15/tc and R 226.18/tc, respectively. Scenario 1a can absorb a higher sugarcane price than scenario 1 due to the lower capital cost because of the smaller cogeneration facilities of scenario 1a. Scenario 2 can absorb a sugarcane price of R 281.81/tc. The reason for this difference is because the integrated plant generates much more revenue than the first generation plants from ethanol sales and it still creates similar revenue from electricity when compared to the 1st generation scenarios (see Table 21). This is reflected in the energy balance where the overall energy efficiency for scenario 2 is 55.09% which is higher than the 39.51% for scenario 1 and the 46.37% for scenario 2. Scenario 2 can absorb a higher sugarcane price even though scenario 2 has a higher capital cost than scenarios 1 and 1a. The sugarcane supply prices that were determined here are still lower than the current sugarcane price given by SASA of 331.55 R /tc. However, it must be noted that this price is determined by the sugar market (SASA, 2012) and in future a separate market for energy cane may determine the price of sugarcane used for the production of ethanol.

The prices determined here for the sugarcane are below the given price of sugarcane even at the low IRR of 15 % for all the scenarios. The IRR must be at least about 30 % for a high risk project that uses new technology such as second generation ethanol, but the inclusion of the mature first generation ethanol production technology in the same plant will serve to lower the risk and thus also decrease

the IRR that would be required to attract investors (Turton et al., 2009a). Sugarcane in Brazil is much cheaper (US$ 19.86/tc or R 163.85/tc) and even though fuel and electricity is less expensive in Brazil (US$ 0.53/L or R 4.37/L and US$ 0.087/kWh or R 0.72/kWh) than in South Africa, the cheap sugarcane results in higher IRR's for 1st generation ethanol plants in Brazil (Macrelli et al., 2012). The tax rate in Brazil is 34 % (Macrelli et al 2012) which is very close to the 35 % assumed in this study.

Scenario 3 was found to be unprofitable at the determined bagasse price, resulting in a negative NPV of R 323.29 million. It was determined that a bagasse price of R 129.73/tb would result in an IRR of 0 %. This means that the plant will break even at this bagasse price. Furthermore it was determined that the for a bagasse price of R 0/tb the IRR would only be 4.55 % which is very low when one considers the high risk of new technology such as 2nd generation bioethanol production. This means that scenario 3 is not viable. It was shown earlier that the integrated scenario 2 is more viable than the 1st generation scenarios 1 and 1a, clearly illustrating the advantage of integration.

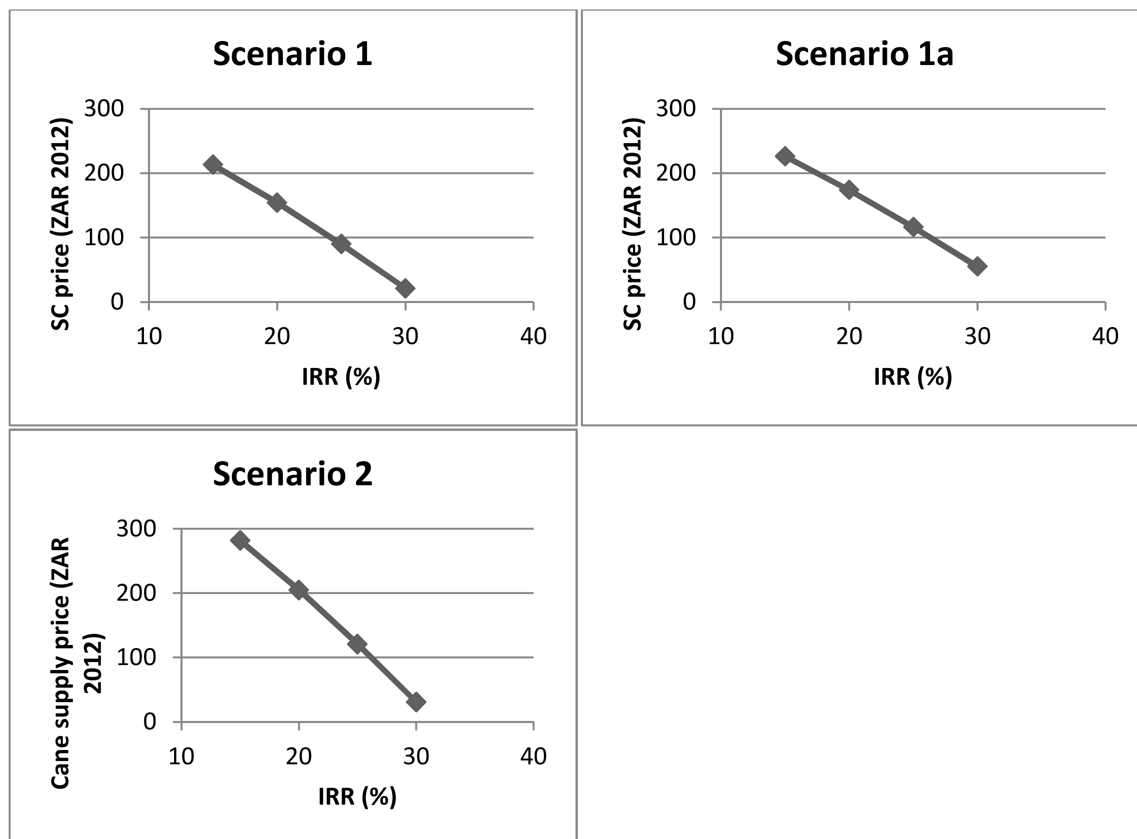

Figure 46: Influence of required IRR values on the demanded cane supply price.

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Figure 47 shows the sensitivity analyses for all scenarios. For scenarios 1 and 1a the cost of sugarcane has the largest effect on the MESP. This is characteristic of a mature technology where the feedstock makes up the largest part (60 – 70 %) of the MESP (Macrelli et al., 2012). By varying the cane price by 50 % above and below its original price the MESP varies from R 9.08/L to R 4.32/L for both scenarios 1 and 1a. Capital cost also has a very large effect on these two scenarios and by varying the capital cost by 50 % above and below its original value the MESP varies from R 8.29/L to R 5.11/L for scenario 1 and from R 8.11/L to R 5.28/L for scenario 1a. The reason for the smaller variation in the MESP of scenario 1a is due to the lower capital cost of this scenario that resulted in a smaller variation in the varied capital cost.

The effect of labour is negligible as can be seen from the sensitivity analyses of scenarios 1 and 1a. This is because only a few operators are required to operate the plant (the labour cost of construction is included in the capital costs). Due to the negligible effect of labour on the MESP for scenario 1 and 1a the cost of labour wasn't included in the sensitivity analyses of scenarios 2 and 3.

For scenario 2 the capital cost and the sugarcane price has a very similar effect on the MESP. This is characteristic of newer technology where the feedstock does not have the biggest effect on the MESP (Macrelli et al., 2012). The increased effect of the capital cost in scenario 2 when compared to scenarios 1 and 1a can be attributed to the higher capital investment required for an integrated facility. By varying the cane price by 50 % above and below its original price the MESP varies from R 8.29/L to R 5.11/L for scenario 2. By varying the capital cost by 50 % above and below its original value the MESP varies from R 8.56/L to R 4.78/L for scenario 2.

The data for the sensitivity analysis of scenario3 was determined at an IRR of 0 %. This was done to be able to perform a sensitivity analysis to determine the effects of the chosen parameters on the MESP, even though this scenario was found to be unprofitable under usual circumstances. In scenario 3 the capital cost has the largest effect on the MESP. By varying the capital cost by 50 % above and below its original value the MESP varies from R 9.79/L to R 3.62/L. Bagasse and enzyme price has very similar effects on the MESP. By varying the enzyme price by 50 % above and below its original price the MESP varies from R 6.98/L to R 6.43/L for scenario 3. By varying bagasse price by 50 % above and below its original price the MESP varies from R 7.13/L to R 6.27/L for scenario 3.

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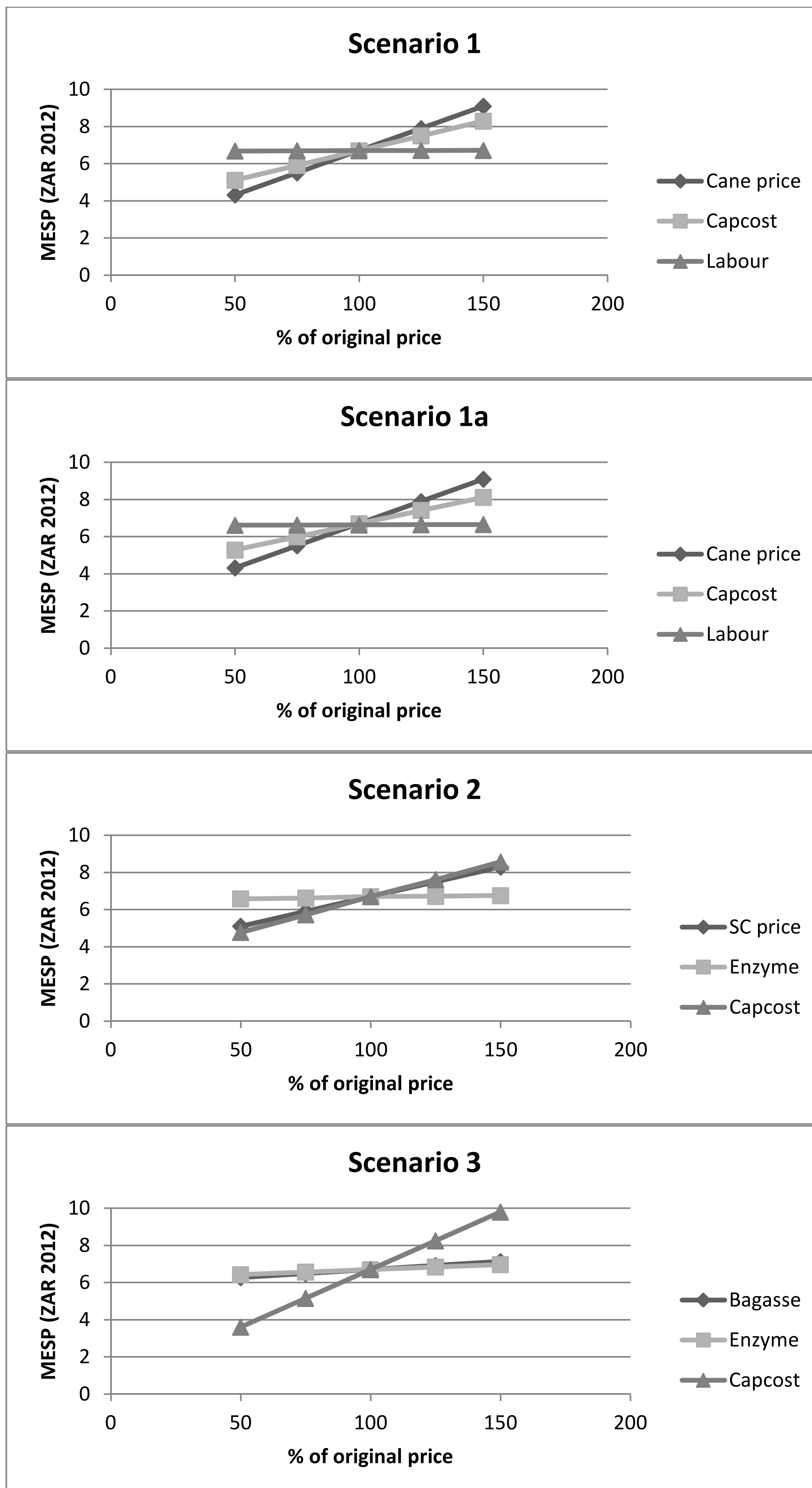

Figure 47: Sensitivity analyses for 1st gen and 1G + 2G.

Due to the large effect that the capital cost and sugarcane price have on the MESP it may seem that the effect of the enzyme cost is negligible. However, the base cost of enzyme that was used is very low. This has the result that the 25 and 50 % changes in the enzyme price is also very low which causes the enzyme price to have a small effect on the MESP in the investigated range of the sensitivity analysis.

However, it was noted that enzyme prices vary widely in literature and there is much uncertainty surrounding enzyme prices. Enzyme prices quoted in literature vary from as low as US$ 0.10 per gallon ethanol produced (US$/gal) to as high as US$ 1.5 (Klein-Marcuschamer et al., 2012) and this wide variation of prices is not reflected in the sensitivity analysis. The effect of such a large variation on the ethanol price can be seen in Figure 48 below for scenarios 2 and 3. The variation of the enzyme price from US$ 0.10/gal to US$ 1.5 US$/gal results in variation of the MESP from R 8.07/L to R 6.59/L for scenario 2 and from R 10.89/L to R 6.48/L for scenario 3. The larger difference in the MESP of scenario 3 caused by the range of enzyme prices is because all of the ethanol produced in scenario 3 is second generation ethanol whereas only some of the ethanol produced in scenario 2 is 2nd generation.

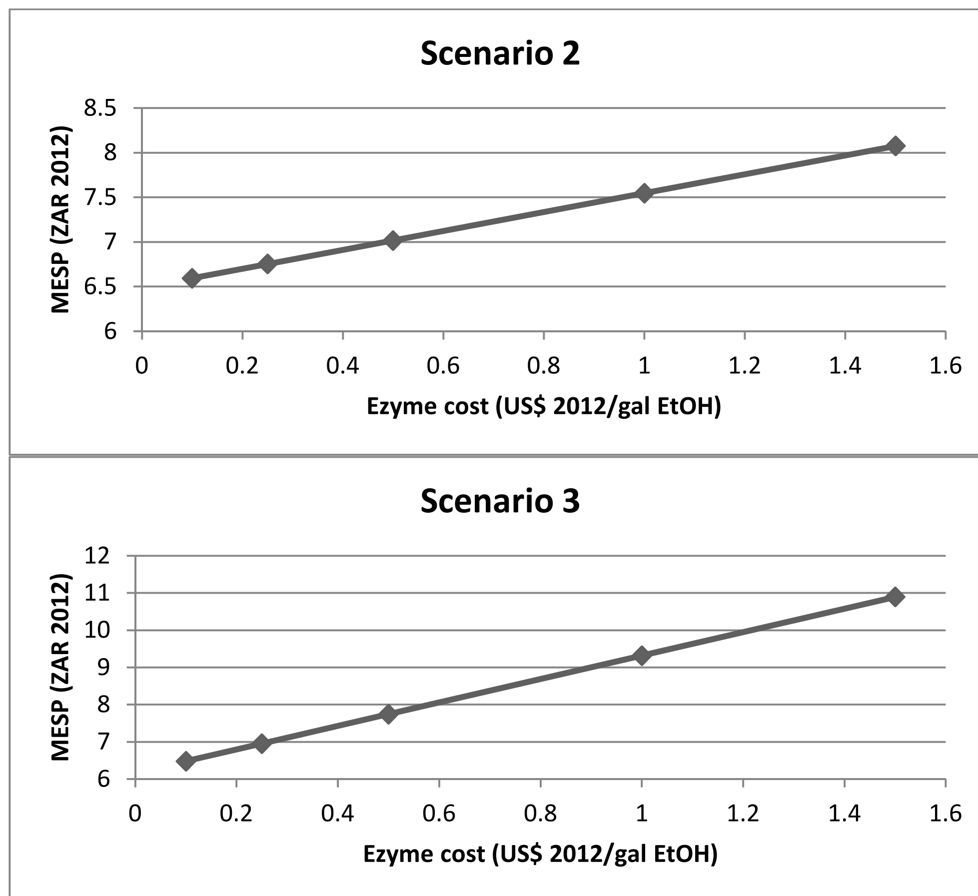

Figure 48: Effect of enzyme costs on the MESP of ethanol from a 1G + 2G plant.

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The current IRR of scenarios 1 and 1a with an electricity price of R 1.835/kWh and the current cane price is 1.618 % (the same IRR was used to be able to determine a bagasse price). The IRR for scenario 2 was 11.66 %. These IRR's are well below the 30 % that would attract investors. At the conditions mentioned here an IRR for scenario 3 could not be determined. Scenario 2 shows the highest IRR even though this scenario produces less electricity and ethanol than scenarios 1a and 3 combined (see Table 21). The higher IRR of scenario 2 can be attributed to the lower capital cost that is a result of integration.

For the scenarios 1 and 1a to achieve an IRR of 30 % under the current conditions the MESP must be R 11.16/L and R 10.67/L, respectively. For scenario 2 to achieve the same IRR the MESP must be R 9.59/L. Scenario 3 requires an MESP of R 16.44/L to achieve an IRR of 30 %. These prices are much higher than the current ethanol price of R 6.70/L, but it shows the effect that integration has on lowering the MESP.

