Production of Bioethanol from Paper Sludge using Simultaneous Saccharification and Fermentation

by

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Abstract

Whereas fuel used for transport and electricity production are mainly fossil–derived, there has recently been an increased focus on bio-fuels due to the impact of fossil derived fuel on the environment as well as the increased energy demand worldwide, concomitant with the depletion of fossil fuel reserves. Paper sludge produced by paper mills are high in lignocellulose and represents a largely untapped feedstock for bio-energy production.

The aim of this study was to determine the composition, fermentability and optimum paper sludge loading and enzyme dosage for producing ethanol from paper sludge. This information was used to develop a model of the process in Aspen Plus®. The mass and energy balances obtained from the Aspen Plus® model were used to develop equipment specifications which were used to source equipment cost data. A techno-economic model was developed from the equipment cost data to assess the economic viability of the simultaneous saccharification and fermentation (SSF) process utilising paper sludge as feedstock.

Nine paper sludge samples obtained from Nampak Tissue (Pty) Ltd. were evaluated in terms of ethanol production and those samples yielding the highest and lowest ethanol titres were selected for optimisation. This allowed for the determination of a range of ethanol concentrations and yields, expressed as percentage of the theoretical maximum, which could be expected on an industrial scale. Response surface methodology was used to obtain quadratic mathematical models to determine the effects of solid loading and cellulase dosage on ethanol production and ethanol yield from paper sludge during anoxic fed-batch fermentations using

Saccharomyces cerevisiae strain MH1000. This approach was augmented with a multi response optimisation approach incorporating a desirability function to determine the optimal solid loading and cellulase dosage in fed-batch SSF cultures. The multi response optimisation revealed that an optimum paper sludge loading of 21% (w/w) and a cellulase loading of 14.5 FPU g^{-1} be used regardless of the paper sludge sample. The fact that one optimal enzyme dosage and paper sludge loading is possible, regardless the paper sludge feed stock, is attractive since the SSF process can be controlled efficiently, while not requiring process alterations to optimize ethanol production when different batches of paper sludge are processed. At the optimum paper sludge loading and cellulase dosage a minimum ethanol concentration of 47.36 g l^{-1} (84.69% of theoretical maximum) can be expected regardless of the paper sludge used.

An economic assessment was conducted to ascertain whether ethanol production from paper sludge using SSF is economically viable. Three scenarios were investigated. In the first scenario revenue was calculated from the ethanol sales linked to the basic fuel price, whereas in the second and third scenarios liquefied petroleum gas (LPG) consumption at the paper mill was replaced with anhydrous and 95% ethanol respectively. In all the cases, paper sludge feed rates of 15, 30 and 50 t d^{-1} were used. The production of ethanol from paper sludge for ethanol sales (scenario 1) resulted in higher IRR and NPV values, as well as shorter payback periods, compared to replacement of LPG at the paper mill (scenarios 2 and 3). At an assumed enzyme cost of $ 0.90 gal^{-1} (R 2.01 $litre^{-1}$), IRR values of 11%, 22% and 30% were obtained at paper sludge feed rates of 15, 30 and 50 t d^{-1}.

A sensitivity analysis performed on the total capital investment and enzyme cost revealed that the SSF process is only economically viable at a paper sludge feed rate of 50 t d^{-1} irrespective of the variation in capital investment. For the SSF process to be economically viable the enzyme costs must be lower than \$ 0.70 gal^{-1} (R 1.56 $litre^{-1}$) and \$ 1.20 gal^{-1} (R 2.68 $litre^{-1}$) for paper sludge feed rates of 30 and 50 t d^{-1} respectively. The SSF process at a paper sludge feed rate of 15 t d^{-1} was not economically viable even assuming a zero enzyme cost.

A Monte Carlo simulation revealed that the SSF process is economically viable at a paper sludge feed rate of 50 t d^{-1} as a mean IRR value of 32% were obtained with a probability of 26% to attain an IRR value lower than 25%. The SSF process at lower paper sludge loadings is not economically viable as probabilities of 70% and 95% were obtained to attain IRR values lower than 25% at paper sludge feed rates of 30 and 15 t d^{-1} respectively.

From this study it can be concluded that paper sludge is an excellent feedstock for ethanol production for the sales of ethanol at a paper sludge feed rate in excess of 50 t d^{-1} with the added environmental benefit of reducing GHG emissions by 42.5%.

Opsomming

Aangesien dat brandstof vir vervoer en energie meestal vanaf fossiel afgeleide bronne kom, is daar onlangs ’n groter fokus op bio-brandstowwe as gevolg van die impak van fossiel afgeleide brandstowwe op die omgewing en 'n verhoogde aanvraag na energie wêreldwyd, gepaardgaande met die uitputting van fossielbrandstof-reserwes. Papier slyk geproduseer deur papier meule is hoog in lignosellulose en verteenwoordig 'n grootliks onontginde grondstof vir etanol produksie.

Die doel van die studie was om vas te stel wat die samestelling, fermenteerbaarheid, optimale papier slyk en ensiem ladings is vir die vervaardiging van etanol uit papier slyk. Die inligting was gebruik om 'n model van die proses in Aspen Plus® te ontwikkel. Die massa-en energiebalanse wat verkry is van die Aspen Plus® model was gebruik om toerusting spesifikasies te ontwikkel wat gebruik was om toerusting kostes te bereken. ‘N tegno-ekonomiese model is ontwikkel om die ekonomiese lewensvatbaarheid van die gelyktydige versuikering en fermentasie proses “SSF” wat gebruik maak van papier slyk as grondstof te assesseer.

Nege papier slyk monsters verkry vanaf Nampak Tissue (Pty) Ltd. is geëvalueer in terme van etanol produksie. Die monsters wat die hoogste en laagste etanol konsentrasies opgelewer het, is geselekteer vir optimalisering omdat dit toegelaat het vir die vasstelling van etanol konsentrasies en opbrengste, uitgedruk as persentasie van die teoretiese maksimum, wat verwag kan word in industrie. Reaksie oppervlak metodologie “RSM” is gebruik om wiskundige modelle te ontwikkel om die impak van papier slyk lading en sellulase dosis op etanol produksie en etanol opbrengs te

assesseer. Die RSM is aangevul met 'n multi effek optimiserings benadering wat 'n wenslikheid funksie inkorporeer om die optimale papier slyk lading en sellulase dosis in gevoerde-enkellading SSF kulture te bepaal. Die multi effek optimalisering het getoon dat 'n optimale papier slyk lading van 21% (w/w) en 'n sellulase dosis van 14.5 FPU g^{-1} gebruik moet word, ongeag van die papier slyk monster. Die feit dat die optimale ensiem dosis en papier slyk lading dieselfde is ongeag die papier slyk monster, is aantreklik aangesien die SSF proses meer doeltreffend beheer kan word omdat proses veranderinge nie nodig is om die proses te optimaliseer nie. By die optimale papier slyk lading en sellulase dosis kan 'n minimum etanol konsentrasie van 47.36 g l^{-1} (84,69% van die teoretiese maksimum) verwag word ongeag van die papier slyk wat gebruik word.

'N ekonomiese evaluasie is gedoen om vas te stel of etanol produksie vanaf papier slyk met behulp van SSF ekonomies lewensvatbaar is. Drie moontlikhede is ondersoek. In die eerste moontlikheid is die inkomste bereken vanaf etanol verkope gekoppel aan die basiese brandstofprys, terwyl in die tweede en derde moontlikhede, LPG by die papier meul vervang is met anhidriese en 95% etanol onderskeidelik. In al die gevalle was daar gebruik gemaak van papier slyk voer tempo’s van 15, 30 en 50 t d^{-1}. Die produksie van etanol uit papier slyk vir verkope (moontlikheid 1) het gelei tot hoër IRR en die NPV waardes, sowel as korter terugverdien tydperke, in vergelyking met die vervanging van LPG by die papier meul (moontlikhede 2 en 3). Met ’n ensiem koste van $ 0.90 gal^{-1} (R 2.01 $litre^{-1}$) is IRR-waardes van 11%, 22% en 30% verkry teen papier slyk voer tempo’s van 15, 30 en 50 t d^{-1} onderskeidelik.

'N sensitiwiteitsanalise uitgevoer op die totale kapitale belegging en ensiem koste het aan die lig gebring dat 'n SSF proses slegs ekonomies lewensvatbaar is op 'n papier

slyk voer tempo van 50 t d^{-1} ongeag van die variasie in die kapitale belegging. Vir die SSF proses om ekonomies lewensvatbaar te wees, moet die ensiem kostes laer wees as \$ 0.70 gal^{-1} (R 1.56 $liter^{-1}$) en \$ 1.20 gal-1 (R 2.68 $liter^{-1}$) vir papier slyk voer tempo's van onderskeidelik 30 en 50 t d^{-1}. Die SSF proses was op 'n papier slyk voer tempo van 15 t d^{-1} nie ekonomies lewensvatbaar nie, selfs teen 'n ensiem koste van nul.

'N Monte Carlo-simulasie het getoon dat die SSF proses ekonomies lewensvatbaar is met 'n papier slyk voer tempo van 50 t d^{-1} omdat 'n gemiddelde IRR-waarde van 32% verkry is met 'n waarskynlikheid van 26% om 'n IRR-waarde laer as 25% te verkry. Die SSF proses teen papier slyk voer tempo's van 30 en 15 t d^{-1} is nie ekonomies lewensvatbaar nie omdat waarskynlikhede van 70% en 95% onderskeidelik verkry is om IRR-waardes laer as 25% te kry.

Daar kan van die studie afgelei word dat papier slyk 'n uitstekende grondstof is vir die produksie van etanol mits 'n papier slyk voer tempo van meer as 50 t d^{-1} bereik kan word. Die produksie van etanol vanaf papier slyk het die bykomende voordeel dat kweekhuis gasse (GHG) met 42.5% verminder word.

Nomenclature

SSF	-	Simultaneous saccharification and fermentation
SHF	-	Separate hydrolysis and fermentation
CBP	-	Consolidated bioprocessing
SSCF	-	Simultaneous saccharification and co-fermentation
CSL	-	Corn steep liquor
RSM	-	Response surface methodology
CCD	-	Central composite design
ANOVA	-	Analysis of variance
NPV	-	Net present value
IRR	-	Internal rate of return
WACC	-	Weighted average cost of capital
GHG	-	Greenhouse gas

Contents

1. Introduction

1.1 Background

According to Seabra *et al.* (2010), 94% of the liquid transportation fuels are derived from oil. Recently there has been an increased focus on bio-fuels due to the impact of fossil fuel consumption on global warming caused by greenhouse gas emissions, the increased energy demand worldwide concomitant with the depletion of fossil fuel reserves (Dias *et al.*, 2011). Lignocellulosic biomass is a renewable resource ideal for energy production, as this sustainable feedstock can be converted to bioethanol by biological conversion.

First generation bioethanol is commercially established and is produced from crops such as corn and sugar cane however it is not sustainable as food crops are used as feedstock (Sims *et al*., 2010). Second generation ethanol is not commercially established but can be produced from sustainable (non-food crop) lignocellulosic biomass that include straw (Tomás-Pejó *et al*., 2008), sugarcane bagasse (da Silveira dos Santos *et al*., 2010), energy crops (Mishima *et al*., 2008) and various forestry and agriculture residues (Sims *et al*., 2010).