For a lower electricity price of R 0.90/kWh (this value was issued to stakeholders in a draft COFIT paper by NERSA in July 2011) the MESP will have to increase to R 11.94/L and R 11.12/L for scenarios 1 and 1a, respectively. For scenario 2 to achieve an IRR of 30 % at the lower electricity price requires an MESP of R 9.95/L. Scenario 3 requires an MESP of R 16.96/L for the same situation.

For the lower electricity price at R 0.90/kWh the current IRR for scenario 2 is 8.34 %. IRR's for the other scenarios could not be calculated at the lower electricity price since the scenarios don't make any profit under the conditions considered.

Under the conditions considered in this study electricity is more expensive than bioethanol on an energy basis. It is counter intuitive to pay more for electricity than for a liquid fuel since liquid fuels are more expensive to manufacture. Internationally ethanol is more expensive than electricity per unit of energy (US$ 26.91/GJ for ethanol and US$ 23.02 for electricity) (Dias et al., 2011b). On an energy basis the current MESP of R 6.70/L is equivalent to a price of 30.04 c/MJ (R 300.40/GJ) while the COFIT price for electricity of R 1.835/kWh is equivalent to 50.97 c/MJ (R 509.70/GJ). If the price of bioethanol and electricity was the same on an energy basis then the MESP would be R 11.37/L which would result in IRR values of 31.00 %, 33.77 %, 39.55 % and 16.64 % for scenarios 1, 1a, 2 and 3, respectively.

Note: The minimum manufacturing price of ethanol could not be determined as the ethanol price was used as an input to determine the sugarcane supply price.

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7.4 Conclusions and recommendations

7.4.1 Modelling

It is concluded that the aim of building a first generation model in Aspen Plus® that is as up to date as possible and that can be used in future for modelling 1st and 2nd generation integrated plant, has been achieved.

Literature only provides the results generated by models and this doesn't allow one to investigate any new parameter changes. To be able to do this a model is needed and it is very time consuming to develop new models. It is recommended that process models be shared between academics and that process data be obtained from industry through partnerships with industry to be able to construct accurate models.

7.4.2 Pinch heat integration

It is concluded that by using pinch technology for heat integration the utility usage is lowered. Especially the hot utility usage, which was lowered from 130459.3 kW to 71885 kW, making more steam available for the generation of power that can be sold to the grid for additional revenue.

It is recommended that pinch heat integration be employed in future biofuel plants to be able to generate more electricity and to save energy to be more environmentally friendly.

7.4.3 Economic analysis

Conclusions:

- Sugarcane must be supplied at a lower price for a 1st generation ethanol plant than for an integrated 1st and 2nd generation ethanol plant to make the production of biofuel from sugarcane economically viable.

- Capital cost and the price of cane has the largest effects on the process economics. The uncertainty that is connected with enzyme prices also has a large effect on the economics.
- The positive effect of integration, that helps to decrease the capital cost, makes the integrated scenario the most viable one. The stand-alone 2nd generation plant is the least viable option.

Recommendations:

- Legislative tools must be used to ensure that sugarcane can be cultivated separately from the sugar industry for biofuel use only. This sugarcane can be cultivated in the suitable land that was identified by Watson (2011). This will create a new market for biofuel sugarcane where the cane price does not depend on the sugar industry. Another advantage of this will be that sugarcane cultivars can be optimised to maximise ethanol production instead of sucrose production.
- More price incentives should be provided to make second generation biofuel production viable. The current Biofuels Strategy was determined for the production of first generation biofuels and the incentives that are offered in this strategy do not consider the higher cost of producing second generation biofuel. Also, since scenarios 1 and 1b are not profitable, it means that even with very high electricity prices, the current biofuels strategy is insufficient to provide economic returns to investors, and acceptable market-competitive cane prices to farmers.

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8. OVERALL CONCLUSIONS

First here follows a summary of the most important conclusions from all the chapters:

- It is concluded from chapter 3 that there is great potential for a recombinant yeast strain to replace a natural hexose fermenting yeast for the fermentation of sucrose in a plant where both 1st and 2nd generation fermentations take place.
- Chapter 4 shows that pressing pre-treated bagasse will sufficiently lower inhibitors in 2nd generation fermentations when using a hardened yeast strain.
- Chapter 5 concluded that a faster feeding strategy better assists yeast to tolerate inhibitors in a 2nd generation fermentation and this also helps to improve productivity by shortening fermentation times and it improves yield by increasing maximum ethanol concentrations.
- Chapter 6 shows that combined 1st and 2nd generation fermentations holds great potential for improving 2nd generation fermentations.
- Chapter 7 concluded that sugarcane is too expensive in South Africa and a separate biofuels market should be created for sugarcane and also that the current incentives that are offered in South Africa are inadequate to make biofuel production attractive to investors.

The conclusions meet the main objectives of the project, as presented in section 2.10 Main project objectives as follows:

- The integration of first and second generation fermentations has great potential to improve second generation ethanol production process by improving the fermentation of cellulose.
- Process integration between first and second generation bioethanol production from sugarcane positively impacts on the process economics making an integrated process economically more viable than any of the stand-alone options. The process economics can be improved by producing table sugar from the part or all of the sucrose like the Brazilian model for ethanol and sugar production in the same facility. Second generation ethanol can still be produced from the bagasse.
- Legislation changes are required to increase the incentives that are offered for the production of second generation biofuels. Currently the same incentives apply to both first and second generation biofuel production in South Africa but it is a lot more expensive to produce second generation biofuels. In the case of sugarcane land must be allocated for the purpose of growing sugarcane for biofuel production and the price of sugarcane for biofuel production must be fixed. South Africa is looking to implement carbon taxes in 2013 or 2014

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at a proposed rate of R 120 per tonne of CO_2 emissions (Reuters, 2012). This will help improve the economics of second generation ethanol from sugarcane bagasse because second generation ethanol provide large GHG emissions savings. Another way to improve the economics through GHG emissions savings is the emissions trading schemes where carbon credits from second generation ethanol can be sold to other producers that require carbon credits (Grubb & Neuhoff, 2006).

Note: Due to time constraints the integrated 1st and 2nd generation processes could not be modelled in detail using the experimental results from this project, thus the process data for these processes were taken from literature. Also the experimental results that were obtained in this project were not optimal and the literature values that were used better reflect what is possible with current technology.

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9. RECOMMENDATIONS AND FUTURE WORK

Further work must be performed on combined 1st and 2nd generation fermentation strategies to improve the ethanol yield and production. It is proposed that combined 1st and 2nd generation experiments be performed where the yeast is first cultivated on sugar syrup in order to obtain a large enough yeast population that will be able to successfully ferment WIS that must be added in a fed batch manner (SSF). It must also be investigated whether feeding sucrose along with the WIS will improve the vigour of the yeast in such fermentations. This will improve process economics for bioethanol production processes where first and second generation feedstocks are available at the same site for bioethanol production. The optimised results from such experiments must then be used to model an integrated 1st and 2nd generation process using process simulation software such as Aspen Plus®.

The uncertainty about enzyme prices must be investigated by performing a techno-economic analysis on the on-site production of enzymes.

Biofuels legislation must be adapted to offer higher incentives for the production of second generation biofuels. Legislation can be used to allocate land for the growing of crops that are dedicated to the production of biofuels. This will create a biofuel market where the feedstock prices used for bioethanol production are independent of the food market. Another advantage of implementing this is that the cultivars can be optimised for biofuel production instead of food production.

Research on biofuels must be done in partnership with industry. This will ensure accurate data for use in process models. An industrial partner can also supply equipment quotes as equipment quotes are not easily found in literature and must be obtained from vendors.

Academic partnerships are also to be encouraged to share data so that biofuel research can progress at a faster pace. Sharing of process models will save a lot of time since it is very time consuming to develop process models.

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11. APPENDICES

APPENDIX A: SAMPLE PREPARATION FOR HPLC ANALYSIS

It is necessary to prepare the samples for HPLC analysis to remove large molecules from the sample such as proteins that can damage the HPLC columns.

First take a sample from the bioreactor or other experiment. Take 1 mL of this sample and centrifuge it at 14 000 rpm for 5 min. Take the supernatant of the centrifuged sample and freeze it away at -20 °C until it must be prepared for HPLC analysis.

Prepare the sample for HPLC analysis according to the following steps:

1. Prepare 1.8 mL of sample at the desired dilution by using distilled water.
2. Add 109 µL 35 % (v/v) perchloric acid (PCA).
3. Incubate on ice for 10 minutes.
4. Add 99 µL 7 M KOH.
5. Incubate overnight on ice.
6. Centrifuge at 14 000 rpm for 10 minutes.
7. Filter the solution using 0.22 µm syringe filters.

The sample is now ready for HPLC analysis

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APPENDIX B: PREPARATION OF YPD PLATES

Prepare the agar mixture according to Table A1 and autoclave mixture. After the mixture has been autoclaved let it cool to 60 °C in an oven. Carefully pour the agar mixture into plastic petri dishes in a laminar flow cabinet using autoclave gloves (approximately 25 mL for each). Let the plates cool and give the agar time to set (approximately 48 hours) and store upside down in a fridge at 4 °C.

Table A1: Ingredients for YPD agar preparation

YPD		
Ingredient	mass %	g/L
Yeats extract	1	10
Peptone powder	2	20
Agar	1.5	15
Glucose	2	20

APPENDIX C: BIOMASS CHARACTERISATION

The biomass characterisation curves were determined from samples that were drawn from the fermentation at 15 hours. The biomass characterisation curves can be seen in in the graphs in Figure A1. In these graphs y1 shows the correlation between the optical density and biomass for the first run of the duplicate fermentations whereas y2 does the same thing for the second run of the duplicate fermentations.

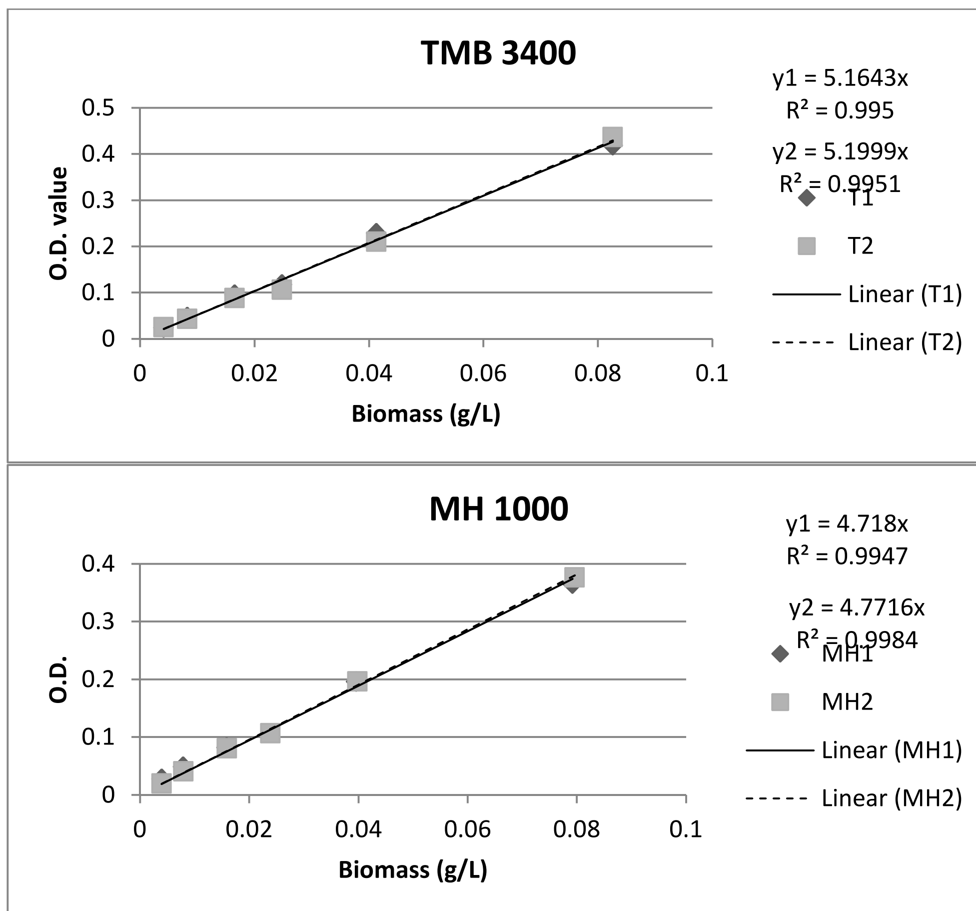

Figure A1: Biomass characterisation curves for TMB 3400 and MH 1000

APPENDIX D: DETERMINATION OF MAXIMUM SPECIFIC GROWTH RATE

The maximum specific growth rates (μ_{max}) for the 1st generation fermentations were determined by fitting Equation A 1 to the data points that were taken during the exponential growth phase. In Equation A 1 X_t is the population size at time t and X_0 is the population size at the start of the exponential growth phase. This method for determining μ_{max} can be seen in Figure A 2.

$$X_t = X_0 e^{(\mu_{max} t)}$$

Equation A 1

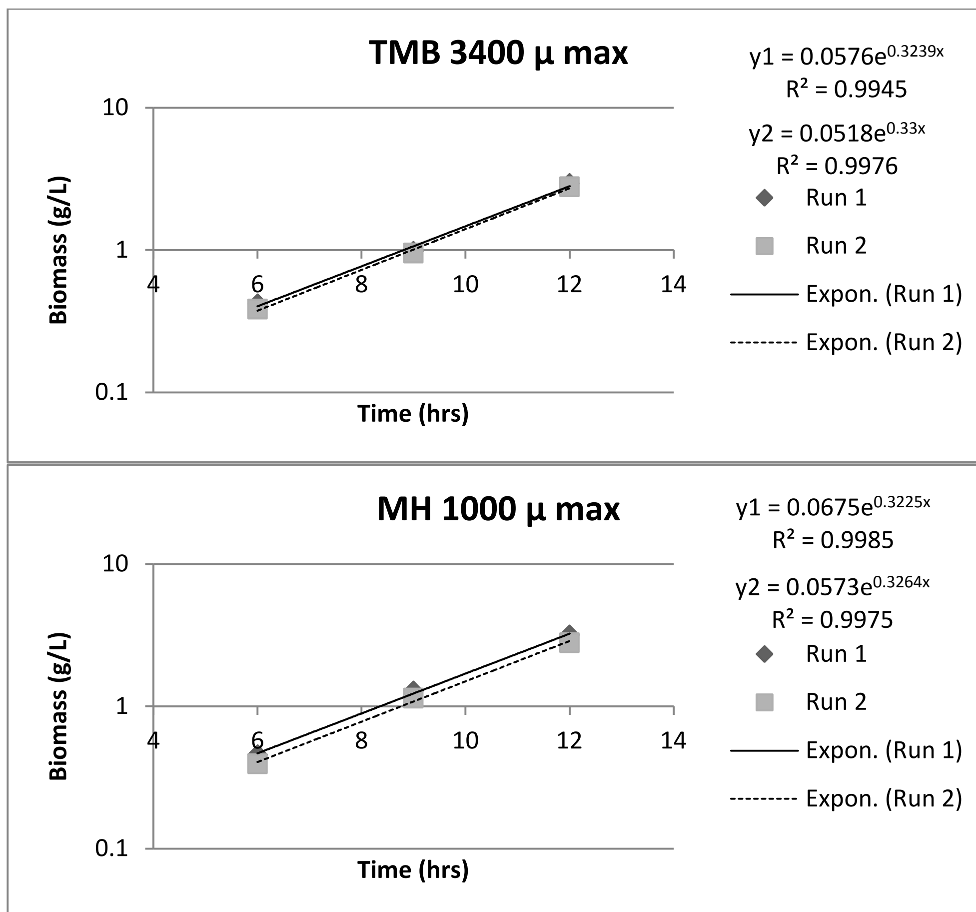

Figure A 2: Determination of maximum specific growth rates for TMB 3400 and MH 1000

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APPENDIX E: MASS BALANCE OF FIRST GENERATION EXPERIMENTS

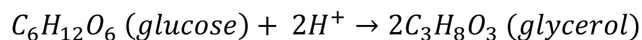
The extra equations used in the mass balances are presented here. The other equations that were used are the conversion of sucrose to glucose and fructose through invertase and the conversion of hexose sugars (glucose or fructose) to ethanol and carbon dioxide through fermentation.

The formation of biomass is given in Equation A 2 (Medina et al., 2010).

$$56\, mmol\ glucose \rightarrow 1\, g\ biomass + 88\, mmol\ EtOH + 95\, mmol\ CO2 + 11\, mmol\ glycerol + 1.7\, mmol\ acetate$$

Equation A 2

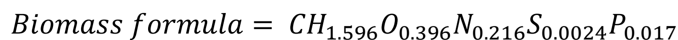
Formation of glycerol is given below in Equation A 3. The $2H^+$came from NADH + H^+. Glycerol formation is required for biomass synthesis and it is produced when the organism is under stress.



$$C_6H_{12}O_6\ (glucose) + 2H^+ \rightarrow 2C_3H_8O_3\ (glycerol)$$

Equation A 3

The biomass formula for *S.cerevisiae* that was used is presented below in Equation A 4 (Nielsen et al., 2003). The molecular weight of S.cerevisiae is 23.59, calculated from Equation A 4



$$Biomass\ formula = CH_{1.596}O_{0.396}N_{0.216}S_{0.0024}P_{0.017}$$

Equation A 4

The mass balances and elemental carbon balances are presented in Table A 2 to Table A 5 for all the first generation fermentation experiments.

Table A 2: Mass balance and elemental carbon balance for the TMB 3400 Run 1.

TMB 3400 1	In (g/L)	Out (g/L)	In (C-moles)	Out (C-moles)
Sucrose	235.34	0.00	8.25	0.00
Glucose	11.10	0.00	0.37	0.00
Fructose	9.11	7.48	0.30	0.25
Ethanol	0.00	113.72	0.00	4.94
Glycerol	0.05	12.08	0.00	0.39
Biomass	0.27	6.15	0.01	0.26
CO2		133.24		0.02
Acetate		0.59	0.00	3.03
H2O	12.39			
Total	268.25	273.26	8.94	8.89
% Balance	101.87		99.46	

Table A 3: Mass balance and elemental carbon balance for the TMB 3400 Run 2.

TMB 3400 2	In (g/L)	Out (g/L)	In (C-moles)	Out (C-moles)
Sucrose	244.08	0.00	8.56	0.00
Glucose	15.15	12.18	0.50	0.41
Fructose	17.11	50.29	0.57	1.67
Ethanol	0.77	85.61	0.03	3.72
Glycerol	0.13	9.17	0.00	0.30
Biomass	0.28	6.35	0.01	0.27
CO2	0.00	106.40		2.42
H2O	12.85	0.00		0.02
Acetate		0.61		
Total	290.36	269.99	9.68	8.78
% Balance	92.98		90.72	

Table A 4: Mass balance and elemental carbon balance for the MH 1000 Run 1.

MH 1000 1	In (g/L)	Out (g/L)	In (C-moles)	Out (C-moles)
Sucrose	222.02	0.00	7.78	0.00
Glucose	15.49	0.00	0.52	0.00
Fructose	17.03	41.26	0.57	1.37
Ethanol	0.74	99.56	0.03	4.32
Glycerol	0.12	11.52	0.00	0.38
Biomass	0.28	5.94	0.01	0.25
CO2		118.09		2.68
H2O	11.69			0.02
Acetate		0.57		
Total	267.34	276.37	8.91	9.01
% Balance	103.38		101.05	

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Table A 5: Mass balance and elemental carbon balance for the MH 1000 Run 2.

MH 1000 2	In (g/L)	Out (g/L)	In (C-moles)	Out (C-moles)
Sucrose	234.19	15.81	8.21	0.55
Glucose	10.59	24.97	0.35	0.83
Fructose	10.09	58.15	0.34	1.94
Ethanol	0.82	57.67	0.04	2.50
Glycerol	0.10	10.09	0.00	0.33
Biomass	0.28	5.52	0.01	0.23
CO2		76.25	0.00	1.73
H2O	11.50			
Acetate		0.53		0.02
Total	267.56	248.45	8.95	8.12
% Balance	92.86		90.75	

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APPENDIX F: RESULTS FOR PRESSING EXPERIMENTS

The results for the pressing experiments are shown here in tabular form (Table A 6 to Table A 9). The moisture contents of the control experiments are shown in Table A 10. The control experiments were performed by drying samples that were not pressed to reduce the moisture content.

Note: All the samples were dried in an oven at 105 °C for 27 hours.

Table A 6: Weights of pre-treated samples before pressing.

Condition	Pressure level	Pressure (MPa)	Sample (g)
1	low	4.768	50.038
	medium	9.536	50.04
	high	14.304	50.031
2	low	4.768	50.029
	medium	9.536	50.021
	high	14.304	50.042
3	low	4.768	50.008
	medium	9.536	49.955
	high	14.304	50.049

Table A 7: Weights of pre-treated samples after pressing.

Condition	Pressure level	Pressure (kPa)	Container (g)	Sample + Container (g)	Sample (g)
1	low	4.768	90.622	119.183	28.5608
	medium	9.536	101.91	130.854	28.9441
	high	14.304	102.171	131.142	28.9706
2	low	4.768	102.955	135.835	32.8796
	medium	9.536	91.227	120.951	29.7236
	high	14.304	92.043	122.311	30.2676
3	low	4.768	97.736	130.786	33.0501
	medium	9.536	103.559	133.835	30.2763
	high	14.304	91.32	121.697	30.377

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Table A 8: Weights of pre-treated samples after drying.

Condition	Pressure level	Pressure (MPa)	Container (g)	Sample + Container (g)	Sample (g)
1	low	4.768	90.622	104.94	14.318
	medium	9.536	101.91	116.645	14.735
	high	14.304	102.171	116.532	14.361
2	low	4.768	102.955	119.59	16.635
	medium	9.536	91.227	106.756	15.529
	high	14.304	92.043	108.019	15.976
3	low	4.768	97.736	113.714	15.978
	medium	9.536	103.559	119.19	15.631
	high	14.304	91.32	107.173	15.853

Table A 9: Moisture content of the pressed samples

Condition	Pressure level	Pressure (MPa)	Pressed sample (g)	Dried sample (g)	Moisture content (wt%)
1	low	4.768	28.561	14.318	49.868
	medium	9.536	28.944	14.735	49.092
	high	14.304	28.971	14.361	50.429
2	low	4.768	32.880	16.635	49.406
	medium	9.536	29.724	15.529	47.755
	high	14.304	30.268	15.976	47.217
3	low	4.768	33.050	15.978	51.655
	medium	9.536	30.276	15.631	48.372
	high	14.304	30.377	15.853	47.812

Table A 10: Moisture content of control samples (samples that were not pressed).

Condition	Container weight (g)	sample befor drying (g)	Sample after drying (g)	Moisture content (wt%)
1	99.337	50.527	15.956	68.44894408
2	105.194	50.317	18.484	63.26417044
3	90.128	50.328	17.814	64.59997615

APPENDIX G: 50 TONNE PRESS

The design of the piston and cylinder set-up was based on a 6" Sch 40 SS pipe that was acquired from a scrap yard. This pipe was used as the cylinder. The design can be seen below in Figure A 3 and a photo of the press and the piston and cylinder set-up can be seen on the next page in Figure A 4. The design and manufacture of the piston and cylinder set-up was done by the Mechanical Engineering work shop at the University of Stellenbosch.

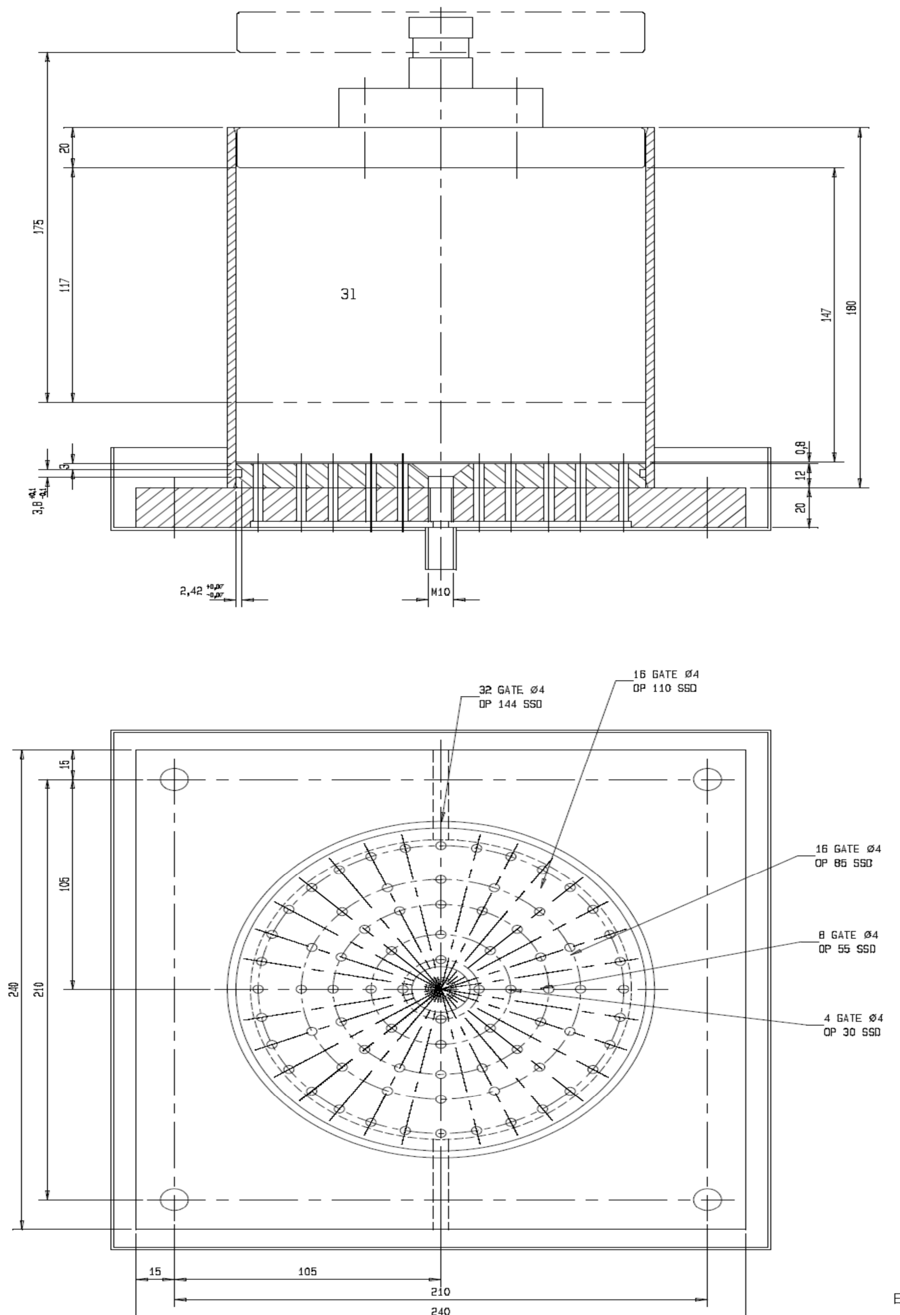

Figure A 3: Design for the piston and cylinder set-up

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Figure A 4: Photo of 50 ton press and the piston and cylinder set-up.

APPENDIX H: MOISTURE CONTENT OF PRESSED WIS USED FOR FERMENTATIONS

The data for the determination of the moisture content (MC%) of the pressed WIS that was used in the 2nd gen and 1st and 2nd gen combined fermentations, are presented here in Table A 11. Control experiments were performed with pre-treated bagasse that was not pressed. The moisture content was calculated using Equation 10.

Table A 11: Moisture content calculations for pre-treated bagasse and pressed WIS.

			Before drying		After drying		
		Container (g)	Sample + container (g)	Sample (g)	Sample + container	Sample (g)	MC %
Wet	W1	89.52	131.78	42.27	104.10	14.58	65.50
	W2	92.05	157.21	65.16	109.77	17.72	72.80
	W3	101.91	143.48	41.57	117.35	15.43	62.87
						avg	67.06
						Stdev	5.15
Pressed	P1	91.07	131.72	40.65	114.73	23.65	41.81
	P2	92.14	136.18	44.04	117.82	25.69	41.68
	P3	98.41	138.20	39.79	121.03	22.63	43.14
						avg	42.21
						Stdev	0.81

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APPENDIX I: INOCULATION CONCENTRATIONS FOR 2ND GEN AND 1ST AND 2ND GEN COMBINATION FERMENTATIONS.

The cell weights in Table A 12 are the wet weights of the cells. The inoculation culture for the 3% WIS/day fermentations were incubated at 37°C instead of 30°C due to problems with the 30°C rotary incubator and this higher incubation temperature led to the lower cell mass. The inoculation concentrations were determined for an initial volume of 3 L.

Table A 12: Concentrations of inoculation cultures for 2nd Gen and 1st and 2nd Gen combination fermentations.

Fermentation	Reactor	Inoculation conc. (g cells/L)
3% WIS/day	1	1.213
	2	0.993
5% WIS/day	1	2.301
	2	1.95
SSF + sucrose	1	1.837
	2	2.237
HSF	1	1.554
SHF	2	2.538

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APPENDIX J: 2ND GENERATION FERMENTATION INOCULATION PREPARATION

Notes:

- If glucose is required for the mineral medium (Table A 13) it must be autoclaved separately.
- After addition of all the components for PBS the pH must be adjusted to 7.4 using 3 M KOH before sterilisation.

Table A 13: Mineral medium

Ingredient	Amount	Unit
Yeast extract	20	g/L
$(NH_4)_2SO_4$	7.5	g/L
KH_2PO_4	3.4	g/L
$MGSO_4.7H_2O$	0.8	g/L
Trace element sol.	1	mL/L
$CaCl_2.H_2O$	0.05	g/L
Citric acid	0.5	g/L
Glucose (if required)	10	g/L

Table A 14: Phosphate buffer solution (PBS)

Ingredient	Amount	Unit
NaCl	8.01	g/L
KCl	0.2	g/L
$Na_2PO_4.2H_2O$	1.45	g/L
KH_2PO_4	0.27	g/L

Cell harvesting steps:

1. Label and weigh six plastic 500 mL centrifuge bottles.
2. Pour 500 mL of culture into each of the six bottles (pour less than 500 mL if the centrifuge bottles are leaky).
3. Centrifuge the bottles at 10 000 rpm for 5 minutes.
4. Decant the supernatant.
5. Add 100 mL of sterile PBS to four of the bottles and shake the bottles to suspend the cells.
6. Transfer the suspension to the remaining two bottles.
7. Centrifuge these two bottles at 10 000 rpm for 5 minutes.
8. Decant the supernatant and add 200 mL of PBS to each bottle. Shake these bottles to suspend the cells and centrifuge at 10 000 rpm for 5 minutes.

9. Decant the supernatant and weigh each bottle. Calculate the wet cell weights by subtracting the weight of the bottles.
10. Add 40 mL of PBS to each bottle and shake to suspend the cells. Transfer this solution to a 50 mL Falcon tube.

This will produce inoculation cells for two reactor fermentations since they are usually performed in duplicate. However, when only one reactor fermentation needs to be performed the procedure above can be performed with six plastic 250 mL centrifuge bottles instead of 500 mL bottles and the rotor of the centrifuge will have to be changed to accommodate the 250 mL bottles.

APPENDIX K: QUALITATIVE GLUCOSE TESTS FOR 2ND GENERATION AND 1ST AND 2ND GENERATION COMBINATION FERMENTATIONS

The graphs in this section show the qualitative glucose tests that were performed with strips that are usually used for testing urine. The value “1” on the graphs indicates the presence of urine while the value “0” indicates the absence of glucose.

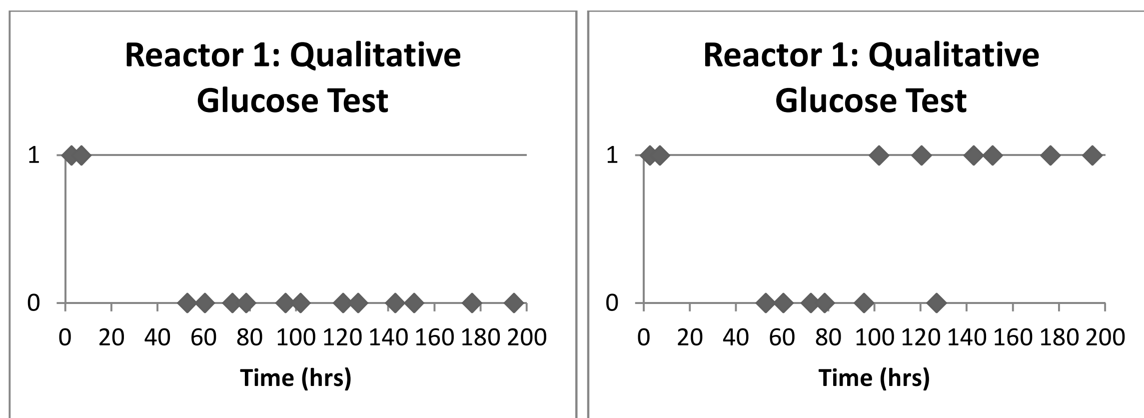

Figure A 5: Qualitative glucose tests for 3 % WIS per day fermentations.