The major drawback with second generation ethanol is that pre-treatment is required to enhance the amenability of the lignocellulosic feedstock for enzymatic hydrolysis. It has been found that the cost associated with pre-treatment could contribute up to 30% of the cost of bioethanol production (Kang *et al*., 2010). However pre-treatment is not required to enhance the amenability of paper sludge for enzymatic hydrolysis, as in the process of paper manufacturing the pulp is extensively chemically treated

(Lynd *et al.,* 2001) and as a result paper sludge is a viable feedstock for bioethanol production.

The increasing population of South Africa will generate increasing amounts of waste in the future. From an environmental perspective the conversion of waste to products is important as it limits the amount of solid waste produced and leads to increased environmental awareness. Waste paper is a major component of household and industrial solid waste streams (van Wyk, 2003), which can be recycled to produce various recycled fibre products such cardboard, roofing materials, newsprint and tissue paper (Biermann, 1993). The manufacturing of recycled fibre products results in the damaging and shortening of pulp fibres. Between 15 and 20% of the pulp feed stock is damaged in the manufacturing process and removed as paper sludge for disposal (Jeffries and Scartman, 1999).

Due to the high moisture content of paper sludge, the cost of transport and disposal is high. Paper sludge disposal costs consists out of two parts, namely landfill charges of R 705 per dry ton and transport costs of R 174 per dry ton (Nampak Tissue, 2011). As a result paper mills are opting to increase co-product production while minimising effluent, which is important for a company to market itself as “green” (Jeffries and Scartman, 1999). The production of bioethanol from paper sludge has the potential to be a cost effective method to reduce transport and disposal costs while providing additional revenue through ethanol sales.

Paper sludge typically contain 50% or more carbohydrates with glucan and xylan as the main components (Lynd *et al*., 2001), making this material a suitable source for conversion to bioethanol. The benefit of using paper sludge for the production of

bioethanol compared to other lignocellulosic feedstock is the negative feed cost associated with the use of paper sludge (Kang *et al*., 2010).

Nampak Tissue (Pty) Ltd. produces various grades of tissue paper derived from recycled fibre, which are sourced from waste office paper, news print and magazines. The waste paper is pulped to obtain individual fibres, which undergo various stages of contaminant removal including different screening stages, hydro-cyclone banks, deinking and washing. The cleaned fibres are bleached and sent to the paper machine where tissue paper is produced. The waste effluent obtained from waste water treatment and the rejects obtained from the different contaminant removal processes are combined and dewatered to produce recycled paper sludge.

In recent times there has been an increased focus on the use of paper sludge as a feedstock for the production of second generation ethanol using SSF. Most of this research was conducted on sludge emanating from the Kraft pulping process (Fan *et al*., 2003; Fan and Lynd, 2006; Kang *et al*., 2010; Zhang and Lynd, 2010). There is a lack of information available on the production of second generation ethanol utilising recycled paper sludge as a feedstock. An economic model is required to assess whether the SSF process is economically viable for the production of bioethanol using paper sludge as a feedstock. To obtain an accurate economic model, information regarding the composition and fermentability of paper sludge as well as the optimum paper sludge and enzyme loadings are required.

1.2 Research Aims

The aim of this study was to determine the composition, fermentability and optimum paper sludge loading and enzyme dosage for producing ethanol from paper sludge obtained from paper recycling operations in South Africa. The information obtained from the experimental work will be used to develop a model of the SSF process in Aspen Plus®. The mass and energy balances obtained in the Aspen Plus® model will be used to develop the equipment specifications required to source equipment cost data. A techno–economic model developed from the equipment cost information will be used to assess the economic viability of the SSF process utilising paper sludge as feedstock.

The following aims and objectives have been identified:

- Determine the chemical composition of paper sludge samples from local paper recycling plants
- Determine the fermentability of paper sludge
- Determine most efficient and economic enzyme dosage and paper sludge loading
- Obtain industrially relevant ethanol concentrations and yields from paper sludge
- Develop a process model
- Complete an economic evaluation of the SSF process

1.3 Research Approach

From the aims it can be seen that this study contains an experimental chapter and a techno-economic chapter. The study approach followed can be seen in Figure 1.1. The process design and economic evaluation are dependent on the results obtained from the experimental section.

This study consists out of four chapters; a literature review, an experimental chapter, a process design/economic evaluation chapter and a chapter summarising the relevant conclusions and recommendations. The experimental and process design/economic evaluation chapters are written in paper format as both chapters can be used as basis for publication. The experimental chapter consists of an abstract, an introduction including the relevant literature, methodology, results, discussion and conclusions. The techno-economic chapter consists of an abstract, an introduction including the relevant literature, with the methodology, results and discussion combined, and conclusions.

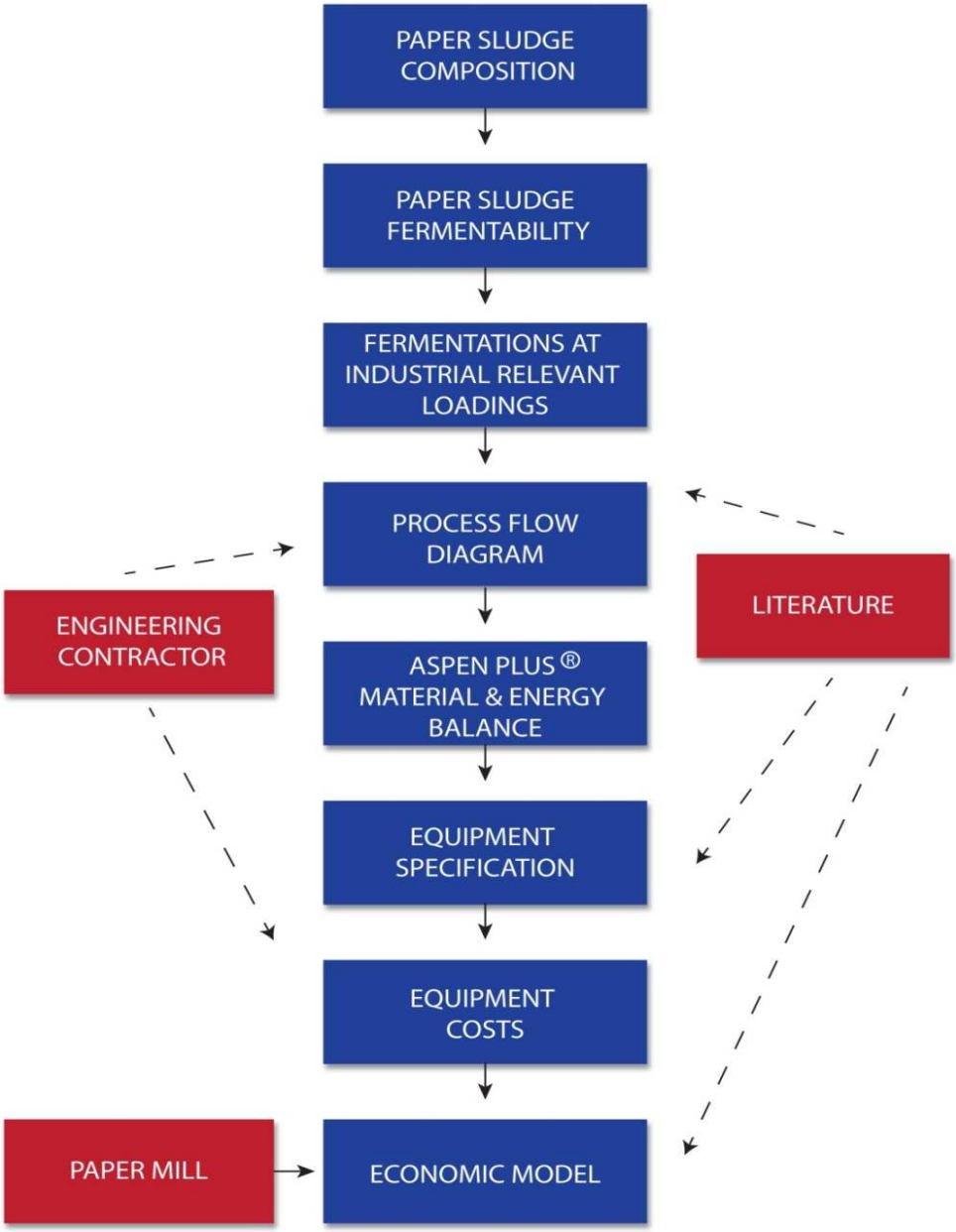

Figure 1.1: Research Approach

2. Literature Review

2.1 Composition of Paper Sludge

Paper sludge emanating from recycled fibre operations consists out of lignocellulose and contaminants such as fillers and ink. The main components of lignocellulosic materials are cellulose, hemi- cellulose and lignin which are intimately associated with each other (Hendriks and Zeeman, 2009). Lignocellulosic biomass typically contains between 35 – 50% cellulose, 20 – 35% hemicellulose, 15 – 25% lignin with the remainder consisting out of ash and extractives (Fan, 2004).

2.1.1 Cellulose

Cellulose is one of the most abundant organic compounds found on earth and is the main component of lignocellulose. Cellulose is a polymer consisting of glucose subunits which is β-1-4-linked and can be seen in Figure 2.1.

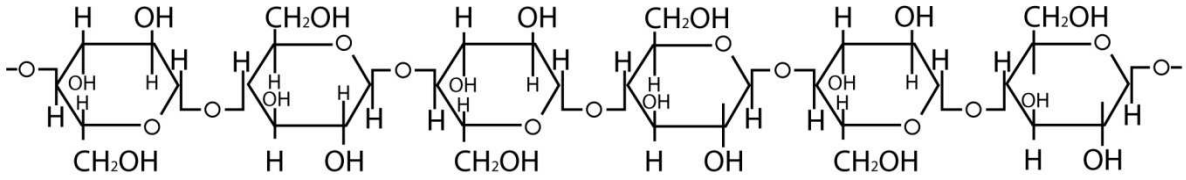

Figure 2.1: Cellulose chain consisting of glucose subunits (redrawn from Fengel and Wegener (2003)).

Intramolecular linkages are formed through hydrogen bonding, which results in an ordered crystalline structure and can be seen in Figure 2.2. Hydrogen bonding is

responsible for chain stiffening and supramolecular structure formation (Fengel and Wegener, 2003).