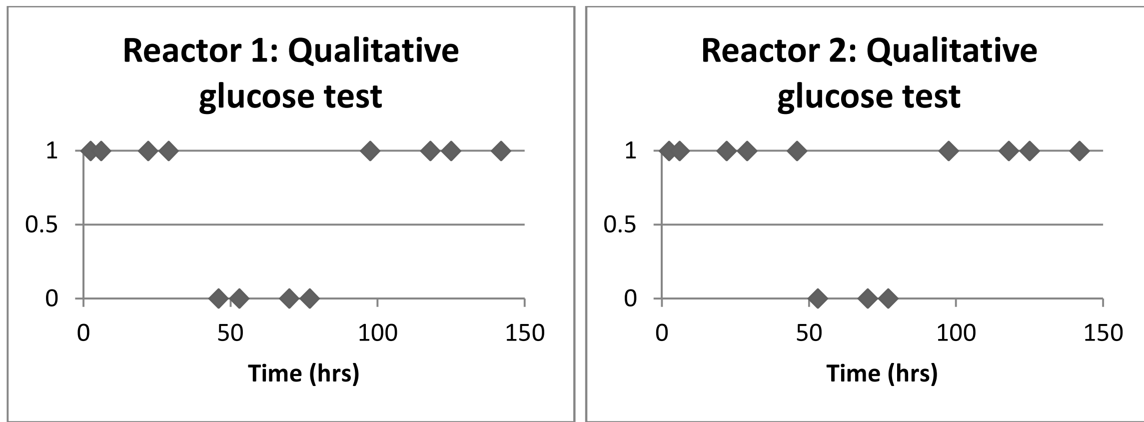

Figure A 6: Qualitative glucose tests for 5 % WIS per day fermentations.

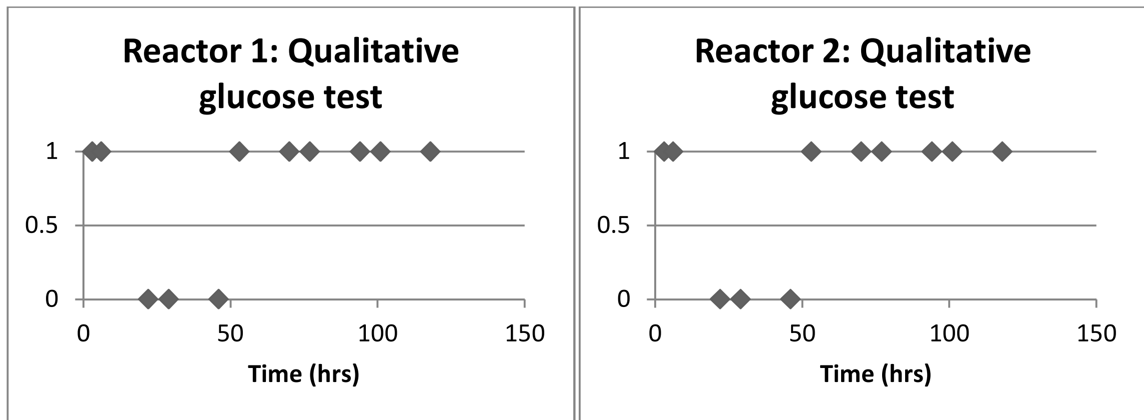

Figure A 7: Qualitative glucose tests for the SSF + sucrose fermentations.

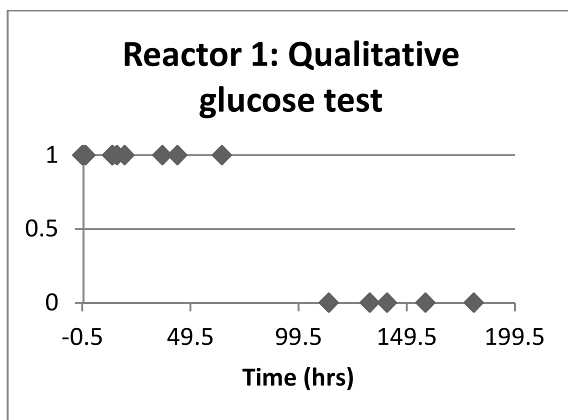

Figure A 8: Qualitative glucose tests for the HSF + sucrose fermentation.

APPENDIX L: CELLOBIOSE CONCENTRATIONS OF WIS FERMENTATIONS

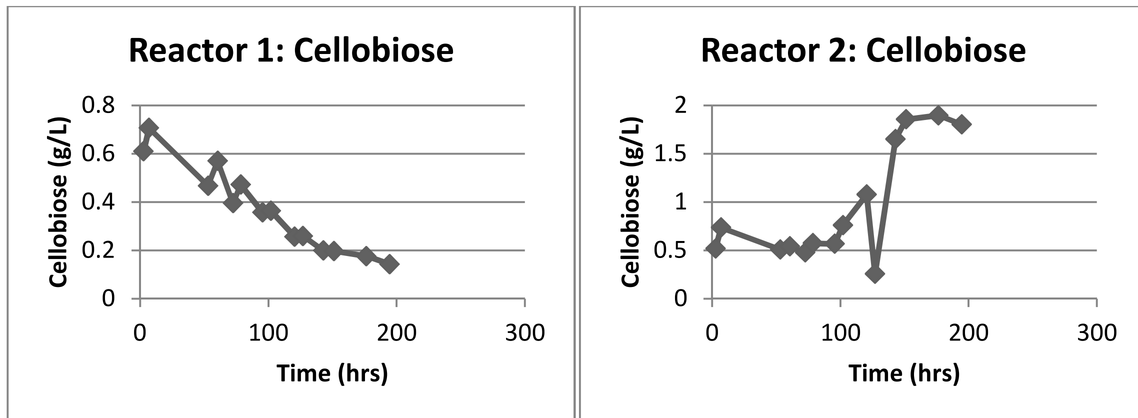

Figure A 9: Cellobiose concentration s for the 3 % WIS per day fermentations.

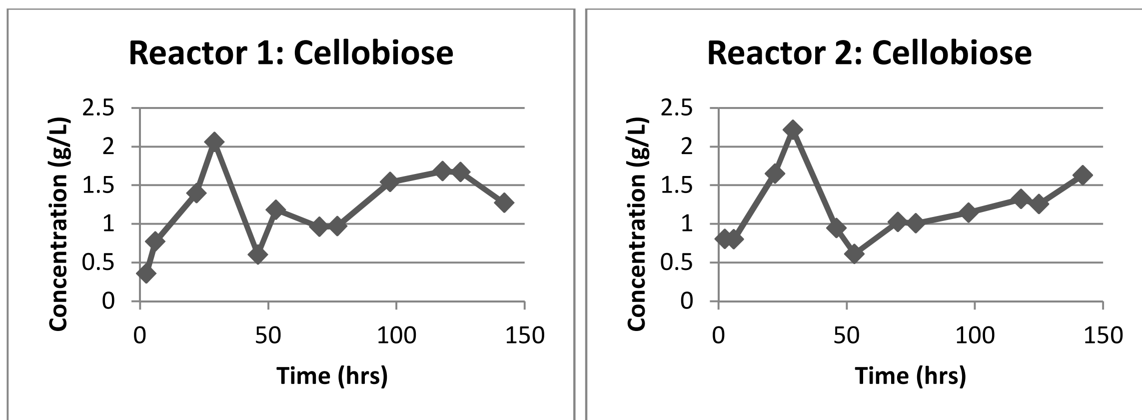

Figure A 10: Cellobiose concentration s for the 5 % WIS per day fermentations.

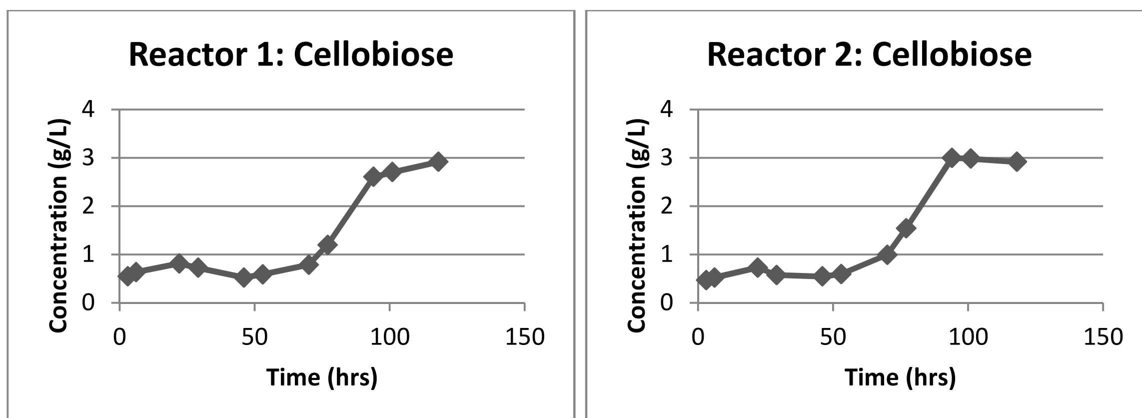

Figure A 11: Cellobiose concentration s for the SSF + sucrose fermentations.

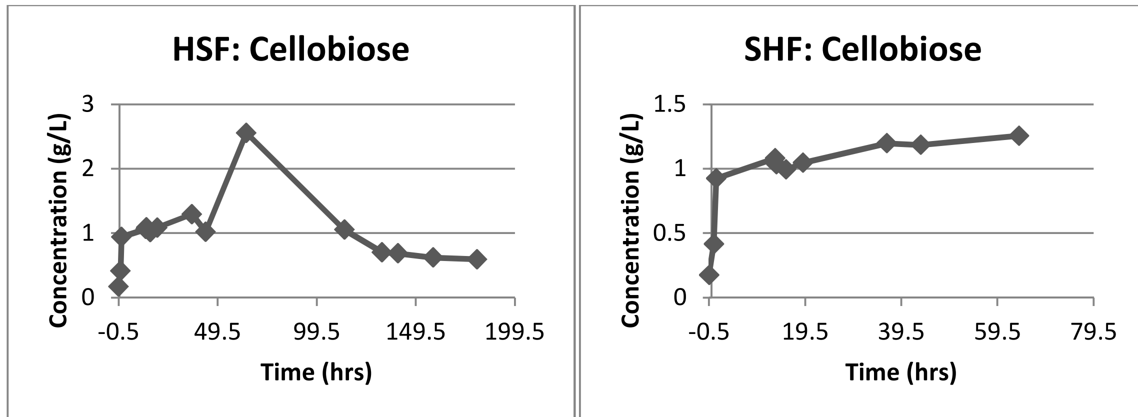

Figure A 12: Cellobiose concentrations for the HSF (left) and SHF (right) fermentations.

APPENDIX M: PERISTALTIC PUMP CALIBRATION

Figure A 13 show the calibration curves for the pumps that were used to feed the sugar syrup into the first and second generation fermentation combinations. Unit 1: Pump 2 was used to feed syrup to reactor 1 and Unit 2: Pump 1 was used to feed syrup to reactor 2.

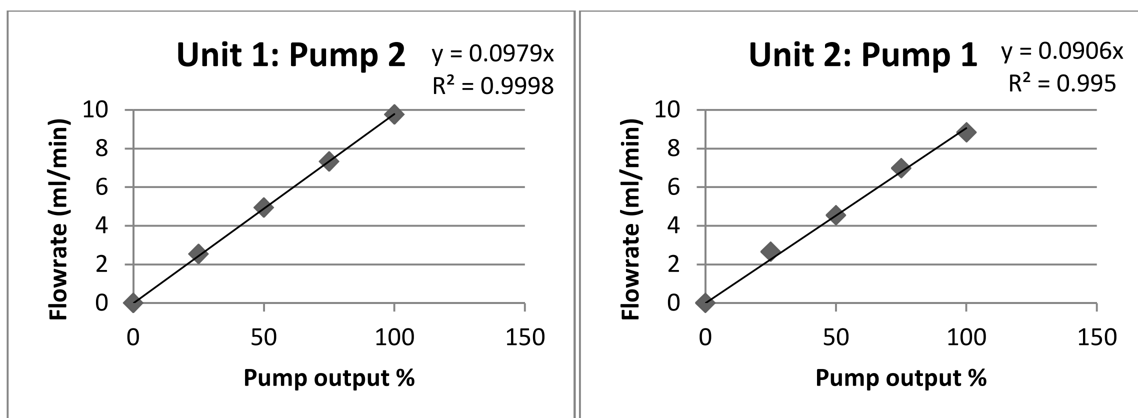

Figure A 13: Calibration curves for the peristaltic pumps responsible for the sugar syrup feed in the 1G + 2G experiments.

The sugar syrup flow rate for the SSF + sucrose fermentations were determined as follows:

The volume of sugar syrup added was approximately 25 % of the original volume of the pure second generation fermentation which was about 4 L. 935 mL of sugar syrup was added. The sugar syrup was added over a period of 48 hours, since this was the time that was needed to feed the remaining WIS. This resulted in a flow rate of 0.325 mL/min.

To obtain this flow rate the calibration curves in Figure A 13 were used to determine the pump output settings that were required. For reactor 1 and rector 2 the pump outputs had to be set to 3.31 % and 3.58 %, respectively.

The sugar syrup flow rates for the HSF + sucrose fermentation was determined as follows:

The sugar syrup was added faster in this experiment due to the poor results from the SSF + sucrose experiments. The flow rate was 1.653 mL/min and a total of 1095 mL of sucrose syrup was added.

To obtain this flow rate the calibration curve in Figure A 13 was used to determine the pump output setting of 16.88 % for reactor 1. This resulted in a feeding time of approximately 11 hours.

The concentrations of the sucrose syrup that was added to each 1G + 2G combination fermentation can be seen in Table A 10.

Table A 15: Concentrations of sugars in sucrose syrup added to the 1G + 2G combination fermentations.

Sucrose syrup concentrations					
experiment	Reactor	Glucose (g/L)	Fructose (g/L)	Sucrose (g/L)	Tot gluc eq. (g/L)
SSF+S	1	18.91	22.46	198.17	249.97
	2	19.52	19.52	176.50	228.44
HSF+S	1	19.22	22.80	187.34	239.21
	Avg.	19.22	21.59	187.34	239.21
	St dev %	1.587	8.35	5.78	4.50

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APPENDIX N: DATA FOR FIRST GENERATION MODEL

Table A 16 shows the sugarcane composition taken from Dias et al (2009). It can be seen from Table A 16 that the dirt was modelled as SiO_2 and the minerals and impurities were modelled as K_2O.

Table A 16: Composition of the sugarcane used in the Aspen Model®

Sugarcane composition	Content (wt%)
Component	
Sucrose	13.3
Cellulose	4.77
Hemicellulose	4.53
Lignin	2.62
Reducing Sugars (glucose)	0.62
Minerals (K_2O)	0.2
Impurities (K_2O)	1.79
Water	71.57
Dirt (SiO_2)	0.6
Total	100

Table A 17 shows the specification that were used for the co-generation system (Dias et al, 2009).

Table A 17: Parameters used for the co-generation system.

Parameters for Co-gen system	
Parameter	Value
Live steam pressure (bar)	90
Boiler thermal efficiency (%)	85
CEST1 isentropic efficiency (%)	72
Other turbine efficiencies (%)	81
Electric generator efficiency (%)	98
Plant electric demand (kWh/tc)	28

Table A 18 shows the pressures and temperatures on the different effects of the MEE (Dias et al, 2009).

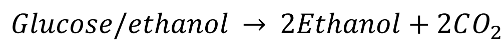
Table A 18: Temperatures and pressures on the different effect of the MEE.

Pressures and temperatures on the MEE		
Effect	Pressure (kPa)	Temperature (°C)
1st	169.6	115.5
2nd	135.4	108.8
3rd	101	100.6
4th	52.9	83.8
5th	20	64.6

Equation A 5 to Equation A 7 are the equations that were used to model the fermenter. The sucrose was assumed to be completely hydrolysed to glucose and fructose (100 % conversion of sucrose) according to the first generation fermentation experiments. The ethanol yield was assumed to be 90.5 % of the theoretical maximum of 0.511 g ethanol per gram of hexose sugar (Dias et al, 2009). This also correlates with the first generation fermentation experiments. The growth of the yeast is presented by Equation A 7. 1.37 % of the hexose sugars are used for the growth of yeast (Dias et al, 2009).

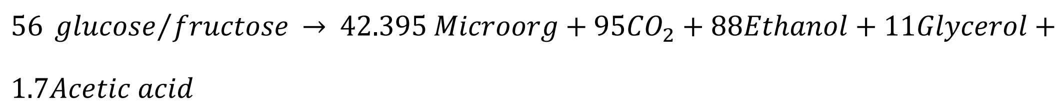
$$Sucrose + H_2O \rightarrow Glucose + Fructose$$

Equation A 5



$$Glucose/ethanol \rightarrow 2Ethanol + 2CO_2$$

Equation A 6



$$56\ glucose/fructose \rightarrow 42.395\ Microorg + 95CO_2 + 88Ethanol + 11Glycerol + 1.7Acetic\ acid$$

Equation A 7

Table A 19 shows the energy contents of the materials that were used to calculate the energy efficiencies of the different processes.

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Table A 19: Energy content values used for process efficiency calculations.

Energy content (Ensinas et al., 2010)	
Cane (MJ/kg)	4.4
Leaves (MJ/kg)	12.96
EtOH (MJ/L)	22.3

APPENDIX O: ASPEN PLUS® STREAM RESULTS

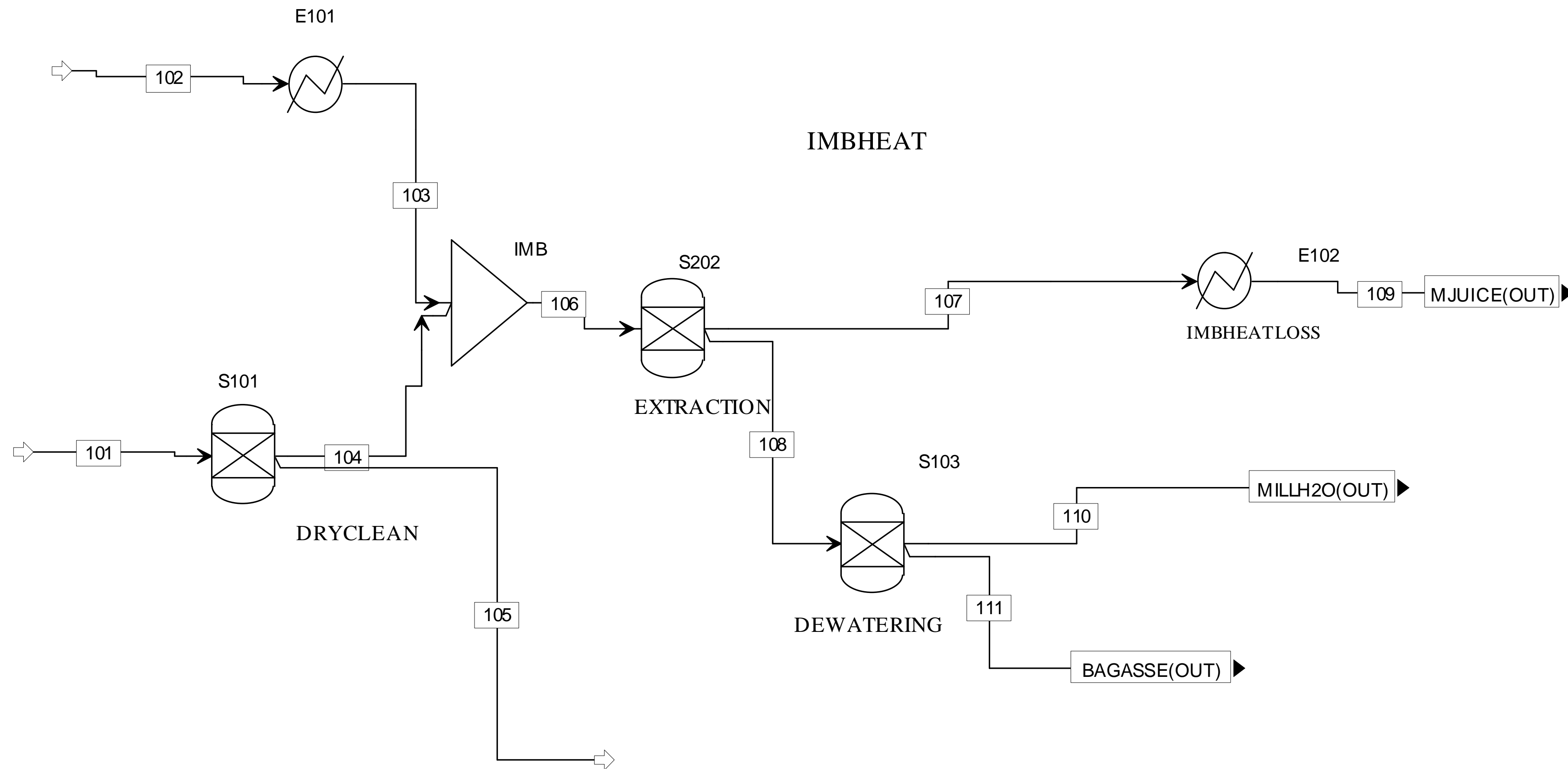

Figure A 14: Area 100: sugarcane cleaning and sucrose extraction

Table A 20: Stream table for Area 100: Sugarcane cleaning and extraction

Cane cleaning and sugar extraction								
Stream #		101	102	104	105	107	109	111
From				S101	S101	S202	E102	S103
To		S101	E101	IMB		E102		
Mass Flow	KG/HR	493000	177874.4	490042	2958	431868.2	431868.2	118903.7
Phase:		Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
Component Mass Flow								
GLUCOSE	KG/HR	3056.6	0	3056.6	0	3026.03	3026.03	30.57
CELLULOS	KG/HR	23516.1	0	23516.1	0	0	0	23516.1
XYLAN	KG/HR	22332.9	0	22332.9	0	0	0	22332.9
LIGNIN	KG/HR	12916.6	0	12916.6	0	0	0	12916.6
H2O	KG/HR	352840.1	177874.4	352840.1	0	354118.2	354118.2	59451.85
SUCROSE	KG/HR	65569	0	65569	0	64913.31	64913.31	655.69
SIO2	KG/HR	2958	0	0	2958	0	0	0
K2O	KG/HR	986	0	986	0	986	986	0
KCL	KG/HR	8824.7	0	8824.7	0	8824.7	8824.7	0
Component Mass Fraction								
GLUCOSE		0.01	0	0.01	0	0.01	0.01	0
CELLULOS		0.05	0	0.05	0	0	0	0.2
XYLAN		0.05	0	0.05	0	0	0	0.19
LIGNIN		0.03	0	0.03	0	0	0	0.11
H2O		0.72	1	0.72	0	0.82	0.82	0.5
SUCROSE		0.13	0	0.13	0	0.15	0.15	0.01
SIO2		0.01	0	0	1	0	0	0
KCL		0.02	0	0.02	0	0.02	0.02	0
Volume Flow	L/MIN	7756.61	2972.99	7572.81	199.18	6978.02	6929.77	1696.14
Temperature	K	298.15	298.15	298.15	298.15	321.17	303.15	321.17
Pressure	BAR	1.01	1.01	1.01	1.01	1.01	1.01	1.01
Vapor Fraction		0	0	0	0	0	0	0
Liquid Fraction		1	1	1	1	1	1	1
Solid Fraction		0	0	0	0	0	0	0
Mass Density	GM/CC	1.06	1	1.08	0.25	1.03	1.04	1.17

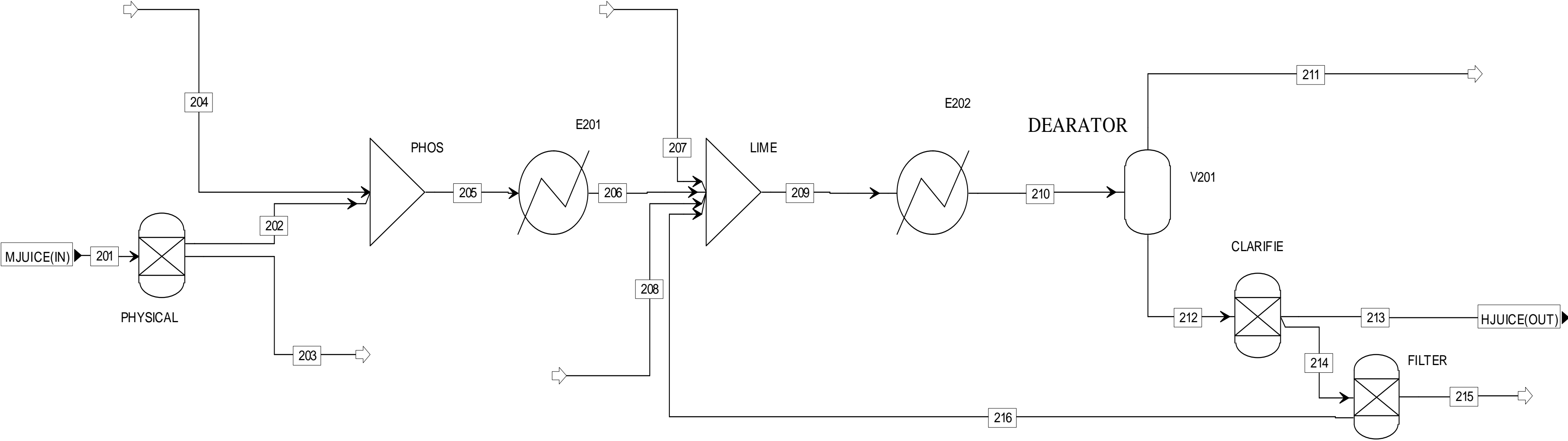

Figure A 15: Area 200: Juice treatment and clarification.

Table A 21: Stream table for Area 200: Juice treatment and clarification.

Juice treatment and clarification									
Stream #		201	202	203	204	205	206	207	208
From			PHYSICAL	PHYSICAL		PHOS	E201		
To		PHYSICAL	PHOS		PHOS	E201	LIME	LIME	LIME
Mass Flow	KG/HR	431868	422058	9810.7	35.5	422093	422093	780.91	15509.8
Phase:		Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
Component Mass Flow									
GLUCOSE	KG/HR	3026.03	3026	0	0	3026	3026.03	0	0
H2O	KG/HR	354118	354118	0	0	354118	354118	0	15509.8
SUCROSE	KG/HR	64913.3	64913	0	0	64913	64913.3	0	0
K2O	KG/HR	986	0	986	0	0	0	0	0
KCL	KG/HR	8824.7	0	8824.7	0	0	0	0	0
LIME	KG/HR	0	0	0	0	0	0	780.91	0
H3PO4	KG/HR	0	0	0	35.5	35.5	35.5	0	0
Component Mass Fraction									
GLUCOSE		0.01	0.01	0	0	0.01	0.01	0	0
H2O		0.82	0.84	0	0	0.84	0.84	0	1
SUCROSE		0.15	0.15	0	0	0.15	0.15	0	0
KCL		0.02	0	0.9	0	0	0	0	0
LIME		0	0	0	0	0	0	1	0
H3PO4		0	0	0	1	0	0	0	0
Volume Flow	L/MIN	6929.77	6724.9	229.95	0.47	6725.1	6849.63	56.34	264.39
Temperature	K	303.15	303.15	303.15	298.15	303.15	343.15	298.15	343.15
Pressure	BAR	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01
Vapor Fraction		0	0	0	0	0	0	0	0
Liquid Fraction		1	1	1	1	1	1	1	1
Solid Fraction		0	0	0	0	0	0	0	0
Mass Density	GM/CC	1.04	1.05	0.71	1.25	1.05	1.03	0.23	0.98

Table A 23: Stream table for Area 200: Juice treatment and clarification (continued).

Juice treatment and clarification									
Stream #		209	210	211	212	213	214	215	216
From		LIME	E202	V201	V201	CLARIFIE	CLARIFIE	FILTER	FILTER
To		E202	V201		CLARIFIE		FILTER		LIME
Mass Flow	KG/HR	440572	440572	0	440572	437556	3015.18	827.4	2187.78
Phase:		Liquid	Liquid	Missing	Liquid	Liquid	Liquid	Liquid	Liquid
Component Mass Flow									
GLUCOSE	KG/HR	3041.16	3041.2	0	3041.2	3026	15.21	0.08	15.13
H2O	KG/HR	371476	371476	0	371476	369619	1857.38	9.29	1848.09
SUCROSE	KG/HR	65237.9	65238	0	65238	64912	326.19	1.63	324.56
K2O	KG/HR	0	0	0	0	0	0	0	0
KCL	KG/HR	0	0	0	0	0	0	0	0
LIME	KG/HR	780.91	780.91	0	780.91	0	780.91	780.91	0
H3PO4	KG/HR	35.5	35.5	0	35.5	0	35.5	35.5	0
Component Mass Fraction									
GLUCOSE		0.01	0.01		0.01	0.01	0.01	0	0.01
H2O		0.84	0.84		0.84	0.84	0.62	0.01	0.84
SUCROSE		0.15	0.15		0.15	0.15	0.11	0	0.15
KCL		0	0		0	0	0	0	0
LIME		0	0		0	0	0.26	0.94	0
H3PO4		0	0		0	0	0.01	0.04	0
Volume Flow	L/MIN	7207.72	7375.6	0	7333.3	7238.6	93.82	57.56	36.19
Temperature	K	343.27	378.15		370.15	370.15	370.15	370.15	370.15
Pressure	BAR	1.01	2.03	1.01	1.01	1.01	1.01	1.01	1.01
Vapor Fraction		0	0		0	0	0	0	0
Liquid Fraction		1	1		1	1	1	1	1
Solid Fraction		0	0		0	0	0	0	0
Mass Density	GM/CC	1.02	1		1	1.01	0.54	0.24	1.01

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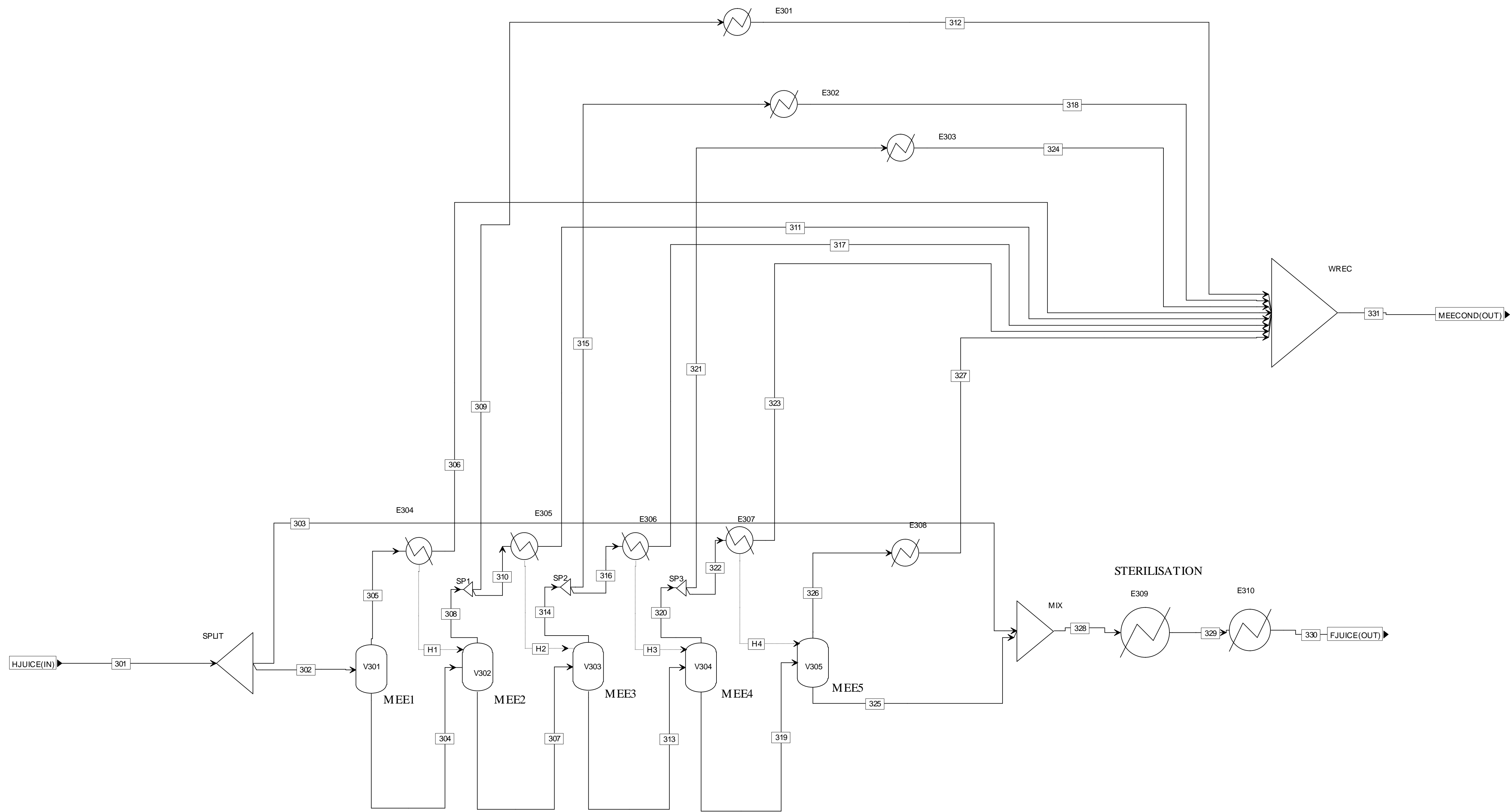

Figure A 16: Area 300: Juice concentration and sterilisation.