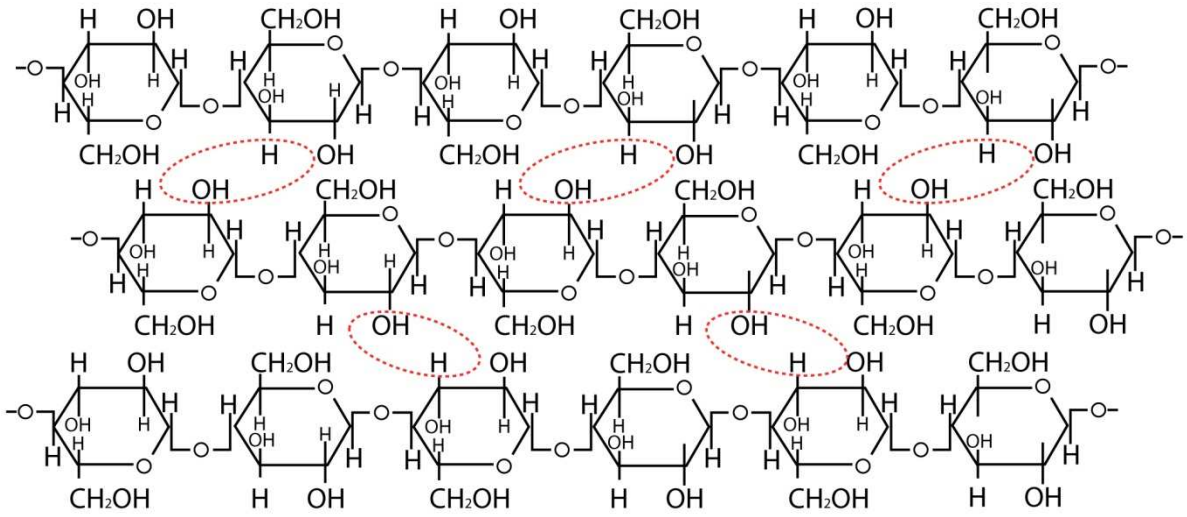

Figure 2.2: The intramolecular linkages of cellulose. Circles with dotted lines indicate intramolecular hydrogen bonding (redrawn from Fengel and Wegener (2003)).

2.1.2 Hemi-cellulose and Lignin

Hemi-cellulose is interwoven with cellulose in the cell walls of plant cells. Hemi-cellulose differs from cellulose as the backbone of hemi-cellulose consists of various sugars, containing mostly xylose. Hemi-cellulose has a lower molecular weight than cellulose and also forms a branched structure. Hemi-cellulose includes xylan, mannan, glucan and, galactan. The sugars that form hemi-cellulose can be divided into pentose, hexose, hexouronic acid and deoxy-hexoses. Second to cellulose, xylan provides the highest quantity of fermentable sugars. Xylan consist out of a homopolymer backbone of xylose linked by β-(1, 4)-glycosidic bonds (Fengel and Wegener, 2003).

After cellulose, lignin is the most abundant substance in the plant kingdom. Lignin is a polymer with a network structure without a distinct primary structure. Lignin is important as it adds rigidity to the cell wall structure, bonds cells together and protects plant tissue from UV and enzyme attack. Lignin is formed through the random polymerisation of phenyl propane units (Fengel and Wegener, 2003).

2.1.3 Enzymatic Hydrolysis

The enzymatic hydrolysis of cellulose resulting in monomeric glucose is carried out with cellulase enzymes, as is illustrated in Figure 2.3. Most of the industrial cellulase cocktails are produced by the fungus *Trichoderma reesei.* Cellulase consists out of three main components including endoglucanases, exoglucanases and β-glucosidases. The components of cellulase work synergistically in the hydrolysis of cellulose to glucose. Endoglucanases act on random sites of the cellulose chain resulting in new polysaccharides. Exoglucanases act on the ends of the polysaccharide chains resulting in either glucose or cellobiose and β-glucosidase act on the cellobiose resulting in glucose monomers (Xiongjun Shoa, 2007). Most commercial cellulase enzymes do not have a high β-glucosidase activity which is essential for the conversion of cellobiose to glucose for fermentation to ethanol. Supplementing cellulase with β-glucosidase can improve the ethanol yield from 60% to 80% of what is theoretically possible (Kádár et al., 2004).

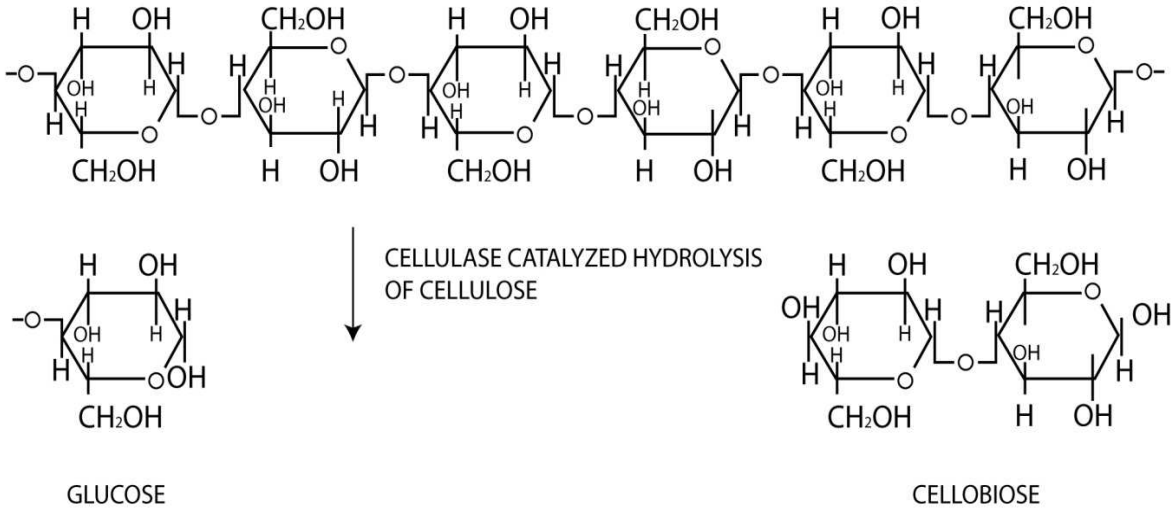

Figure 2.3: The enzymatic hydrolysis of cellulose (redrawn from van Wyk (2002))

2.2 Overview of technology used for second generation ethanol production from lignocellulosic biomass

The production of ethanol from lignocellulosic biomass consists out of the following process steps; pre-treatment, enzymatic hydrolysis, fermentation and product recovery. An overview of the production of bioethanol from lignocellulose can be seen in Figure 2.4

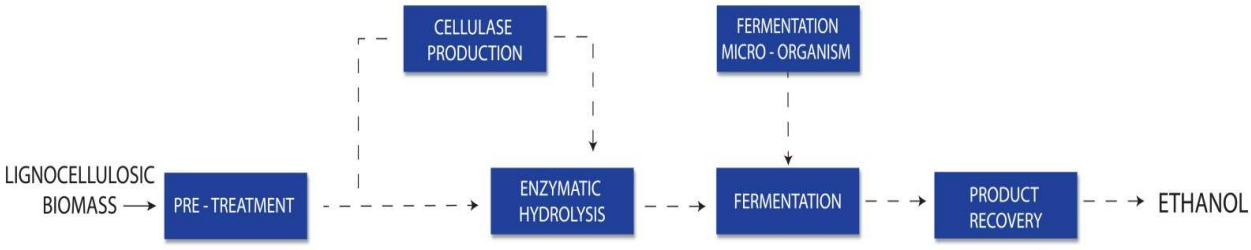

Figure 2.4: Summary of the biomass to bioethanol process (redrawn from Fan (2004)).

2.2.1 Pre-treatment

One of the main challenges of producing ethanol from lignocellulosic feedstock in general is that pre-treatment is required to increase the digestibility of cellulose and to maximise the sugar yield from hemi-cellulose (Cruz *et al*., 2011). However, most heat- and acid-based pre-treatment processes generate unwanted inhibitory by-products such as furans and phenolic compounds which negatively impacts yeast performance (Parawira and Tekere, 2011). Typically, such pre-treatment processes are carried out using steam explosion, dilute acid treatment, organosolv or sulphite pre-treatment (Zhu and Pan, 2010). The pre-treatment step contributes up to 30% of the total production cost of the bioethanol process (Fan, 2004). However pre-treatment is not required to enhance the amenability of paper sludge for enzymatic hydrolysis since it is already processed in the paper manufacturing step (Kang et al., 2010).

2.2.2 Saccharification and Fermentation

The saccharification and fermentation sub-processes can be achieved with different process configurations. An overview of different methods used to hydrolyse and ferment lignocellulosic biomass is presented in Figure 2.5.

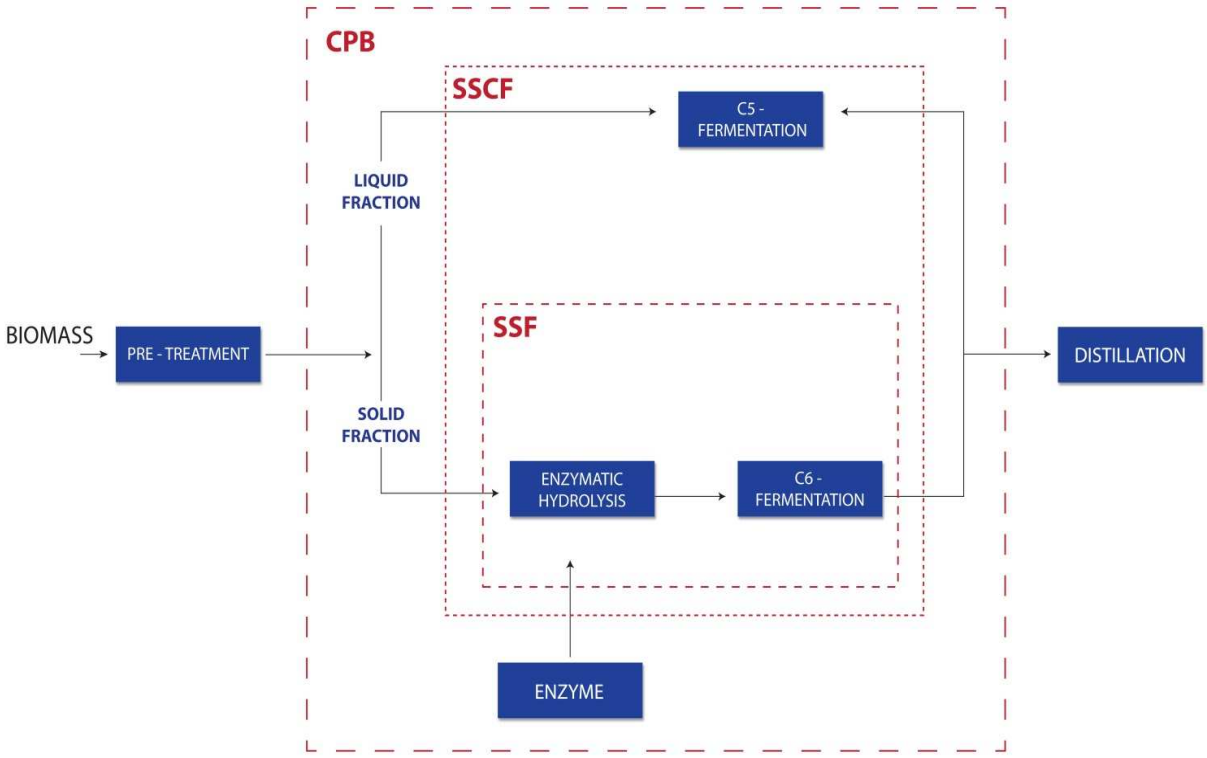

Figure 2.5: Lignocellulosic saccharification and fermentation process configurations (redrawn from Gírio et al. (2010))

During simultaneous saccharification and fermentation (SSF) cellulase enzymes are added from an external source to the substrate. The enzymatic hydrolysis (saccharification) and fermentation occurs simultaneously. A SSF process utilising a fermenting microorganism which can ferment both hexose and pentose sugars is known as simultaneous saccharification and co-fermentation (SSCF). Conversely in the separate hydrolysis and fermentation (SHF) of a substrate to ethanol, the enzymatic hydrolysis step precedes the fermentation of the hydrolysed substrate. Consolidated bioprocessing (CBP) is a process where the necessary enzymes are produced, the hydrolysis and the fermentation of sugars (hexose and pentose) to ethanol occurs in one step (Gírio et al., 2010). The use of CBP technology can eliminate the requirement for the addition of enzymes and therefore significantly improve the economic viability of producing ethanol from paper sludge.