Table A 22: Stream table for Area 300: Juice concentration and sterilisation

Juice concentration and sterilisation													
Stream #		301	302	303	304	305	306	307	308	309	310	311	312
From			SPLIT	SPLIT	V301	V301	E304	V302	V302	SP1	SP1	E305	E301
To		SPLIT	V301	MIX	V302	E304	WREC	V303	SP1	E301	E305	WREC	WREC
Mass Flow	KG/HR	437556.3	196900.3	240656	165714.5	31185.84	31185.84	132901	32813.52	3281.35	29532.17	29532.17	3281.35
Phase:		Liquid	Liquid	Liquid	Liquid	Vapor	Liquid	Liquid	Vapor	Vapor	Vapor	Liquid	Liquid
Component Mass Flow													
GLUCOSE	KG/HR	3025.96	1361.68	1664.28	1361.68	0	0	1361.68	0	0	0	0	0
H2O	KG/HR	369618.7	166328.4	203290.3	135142.6	31185.81	31185.81	102329.1	32813.48	3281.35	29532.13	29532.13	3281.35
SUCROSE	KG/HR	64911.68	29210.26	35701.42	29210.22	0.04	0.04	29210.18	0.04	0	0.04	0.04	0
Component Mass Fraction													
GLUCOSE		0.01	0.01	0.01	0.01	0	0	0.01	0	0	0	0	0
H2O		0.84	0.84	0.84	0.82	1	1	0.77	1	1	1	1	1
SUCROSE		0.15	0.15	0.15	0.18	0	0	0.22	0	0	0	0	0
Volume Flow	L/MIN	7238.62	3257.38	3981.24	2753.84	543782.5	549.01	2164.97	705636.8	70563.68	635073.1	517.04	57.45
Temperature	K	370.15	370.15	370.15	388.65	388.65	388.27	381.97	381.97	381.97	381.97	381.49	381.49
Pressure	BAR	1.01	1.01	1.01	1.7	1.7	1.7	1.35	1.35	1.35	1.35	1.35	1.35
Vapor Fraction		0	0	0	0	1	0	0	1	1	1	0	0
Liquid Fraction		1	1	1	1	0	1	1	0	0	0	1	1
Solid Fraction		0	0	0	0	0	0	0	0	0	0	0	0
Mass Density	GM/CC	1.01	1.01	1.01	1	0	0.95	1.02	0	0	0	0.95	0.95

Table A 24: Stream table for Area 300: Juice concentration and sterilisation (continued)

Juice concentration and sterilisation													
Stream #		313	314	315	316	317	318	319	320	321	322	323	324
From		V303	V303	SP2	SP2	E306	E302	V304	V304	SP3	SP3	E307	E303
To		V304	SP2	E302	E306	WREC	WREC	V305	SP3	E303	E307	WREC	WREC
Mass Flow	KG/HR	101870.2	31030.81	3103.08	27927.73	27927.73	3103.08	71896.37	29973.8	2997.38	26976.42	26976.42	2997.38
Phase:		Liquid	Vapor	Vapor	Vapor	Liquid	Liquid	Liquid	Vapor	Vapor	Vapor	Liquid	Liquid
Component Mass Flow													
GLUCOSE	KG/HR	1361.68	0	0	0	0	0	1361.68	0	0	0	0	0
H2O	KG/HR	71298.35	31030.77	3103.08	27927.69	27927.69	3103.08	41324.58	29973.77	2997.38	26976.39	26976.39	2997.38
SUCROSE	KG/HR	29210.14	0.04	0	0.04	0.04	0	29210.1	0.03	0	0.03	0.03	0
Component Mass Fraction													
GLUCOSE		0.01	0	0	0	0	0	0.02	0	0	0	0	0
H2O		0.7	1	1	1	1	1	0.57	1	1	1	1	1
SUCROSE		0.29	0	0	0	0	0	0.41	0	0	0	0	0
Volume Flow	L/MIN	1611.98	876913.5	87691.35	789222.2	485.77	53.97	1078.77	1548916	154891.6	1394024	463.55	51.51
Temperature	K	373.71	373.71	373.71	373.71	373.06	373.06	356.88	356.88	356.88	356.88	355.88	355.88
Pressure	BAR	1.01	1.01	1.01	1.01	1.01	1.01	0.53	0.53	0.53	0.53	0.53	0.53
Vapor Fraction		0	1	1	1	0	0	0	1	1	1	0	0
Liquid Fraction		1	0	0	0	1	1	1	0	0	0	1	1
Solid Fraction		0	0	0	0	0	0	0	0	0	0	0	0
Mass Density	GM/CC	1.05	0	0	0	0.96	0.96	1.11	0	0	0	0.97	0.97

Table A 24: Stream table for Area 300: Juice concentration and sterilisation (continued)

Juice concentration and sterilisation								
Stream #		325	326	327	328	329	330	331
From		V305	V305	E308	MIX	E309	E310	WREC
To		MIX	E308	WREC	E309	E310		
Mass Flow	KG/HR	43524.78	28371.59	28371.59	284180.8	284180.8	284180.8	153375.6
Phase:		Liquid	Vapor	Liquid	Liquid	Liquid	Liquid	Mixed
Component Mass Flow								
GLUCOSE	KG/HR	1361.68	0	0	3025.96	3025.96	3025.96	0
H2O	KG/HR	12953.04	28371.55	28371.55	216243.3	216243.3	216243.3	153375.4
SUCROSE	KG/HR	29210.06	0.04	0.04	64911.48	64911.48	64911.48	0.2
Component Mass Fraction								
GLUCOSE		0.03	0	0	0.01	0.01	0.01	0
H2O		0.3	1	1	0.76	0.76	0.76	1
SUCROSE		0.67	0	0	0.23	0.23	0.23	0
Volume Flow	L/MIN	584.4	3657688	480.97	4566.34	4693.45	4420.17	1190679
Temperature	K	335.83	335.83	333.17	366.87	403.15	305.15	333.17
Pressure	BAR	0.2	0.2	0.2	1.01	3.04	1.01	0.2
Vapor Fraction		0	1	0	0	0	0	0.06
Liquid Fraction		1	0	1	1	1	1	0.94
Solid Fraction		0	0	0	0	0	0	0
Mass Density	GM/CC	1.24	0	0.98	1.04	1.01	1.07	0

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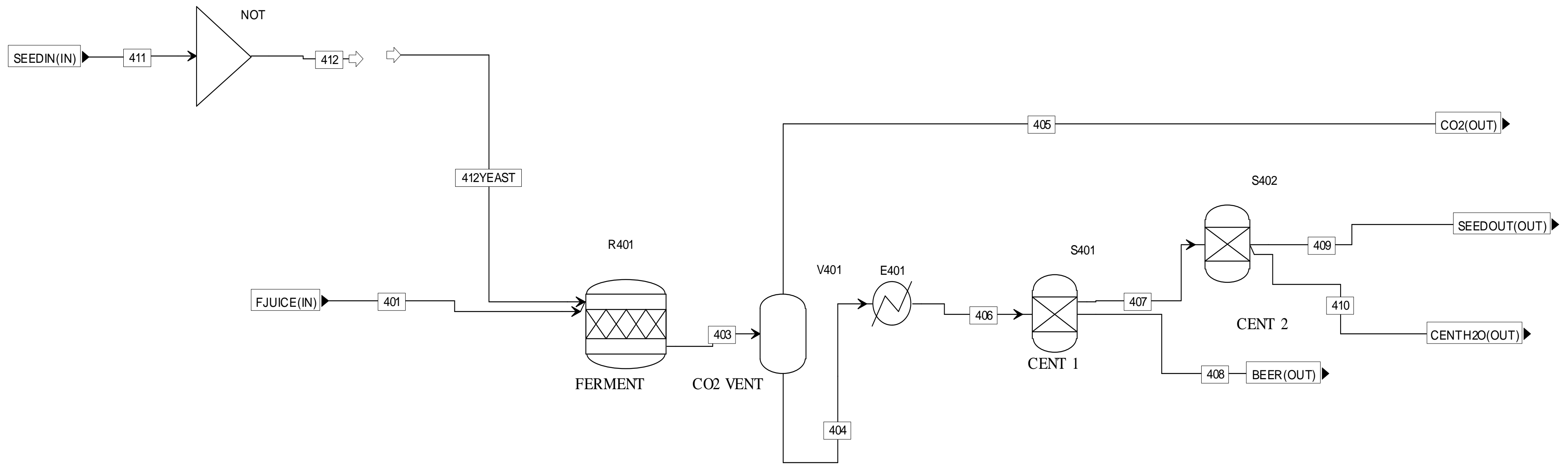

Figure A 17 Area 400: Fermentation

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Table A 23: Stream table for Area 400: Fermentation

Fermentation							
Stream#		401	403	404	405	406	407
From			R 401	V401	V401	E401	S401
To		R 401	V401	E401		S401	S402
Mass Flow	KG/HR	284180.8	290908.6	258220.7	32687.89	258220.7	5389.61
Phase:		Liquid	Mixed	Liquid	Vapor	Liquid	Liquid
Component Mass Flow							
GLUCOSE	KG/HR	3025.96	3484.63	3484.63	0	3484.63	0
MICROORG	KG/HR	0	1883	1883	0	1883	1883
ETHANOL	KG/HR	0	33062.99	32107.18	955.81	32107.18	0
H2O	KG/HR	216243.3	217669	217098.5	570.56	217098.5	3497
CO2	KG/HR	0	31587.94	426.43	31161.51	426.43	0
AACID	KG/HR	0	0.94	0.94	0	0.94	0
GLYCEROL	KG/HR	0	9.32	9.32	0	9.32	0
SUCROSE	KG/HR	64911.48	0	0	0	0	0
FRUCTOSE	KG/HR	0	3201.11	3201.11	0	3201.11	0
Component Mass Fraction							
GLUCOSE		0.01	0.01	0.01	0	0.01	0
MICROORG		0	0.01	0.01	0	0.01	0.35
H2O		0.76	0.75	0.84	0.02	0.84	0.65
CO2		0	0.11	0	0.95	0	0
SUCROSE		0.23	0	0	0	0	0
FRUCTOSE		0	0.01	0.01	0	0.01	0
Volume Flow	L/MIN	4420.17	322672.8	4579.86	287054.9	4561.68	226.07
Temperature	K	305.15	305.15	305.36	305.36	297.15	297.15
Pressure	BAR	1.01	1.01	1.11	1.11	1.01	1.01
Vapor Fraction		0	0.06	0	1	0	0
Liquid Fraction		1	0.94	1	0	1	1
Solid Fraction		0	0	0	0	0	0
Mass Density	GM/CC	1.07	0.02	0.94	0	0.94	0.4

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Table A 25: Stream table for Area 400: Fermentation (continued)

Fermentation							
Stream#		408	409	410	411	412	412YEAST
From		S401	S402	S402		NOT	
To					NOT		R 401
Mass Flow	KG/HR	252831.1	2699.61	2690	7410.14	7410.14	6725
Phase:		Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
Component Mass Flow							
GLUCOSE	KG/HR	3484.63	0	0	0	0	0
MICROORG	KG/HR	0	1883	0	1791.3	1791.3	1883
ETHANOL	KG/HR	32107.18	0	0	0	0	0
H2O	KG/HR	213601.5	807	2690	5609.7	5609.7	4842
CO2	KG/HR	426.43	0	0	0	0	0
AACID	KG/HR	0.94	0	0	0	0	0
GLYCEROL	KG/HR	9.32	0	0	0	0	0
SUCROSE	KG/HR	0	0	0	0	0	0
FRUCTOSE	KG/HR	3201.11	0	0	0	0	0
Component Mass Fraction							
GLUCOSE		0.01	0	0	0	0	0
MICROORG		0	0.7	0	0.24	0.24	0.28
H2O		0.84	0.3	1	0.76	0.76	0.72
CO2		0	0	0	0	0	0
SUCROSE		0	0	0	0	0	0
FRUCTOSE		0.01	0	0	0	0	0
Volume Flow	L/MIN	4335.49	181.12	44.95	253.39	253.39	250.66
Temperature	K	297.15	297.15	297.15	297.89	297.89	305.15
Pressure	BAR	1.01	1.01	1.01	1.01	1.01	1.01
Vapor Fraction		0	0	0	0	0	0
Liquid Fraction		1	1	1	1	1	1
Solid Fraction		0	0	0	0	0	0
Mass Density	GM/CC	0.97	0.25	1	0.49	0.49	0.45

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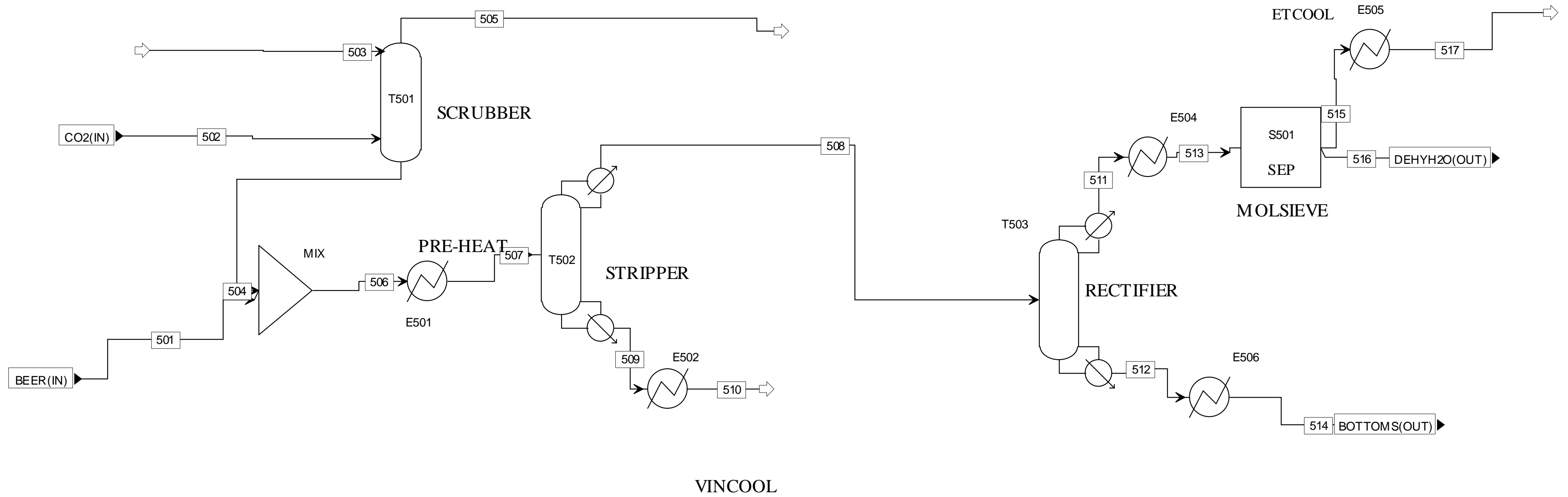

Figure A 18: Area 500: Separation

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Table A 24: Stream table for separation section