The major disadvantage of SHF compared to SSF resides in the feedback inhibition on cellulase enzymes by accumulated glucose, which results in low ethanol yields. Glucose inhibition severely impacts cellulase performance at glucose concentrations in excess of 20 g l^{-1} (Xiao *et al.,* 2004). On the other hand, in SSF the fermenting micro-organism converts the glucose to ethanol as soon as it is hydrolysed, therefore eliminating glucose inhibition resulting in higher ethanol yields (Lin and Tanaka, 2005). Consequently SSF was used in this study to produce ethanol using paper sludge as a feedstock.

Xylan is the main hemi-cellulose component in paper sludge and the fermentation of both glucose and xylose (derived by the hydrolysis of xylan) can increase the ethanol yield. Glucose and xylose competitively inhibits the uptake of each other, but as the sugar concentrations in SSCF is low, the competitive inhibition is negated *(*Zhang *et al.*, 2009).

Most lignocellulosic materials are nutrient poor and as a result additional nutrients are required to obtain high ethanol yields in SSF, but the addition of nutrients leads to an increase of the production cost of bioethanol. A low cost medium consisting of 0.3% (w/w) CSL and $MgSO_4.7H_2O$ provides the necessary nutrients with the added benefit that the required components are available on industrial scale (Kadam and Newman, 1997).

The cost of cellulase production has a major cost implication in the production of ethanol from paper sludge. Enzyme cost estimates found in literature varied considerably and a summary of enzyme costs per unit of ethanol produced can be seen in Table 2.1.

Table 2.1: Summary of the enzyme costs found in literature

Enzyme Cost	**Source**
\$ 0.10 gal^{-1} (R 0.22 l^{-1})	(Aden and Foust, 2009)
\$ 0.30 gal^{-1} (R 0.66 l^{-1})	(Lynd *et al*., 2008)
\$ 0.40 gal^{-1} (R 0.88 l^{-1})	(Kazi *et al*., 2010)
\$ 0.68 gal^{-1} (R 1.50 l^{-1})	(Klein-Marcuschamer *et al*., 2012)
\$ 1.47 gal^{-1} (R 3.28 l^{-1})	(Klein-Marcuschamer *et al*., 2012)

The development and use of CBP yeasts can drastically improve the cost implication of enzyme production as the fermenting organism produces cellulase while fermenting hexose and pentose sugars to ethanol (van Zyl *et al*., 2007).

Different fermenting micro-organisms can be used to produce ethanol from paper sludge. The most relevant micro-organisms are *Escherichia coli, Zymomonas mobilis, Saccharomyces cerevisiae* and *Pichia stipitis.* The characteristics of the different micro-organisms (wild type) can be seen in Table 2.2.

Table 2.2: Advantages and disadvantages of different fermenting micro-organisms (redrawn from Gírio et al. (2010))

Characteristics	Micro-organism			
	E. coli	*Z. mobilis*	*S. cerevisiae*	*P. stipitis*
Glucose Fermentation	+	+	+	+
Other C6 Utilisation	+	-	+	+
C5 Utilisation	+	-	-	+
Anaerobic Fermentation	+	+	+	-
Ethanol Productivity from Glucose	-	+	+	W
Ethanol Tolerance	W	W	+	W
Inhibitor Tolerance	W	W	+	W
Osmotolerance	-	-	+	W
Acidic pH range	-	-	+	W

.+ Positive, - Negative, W weak

As seen in Table 2.2, *S. cerevisiae* outperforms the other microorganisms in all characteristics except in the utilisation of pentose sugars. *E. coli* ATCC-55124 readily ferments xylose and other pentose sugars but it has a low ethanol tolerance as a maximum ethanol concentration of approximately 40 g l^{-1} can be expected (Kang *et al*., 2010). In the fermentation of corn a final ethanol concentration of up to 130 g l^{-1} can be expected when using wild type *S. cerevisiae* but this microorganism cannot ferment pentose sugars (Taylor *et al., 2010). Z. mobilis* 8b has a low ethanol tolerance similar to that of *E. coli* as a maximum ethanol concentration of 40 g l^{-1} was obtained when using paper sludge as a feedstock (Zhang and Lynd, 2010). It should be noted that although wild type *S. cerevisiae* cannot ferment pentose sugars it can be engineered to express xylose isomerase, imparting the ability to utilise xylose in addition to glucose as carbon source. As an example, a recombinant strain *S. cerevisiae* with the capability to ferment pentose sugars such as xylose to ethanol was used by Zhang and Lynd (2010).

2.3 Previous work on the production of ethanol from paper sludge

SSF experiments used for the production of ethanol from paper sludge can be carried out using batch, fed-batch or semi-continuous strategies. Fed-batch and semi-continuous fermentations produce higher ethanol yields compared to batch fermentations as high solid loadings can be fed, which is impractical when using a batch strategy due to the high viscosity that such a high loading might entail (Ballesteros *et al.,* 2002). Fed-batch and semi-continuous SSF cultivations provides a practical alternative in that fermentable solids can be fed incrementally to obtain an

overall high solid loading, while avoiding negative viscosity effects (Olofsson *et al*., 2008).