Separation								
Stream #		501	502	503	504	505	506	507
From					T501	T501	MIX	E501
To		MIX	T501	T501	MIX		E501	T502
Mass Flow	KG/HR	252831.1	32687.89	79736.31	80938.37	31485.83	333769.4	333769.4
Phase:		Liquid	Vapor	Liquid	Mixed	Vapor	Liquid	Mixed
Component Mass Flow								
GLUCOSE	KG/HR	3484.63	0	0	0	0	3484.63	3484.63
ETHANOL	KG/HR	32107.18	955.81	0	954.79	1.02	33061.97	33061.97
H_2O	KG/HR	213601.5	570.56	79736.31	79877.32	429.55	293478.8	293478.8
CO_2	KG/HR	426.43	31161.51	0	106.25	31055.26	532.68	532.68
AACID	KG/HR	0.94	0	0	0	0	0.94	0.94
GLYCEROL	KG/HR	9.32	0	0	0	0	9.32	9.32
FRUCTOSE	KG/HR	3201.11	0	0	0	0	3201.11	3201.11
Component Mass Fraction								
GLUCOSE		0.01	0	0	0	0	0.01	0.01
ETHANOL		0.13	0.03	0	0.01	0	0.1	0.1
H_2O		0.84	0.02	1	0.99	0.01	0.88	0.88
CO_2		0	0.95	0	0	0.99	0	0
FRUCTOSE		0.01	0	0	0	0	0.01	0.01
Volume Flow	L/MIN	4335.49	287054.9	1332.71	1359.44	296392.2	5694.7	13931.15
Temperature	K	297.15	305.36	298.15	302.22	298.62	298.33	345.15
Pressure	BAR	1.01	1.11	1.01	1.01	1.01	1.01	1.01
Vapor Fraction		0	1	0	0	1	0	0
Liquid Fraction		1	0	1	1	0	1	1
Solid Fraction		0	0	0	0	0	0	0
Mass Density	GM/CC	0.97	0	1	0.99	0	0.98	0.4

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Table A 26: Stream table for separation section (continued)

Separation								
Stream #		508	509	510	511	512	513	514
From		T502	T502	E502	T503	T503	E504	E506
To		T503	E502		E504	E506	S501	
Mass Flow	KG/HR	60094.7	273674.7	273674.7	36367.87	23726.83	36367.87	23726.83
Phase:		Vapor	Liquid	Liquid	Vapor	Liquid	Vapor	Liquid
Component Mass Flow								
GLUCOSE	KG/HR	0	3484.63	3484.63	0	0	0	0
ETHANOL	KG/HR	33052.08	9.89	9.89	33040.21	11.86	33040.21	11.86
H_2O	KG/HR	26509.9	266968.9	266968.9	2794.98	23714.93	2794.98	23714.93
CO_2	KG/HR	532.68	0	0	532.68	0	532.68	0
AACID	KG/HR	0.04	0.9	0.9	0	0.04	0	0.04
GLYCEROL	KG/HR	0	9.32	9.32	0	0	0	0
FRUCTOSE	KG/HR	0	3201.11	3201.11	0	0	0	0
Component Mass Fraction								
GLUCOSE		0	0.01	0.01	0	0	0	0
ETHANOL		0.55	0	0	0.91	0	0.91	0
H_2O		0.44	0.98	0.98	0.08	1	0.08	1
CO_2		0.01	0	0	0.01	0	0.01	0
FRUCTOSE		0	0.01	0.01	0	0	0	0
Volume Flow	L/MIN	727667.2	4822.78	4595.06	313363.2	417.5	344194.2	397.84
Temperature	K	375.16	388.22	308.15	359.13	387.46	393.15	308.15
Pressure	BAR	1.55	1.69	1.01	1.38	1.65	1.38	1.01
Vapor Fraction		1	0	0	1	0	1	0
Liquid Fraction		0	1	1	0	1	0	1
Solid Fraction		0	0	0	0	0	0	0
Mass Density	GM/CC	0	0.95	0.99	0	0.95	0	0.99

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Table A 26: Stream table for separation section (continued)

Separation				
Stream #		515	516	517
From		S501	S501	E505
To		E505		
Mass Flow	KG/HR	33272.48	3095.4	33272.48
Phase:		Vapor	Vapor	Liquid
Component Mass Flow				
GLUCOSE	KG/HR	0	0	0
ETHANOL	KG/HR	33040.21	0	33040.21
H2O	KG/HR	232.26	2562.71	232.26
CO2	KG/HR	0	532.68	0
AACID	KG/HR	0	0	0
GLYCEROL	KG/HR	0	0	0
FRUCTOSE	KG/HR	0	0	0
Component Mass Fraction				
GLUCOSE		0	0	0
ETHANOL		0.99	0	0.99
H2O		0.01	0.83	0.01
CO2		0	0.17	0
FRUCTOSE		0	0	0
Volume Flow	L/MIN	299541.2	60451.11	716.95
Temperature	K	388.71	393.15	308.15
Pressure	BAR	1.29	1.38	1.28
Vapor Fraction		1	1	0
Liquid Fraction		0	0	1
Solid Fraction		0	0	0
Mass Density	GM/CC	0	0	0.77

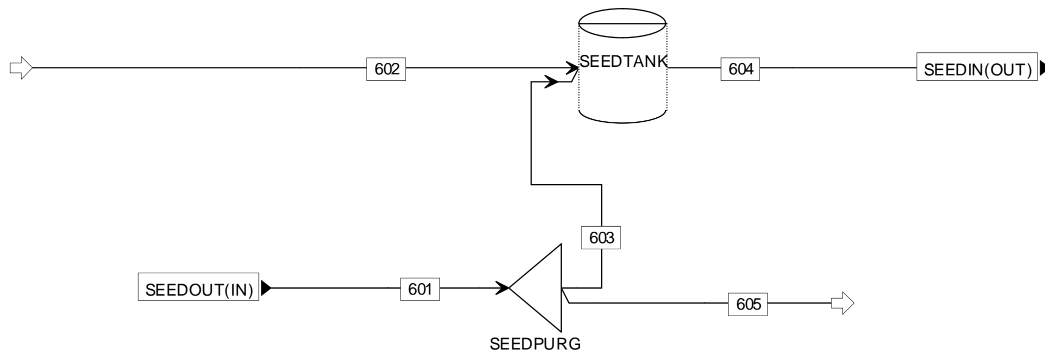

Figure A 19: Area 600: Yeast recycling.

Table A 25: Stream table for yeast recycling section

Yeast Recycle						
Stream#		601	602	603	604	605
From				SEEDPURG	SEEDTANK	SEEDPURG
To		SEEDPURG	SEEDTANK	SEEDTANK		
Mass Flow	KG/HR	2699.61	4842	2568.14	7410.14	131.47
Phase:		Liquid	Liquid	Liquid	Liquid	Liquid
Component Mass Flow						
MICROORG	KG/HR	1883	0	1791.3	1791.3	91.7
H2O	KG/HR	807	4842	767.7	5609.7	39.3
Component Mass Fraction						
MICROORG		0.7	0	0.7	0.24	0.7
H2O		0.3	1	0.3	0.76	0.3
Volume Flow	L/MIN	181.12	80.93	172.3	253.39	8.82
Temperature	K	297.15	298.15	297.15	297.89	297.15
Pressure	BAR	1.01	1.01	1.01	1.01	1.01
Vapor Fraction		0	0	0	0	0
Liquid Fraction		1	1	1	1	1
Solid Fraction		0	0	0	0	0
Mass Density	GM/CC	0.25	1	0.25	0.49	0.25

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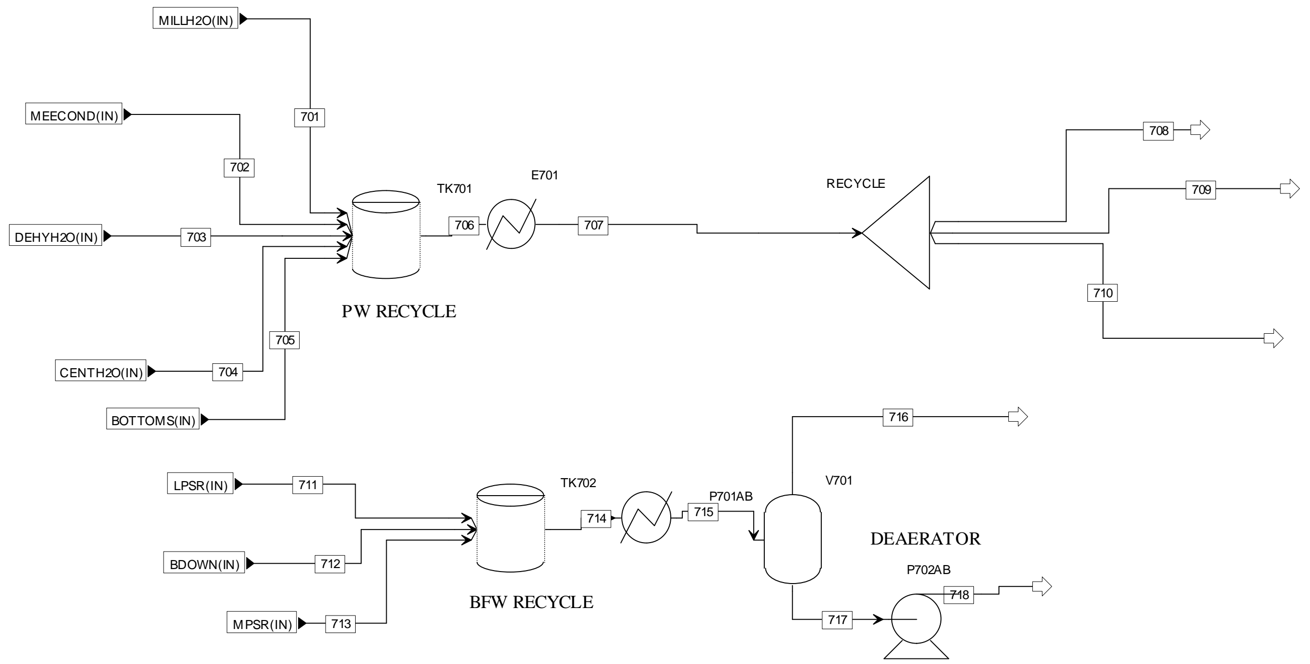

Figure A 20: Area 700: Water treatment.

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Table A 26: Stream table for Area 700: Water treatment

Water treatment										
Stream #		701	702	703	704	705	706	707	708	709
From							TK701	E701	RECYCLE	RECYCLE
To		TK701	TK701	TK701	TK701	TK701	E701	RECYCLE		
Mass Flow	KG/HR	117144.5	153375.6	3095.4	2690	23726.83	300032.2	300032.2	17787.4	279713.3
Phase:		Liquid	Mixed	Vapor	Liquid	Liquid	Mixed	Mixed	Mixed	Mixed
Component Mass Flow										
ETHANOL	KG/HR	0	0	0	0	11.86	11.86	11.86	0.7	11.06
H2O	KG/HR	117144.5	153375.4	2562.71	2690	23714.93	299487.5	299487.5	17755.1	279205.4
CO2	KG/HR	0	0	532.68	0	0	532.68	532.68	31.58	496.61
AACID	KG/HR	0	0	0	0	0.04	0.04	0.04	0	0.04
SUCROSE	KG/HR	0	0.2	0	0	0	0.2	0.2	0.01	0.18
Component Mass Fraction										
H2O		1	1	0.83	1	1	1	1	1	1
CO2		0	0	0.17	0	0	0	0	0	0
Volume Flow	L/MIN	1974.19	1190679	60451.11	44.95	397.84	1126102	5993.98	355.35	5588.05
Temperature	K	321.17	333.17	393.15	297.15	308.15	332.63	298.15	298.15	298.15
Pressure	BAR	1.01	0.2	1.38	1.01	1.01	0.2	1.01	1.01	1.01
Vapor Fraction		0	0.06	1	0	0	0.03	0	0	0
Liquid Fraction		1	0.94	0	1	1	0.97	1	1	1
Solid Fraction		0	0	0	0	0	0	0	0	0
Mass Density	GM/CC	0.99	0	0	1	0.99	0	0.83	0.83	0.83

Table A 28: Stream table for Area 700: Water treatment (continued)

Water treatment										
Stream #		710	711	712	713	714	715	716	717	718
From		RECYCLE				TK702	P701AB	V701	V701	P702AB
To			TK702	TK702	TK702	P701AB	V701		P702AB	
Mass Flow	KG/HR	2531.56	213657	0	25143	238800	238800	0	238800	238800
Phase:		Mixed	Vapor	Missing	Vapor	Vapor	Liquid	Missing	Liquid	Liquid
Component Mass Flow										
ETHANOL	KG/HR	0.1	0	0	0	0	0	0	0	0
H2O	KG/HR	2526.97	213657	0	25143	238800	238800	0	238800	238800
CO2	KG/HR	4.49	0	0	0	0	0	0	0	0
AACID	KG/HR	0	0	0	0	0	0	0	0	0
SUCROSE	KG/HR	0	0	0	0	0	0	0	0	0
Component Mass Fraction										
H2O		1	1		1	1	1		1	1
CO2		0	0		0	0	0		0	0
Volume Flow	L/MIN	50.57	6004812	0	642535.7	3548880	4227.38	0	4239.61	4223.21
Temperature	K	298.15	412.91		376.1	410.47	394.95		398.15	399.55
Pressure	BAR	1.01	1.12	90	1.12	2.1	4	2.5	2.5	95
Vapor Fraction		0	1		1	1	0		0	0
Liquid Fraction		1	0		0	0	1		1	1
Solid Fraction		0	0		0	0	0		0	0
Mass Density	GM/CC	0.83	0		0	0	0.94		0.94	0.94

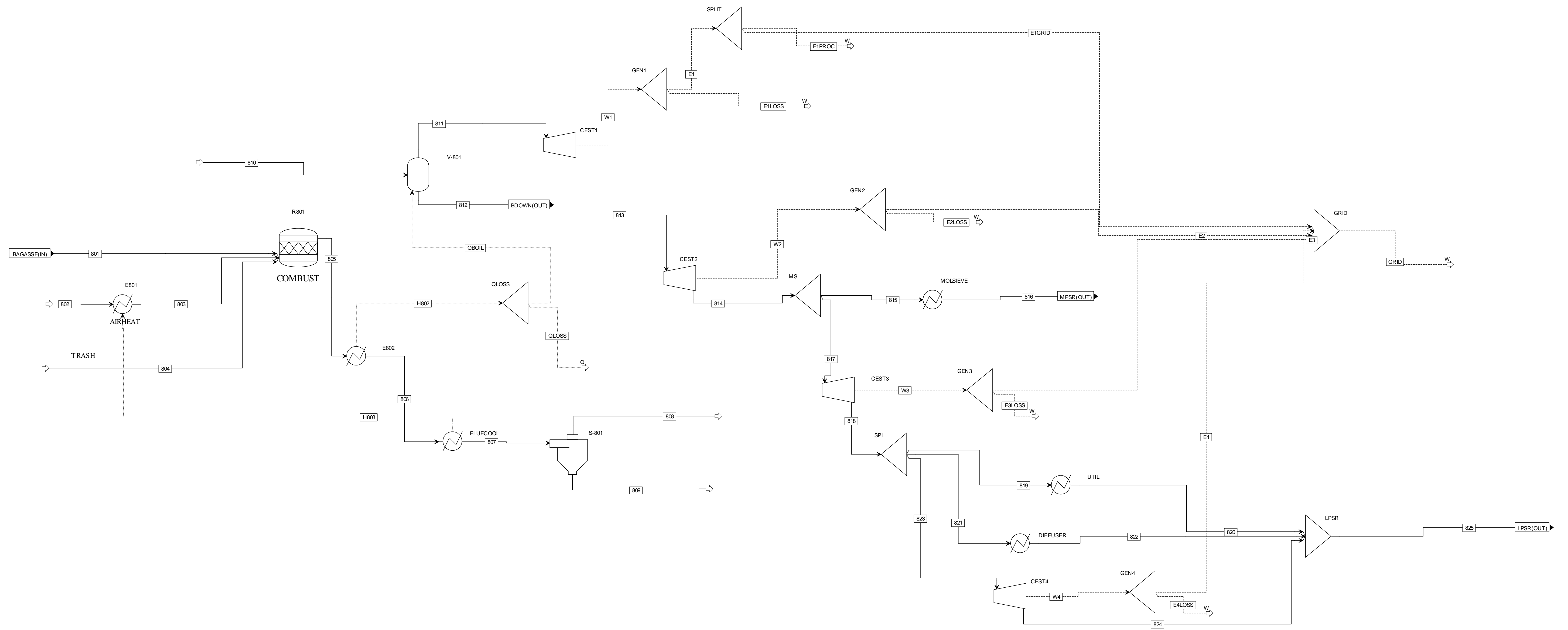

Figure A 21: Area 800: Cogeneration of heat and power

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Table A 27: Stream table for Area 800: Co-generation of steam and power

Cogeneration of heat and power								
Stream#		801	802	803	804	805	806	807
From				E801		R 801	E802	FLUECOOL
To		R 801	E801	R 801	R 801	E802	FLUECOOL	S-801
Mass Flow	KG/HR	118903.7	916000	916000	81200	1116104	1116104	1116104
Phase:		Liquid	Vapor	Vapor	Liquid	Mixed	Mixed	Mixed
Component Mass Flow								
GLUCOSE	KG/HR	30.57	0	0	0	30.57	30.57	30.57
CELLULOS	KG/HR	23516.1	0	0	27619.58	51135.68	51135.68	51135.68
XYLAN	KG/HR	22332.9	0	0	26229.92	48562.82	48562.82	48562.82
LIGNIN	KG/HR	12916.6	0	0	15170.5	28087.1	28087.1	28087.1
H2O	KG/HR	59451.85	0	0	12180	71631.85	71631.85	71631.85
N2	KG/HR	0	702648	702648	0	702648	702648	702648
O2	KG/HR	0	213352	213352	0	213352	213352	213352
SUCROSE	KG/HR	655.69	0	0	0	655.69	655.69	655.69
Component Mass Fraction								
CELLULOS		0.2	0	0	0.34	0.05	0.05	0.05
XYLAN		0.19	0	0	0.32	0.04	0.04	0.04
LIGNIN		0.11	0	0	0.19	0.03	0.03	0.03
H2O		0.5	0	0	0.15	0.06	0.06	0.06
N2		0	0.77	0.77	0	0.63	0.63	0.63
O2		0	0.23	0.23	0	0.19	0.19	0.19
Volume Flow	L/MIN	1696.14	12940030	25087630	993.2	57838250	25561640	15924810
Temperature	K	321.17	298.15	577.63	298.15	1143.15	523.15	333.15
Pressure	BAR	1.01	1.01	1.01	1.01	0.98	1.01	1.01
Vapor Fraction		0	1	1	0	0.98	0.98	0.96
Liquid Fraction		1	0	0	1	0.02	0.02	0.04
Solid Fraction		0	0	0	0	0	0	0
Mass Density	GM/CC	1.17	0	0	1.36	0	0	0

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Table A 29: Stream table for Area 800: Co-generation of steam and power (continued)

Cogeneration of heat and power								
Stream#		808	809	810	811	812	813	814
From		S-801	S-801		V-801	V-801	CEST1	CEST2
To				V-801	CEST1		CEST2	MS
Mass Flow	KG/HR	1116104	0	238800	238800	0	238800	238800
Phase:		Mixed	Missing	Liquid	Vapor	Missing	Vapor	Vapor
Component Mass Flow								
GLUCOSE	KG/HR	30.57	0	0	0	0	0	0
CELLULOS	KG/HR	51135.68	0	0	0	0	0	0
XYLAN	KG/HR	48562.82	0	0	0	0	0	0
LIGNIN	KG/HR	28087.1	0	0	0	0	0	0
H2O	KG/HR	71631.85	0	238800	238800	0	238800	238800
N2	KG/HR	702648	0	0	0	0	0	0
O2	KG/HR	213352	0	0	0	0	0	0
SUCROSE	KG/HR	655.69	0	0	0	0	0	0
Component Mass Fraction								
CELLULOS		0.05		0	0		0	0
XYLAN		0.04		0	0		0	0
LIGNIN		0.03		0	0		0	0
H2O		0.06		1	1		1	1
N2		0.63		0	0		0	0
O2		0.19		0	0		0	0
Volume Flow	L/MIN	15924810	0	4223.04	150671.6	0	525721.7	1475020
Temperature	K	333.15		399.5	799.13		624.1	491.82
Pressure	BAR	1.01		95	90	90	21	6
Vapor Fraction		0.96		0	1		1	1
Liquid Fraction		0.04		1	0		0	0
Solid Fraction		0		0	0		0	0
Mass Density	GM/CC	0		0.94	0.03		0.01	0

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Table A 29: Stream table for Area 800: Co-generation of steam and power (continued)

Cogeneration of heat and power								
Stream#		815	816	817	818	819	820	821
From		MS	MOLSIEVE	MS	CEST3	SPL	UTIL	SPL
To		MOLSIEVE		CEST3	SPL	UTIL	LPSR	DIFFUSER
Mass Flow	KG/HR	25143	25143	213657	213657	111121	111121	33524
Phase:		Vapor	Vapor	Vapor	Vapor	Vapor	Vapor	Vapor
Component Mass Flow								
GLUCOSE	KG/HR	0	0	0	0	0	0	0
CELLULOS	KG/HR	0	0	0	0	0	0	0
XYLAN	KG/HR	0	0	0	0	0	0	0
LIGNIN	KG/HR	0	0	0	0	0	0	0
H2O	KG/HR	25143	25143	213657	213657	111121	111121	33524
N2	KG/HR	0	0	0	0	0	0	0
O2	KG/HR	0	0	0	0	0	0	0
SUCROSE	KG/HR	0	0	0	0	0	0	0
Component Mass Fraction								
CELLULOS		0	0	0	0	0	0	0
XYLAN		0	0	0	0	0	0	0
LIGNIN		0	0	0	0	0	0	0
H2O		1	1	1	1	1	1	1
N2		0	0	0	0	0	0	0
O2		0	0	0	0	0	0	0
Volume Flow	L/MIN	155303.2	642535.7	1319716	2691802	1399981	2839725	422359
Temperature	K	491.82	376.1	491.82	415.02	415.02	376.1	415.02
Pressure	BAR	6	1.12	6	2.5	2.5	1.12	2.5
Vapor Fraction		1	1	1	1	1	1	1
Liquid Fraction		0	0	0	0	0	0	0
Solid Fraction		0	0	0	0	0	0	0
Mass Density	GM/CC	0	0	0	0	0	0	0

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Table A 29: Stream table for Area 800: Co-generation of steam and power (continued)

Cogeneration of heat and power					
Stream#		822	823	824	825
From		DIFFUSER	SPL	CEST4	LPSR
To		LPSR	CEST4	LPSR	
Mass Flow	KG/HR	33524	69012	69012	213657
Phase:		Vapor	Vapor	Mixed	Vapor
Component Mass Flow					
GLUCOSE	KG/HR	0	0	0	0
CELLULOS	KG/HR	0	0	0	0
XYLAN	KG/HR	0	0	0	0
LIGNIN	KG/HR	0	0	0	0
H2O	KG/HR	33524	69012	69012	213657
N2	KG/HR	0	0	0	0
O2	KG/HR	0	0	0	0
SUCROSE	KG/HR	0	0	0	0
Component Mass Fraction					
CELLULOS		0	0	0	0
XYLAN		0	0	0	0
LIGNIN		0	0	0	0
H2O		1	1	1	1
N2		0	0	0	0
O2		0	0	0	0
Volume Flow	L/MIN	1487912	869461.9	1729735	6004812
Temperature	K	649.25	415.02	376.06	412.91
Pressure	BAR	1.12	2.5	1.12	1.12
Vapor Fraction		1	1	0.98	1
Liquid Fraction		0	0	0.02	0
Solid Fraction		0	0	0	0
Mass Density	GM/CC	0	0	0	0

APPENDIX P: ASPEN PLUS® UNIT OPERATION SUMMARIES

Table A 28: Unit operation summary for Area 100: Sugarcane cleaning and sucrose extraction

Area 100		
Name	E101	E102
Specified pressure [atm]	1	1
Specified temperature [C]	98	30
Specified vapor fraction		
Calculated pressure [bar]	1.01325	1.01325
Calculated temperature [K]	371.15	303.15
Calculated vapor fraction	0	0
Calculated heat duty [kW]	15105.5	-7905.59
Net duty [kW]	15105.5	-7905.59

Table A 29: Unit operation summary for Area 200: Juice treatment and clarification.

Area 200		
Name	E201	E202
Specified pressure [atm]	1	2
Specified temperature [C]	70	105
Specified vapor fraction		
Calculated pressure [bar]	1.01325	2.0265
Calculated temperature [K]	343.15	378.15
Calculated vapor fraction	0	0
Calculated heat duty [kW]	17414.1	16058.8
Net duty [kW]	17414.1	16058.8

Table A 30: Unit operation summary for Area 300: Juice concentration and sterilisation

Area 300							
Name	E301	E302	E303	E304	E305	E306	E307
Specified pressure [atm]	1.33629	0.996793	0.522082	1.67382	1.33629	0.996793	0.522082
Specified temperature [C]							
Specified vapor fraction	0	0	0	0	0	0	0
Calculated pressure [bar]	1.354	1.01	0.529	1.696	1.354	1.01	0.529
Calculated temperature [K]	381.488	373.058	355.877	388.267	381.488	373.058	355.877
Calculated vapor fraction	0	0	0	0	0	0	0
Calculated heat duty [kW]	-2048.2	-1954.95	-1923.22	-19319.2	-18433.8	-17594.5	-17309
Net duty [kW]	-2048.2	-1954.95	-1923.22	-19319.2	-18433.8	-17594.5	-17309

Table A 32: Unit operation summary for Area 300: Juice concentration and sterilisation (continued)

Area 300			
Name	E308	E309	E310
Specified pressure [atm]	0.197385	3	1
Specified temperature [C]		130	32
Specified vapor fraction	0		
Calculated pressure [bar]	0.2	3.03975	1.01325
Calculated temperature [K]	333.173	403.15	305.15
Calculated vapor fraction	0	0	0
Calculated heat duty [kW]	-18649.9	10223.2	-27277.2
Net duty [kW]	-18649.9	10223.2	-27277.2

Table A 31: Unit operation summary for Area 400: Fermentation

Area 400	
Name	E401
Specified pressure [atm]	1
Specified temperature [C]	24
Specified vapor fraction	
Calculated pressure [bar]	1.01325
Calculated temperature [K]	297.15
Calculated vapor fraction	0
Calculated heat duty [kW]	-2333.47
Net duty [kW]	-2333.47

Table A 32: Unit operation summary for Area 500: Separation

Area 500					
Name	E501	E502	E503	E504	E505
Specified pressure [atm]	1	1	1.36195	1.26	1
Specified temperature [C]	72	35	120	35	35
Specified vapor fraction					
Calculated pressure [bar]	1.01325	1.01325	1.38	1.2767	1.01325
Calculated temperature [K]	345.15	308.15	393.15	308.15	308.15
Calculated vapor fraction	0.00101192	0	1	0	0
Calculated heat duty [kW]	17651.7	-25095.3	586.495	-9771.48	-2193.59
Net duty [kW]	17651.7	-25095.3	586.495	-9771.48	-2193.59

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Table A 33: Unit operation summary for Area 700: Water treatment.

Area 700		
Name	E701	P701AB
Specified pressure [atm]	1	3.94769
Specified temperature [C]	25	121.8
Specified vapor fraction		
Calculated pressure [bar]	1.01325	4
Calculated temperature [K]	298.15	394.95
Calculated vapor fraction	0.00014507	0
Calculated heat duty [kW]	-17658.5	-148754
Net duty [kW]	-17658.5	-148754

Table A 34: Unit operation summary for Area 800: Cogeneration

Area 800						
Name	DIFSTEAM	E801	E802	FLUECOOL	MOLSIEVE	UTIL
Specified pressure [atm]	1.10856	1	1	1	1.10856	1.10856
Specified temperature [C]	376.1		250	60	102.95	102.95
Specified vapor fraction						
Calculated pressure [bar]	1.12325	1.01325	1.01325	1.01325	1.12325	1.12325
Calculated temperature [K]	649.25	577.626	523.15	333.15	376.1	376.1
Calculated vapor fraction	1	1	0.976257	0.955727	1	1
Calculated heat duty [kW]	4358.41	73209.7	-227825	-73210	-1490.23	-2186.41
Net duty [kW]	4358.41	0	-227825	-73210	-1490.23	-2186.41

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APPENDIX Q: HENSAD PRINTOUTS

Below is a summary report that was generated by the HENSAD software of the heat integration problem.

Table of Results

Minimum Temperature Approach = 10℃

Hot Stream Data

Mass Flow kg/s	Cp kJ/kg/℃	Temp In ℃	Temp Out ° C	Stream Enthalpy kW	Film Heat Transf. Coef W/m2/℃
.9114	4884.	108.8	108.3	2048.	1000.
.8619	3436.	100.5	99.91	1955.	1000.
.8326	2309.	83.73	82.73	1923.	1000.
7.880	954.2	62.68	60.20	18649	1000.
78.93	3.525	130.0	32.00	27277	560.0
71.72	4.065	32.00	24.00	2333.	560.0
125.9	41.54	105.9	102.1	19887	1000.
76.02	4.152	115.0	35.00	25255	560.0
6.590	4.197	114.3	35.00	2194.	560.0
37.07	1098.	86.60	85.97	25648	1000.
9.242	13.05	116.0	35.00	9770.	560.0

Cumulative Hot Stream Energy Available = 136941.4 kW

Cold Stream Data

Mass Flow kg/s	Cp kJ/kg/℃	Temp In ℃	Temp Out ° C	Stream Enthalpy kW	Film Heat Transf. Coef W/m2/℃
117.2	3.713	30.00	70.00	-17414	560.0
122.3	3.749	70.00	105.0	-16058	560.0
78.93	3.569	93.72	130.0	-10222	560.0
92.71	4.072	25.00	72.00	-17743	560.0
101.6	1826.	114.7	115.0	-57531	1000.
11.69	1888.	113.7	114.3	-11488	1000.

Cumulative Cold Stream Energy Available = -130459.3 kW

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Data for Generating Temperature Interval Diagram

Number of Temperature Intervals = 27

Interval	Temperature Range ℃	℃	Excess Heat kW	Cummulative Q k W
A	140.0	130.0	-2817.	-2817.
B	130.0	125.0	-16.97	-2834.
C	125.0	124.7	-57532	-60366
D	124.7	124.3	-1.549	-60368
E	124.3	123.7	-11490	-71858
F	123.7	116.0	-26.82	-71885
G	116.0	115.0	117.1	-71768
H	115.0	114.3	-18.17	-71786
I	114.3	108.8	9.385	-71776
J	108.8	108.3	2048.	-69728
K	108.3	105.9	4.163	-69724
L	105.9	103.7	11413	-58310
M	103.7	102.1	8937.	-49373
N	102.1	100.5	433.7	-48939
O	100.5	99.91	2142.	-46797
P	99.91	86.60	3773.	-43024
Q	86.60	85.97	25826	-17197
R	85.97	83.73	635.0	-16562
S	83.73	82.73	2206.	-14355
T	82.73	82.00	206.9	-14148
U	82.00	80.00	-188.0	-14337
V	80.00	62.68	-1222.	-15559
W	62.68	60.20	18474	2915.
X	60.20	40.00	-1425.	1490.
Y	40.00	35.00	1823.	3314.
Z	35.00	32.00	835.0	4149.
Z	32.00	24.00	2333.	6482.

Pinch Temperature - Hot = 116℃
Pinch Temperature - Cold = 106℃

Hot Utility Requirement = 71885 kW
Cold Utility Requirement = 78367 kW

Minimum Number of Exchanger Required to Accomplish Minimum Utility Loads
In Special Circumstances the Minimum Required may be Lower than Indicated Below

Number Above the Pinch =4
Number Below the Pinch =14

Data for Composite Enthalpy - Temperature Diagram

Temperature ℃	Hot Stream Enthalpy kW	Temperature ℃	Cold Stream Enthalpy kW
24.00	.0000	14.00	78367
32.00	2333.	22.00	78367
35.00	3168.	25.00	78367

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40.00	6879.	30.00	80255
60.20	21874	50.20	96675
62.68	42365	52.68	98691
80.00	55222	70.00	11277
82.00	56706	72.00	11444
82.73	57248	72.73	11477
83.73	59913	73.73	11523
85.97	61576	75.97	11626
86.60	87692	76.60	11655
99.91	97572	89.91	12266
100.5	10001	90.57	12296
102.1	10115	92.10	12366
103.7	11083	93.72	12440
105.9	12386	95.90	12602
108.3	12567	98.34	12783
108.8	12806	98.80	12817
114.3	13214	104.3	13224
115.0	13264	105.0	13276
116.0	13304	106.0	13304
123.7	13521	113.7	13523
124.3	13535	114.3	14687
124.7	13548	114.7	14700
125.0	13556	115.0	20461
130.0	13694	120.0	20600
140.0	13694	130.0	20882

Heat Transfer Area for Process Exchangers in Network = 9651   m2