For SSF of any lignocellulosic substrate to be economically viable, it is critical to produce ethanol at concentrations in excess of 40 g l^{-1} as the distillation process at lower concentrations would be highly energy intensive, making such a process not viable (Fan *et al*., 2003). To attain ethanol concentrations in excess of 40 g l^{-1} a fed-batch or semi-continuous strategy should be used to incrementally increase the solid loading. In this study a fed-batch protocol was used in the experimental chapter although a semi-continuous process was used in the process modelling and economic chapter. The motive for using a fed-batch protocol for experimental work was that a fed-batch protocol is easier to operate, requires less supervision and complex equipment. It is preferable to use a semi-continuous fermentation process in industry as smaller process volumes are required coupled without the downtime experienced with a batch or fed-batch process (Li *et al*., 2011). It is assumed that there will be no significant difference in performance between a semi-continuous process and a fed-batch process. According to Çaylak and Sukan (1998) there is less than 5% difference in fermentation performance, based on ethanol yield, between fed-batch and semi-continuous fermentations. For screening work a batch protocol was used as batch experiments are simpler to use. Once the optimal operating parameters were determined, a fed-batch protocol was used to obtain industrially relevant ethanol concentrations.

Ethanol concentrations in excess of 40 g l^{-1} were obtained using a fed-batch protocol with paper sludge as feedstock in SSF by Fan *et al.* (2003), Kang *et al.* (2010) and Zhang *et al.* (2009). In SSCF batch experiments, conversions of paper sludge to

ethanol of 51% were obtained at a solids concentration of 178 g l^{-1} (Marques *et al.,* 2008) with a maximum ethanol concentration of 19 g l^{-1}. The low conversion obtained in batch experiments can be attributed to mixing difficulties at high solid loadings (Ballesteros *et al.*, 2002). Mixing is required in the reactor to obtain high ethanol conversions. The mixing energy required to mix unreacted paper sludge exponentially increases with solids content. However, when working with a fed-batch protocol, no substantial increase in mixing energy is required since the low concentrations of paper sludge fed intermittently are hydrolysed which results in a reduction in mixing energy required.

The SSF process is normally operated at temperatures ranging between 34 ℃ and 37 ℃, which is a compromise between the optimal te mperatures for the enzymatic hydrolysis of paper sludge and the fermentation of sugars released. The optimum temperature for fermentation by yeast is 30 ℃, whe reas the optimum temperature for enzymatic hydrolysis is 50℃ (Olofsson *et al*., 2008). By operating the SSCF process at lower temperatures, inhibition effects on the yeast is reduced as the operating temperature is close to the optimum temperature for the yeast.

New legislation will force the paper industry to reduce the water content in the paper sludge prior to disposal which can only be achieved by drying, which will result in increased energy costs. An additional benefit of SSF is that it improves the dewaterability of paper sludge (Jeffries and Scartman, 1999) which leads to a reduction of the volume of waste disposed of which reduces disposal costs as well as the energy required in the drying of paper sludge .

2.4 Economic evaluation of simultaneous saccharification and fermentation using paper sludge as feedstock to produce ethanol

For an investor or an organisation to consider an investment in a new project or the expansion of current operations, the economic viability of the project should be proven beyond reasonable doubt. Such economic viability can be determined by using economic modelling techniques, based on financial statements, where resulting cash flows will show whether or not proceeds will sustain the investment with a reasonable rate of return. Key economic indicators such as the internal rate of return (IRR), net present value (NPV), payback period and discounted payback period are often used as a measure of economic viability (Perry and Green, 2008).

The IRR can be defined as the discount rate that results in a NPV value of zero, implying that it is the maximum possible rate of return on an investment (Seider *et al*., 2004). Therefore, from an investment point of view the IRR should always be greater than the rate of return required by an investor. The NPV can be defined as the sum of all the discounted cash flows over the life of a project at a fixed discount rate (Amigun *et al*., 2011).

However, economic analyses that use deterministic estimates in calculating key economic indicators neglect uncertainty and risk in the model. The use of probability distributions quantifies the possibility of economic success and risk of failure (Amigun *et al*., 2011). A probabilistic method such as the Monte Carlo analysis provides a powerful tool to model the uncertainty in the input by accounting for the uncertainties characterised by probability distributions. The response obtained from the Monte

Carlo analysis results in a probability distribution of key economic indicators (Petersen, 2012), thus providing an assessment of the probability of success.

IRR values in excess of 20% can be expected for a SSF process utilising paper sludge as a feedstock (Fan, 2004). The method used by Fan, (2004) was to assume a revenue from the sales of ethanol, various fixed and operating costs and to calculate the affordable capital investment assuming a IRR of 20%.

There is a lack of information available in literature where a Monte Carlo analysis was used to calculate the economic viability of the production of ethanol from paper sludge. Monte Carlo analyses were used to model the risk of producing ethanol from wheat (Richardson *et al.*, 2007) and triticale (Amigun *et al.,* 2011) in South Africa. For a bioethanol plant with a capacity of 103 million m^3, a probability of 97% was obtained to achieve a positive NPV at a discount rate of 25% (Richardson *et al.*, 2007).

3. Optimisation of simultaneous saccharification and fermentation with paper sludge as feedstock using response surface methodology

Abstract

Recently, there has been an increased focus on bio-fuels due to the impact of fossil derived fuel on the environment as well as the increased energy demand worldwide, concomitant with the depletion of fossil fuel reserves. Paper sludge produced by paper mills are derived from lignocellulosic material and can be used as feedstock to produce ethanol. The results presented in this study differed from published results as the paper sludge used in this study emanated from recycled fibre operations. In this study, response surface methodology was used to obtain quadratic mathematical models to investigate the effects of solid loading and cellulase dosage on ethanol production and ethanol yield from paper sludge during fed-batch fermentations using *Saccharomyces cerevisiae* strain MH1000. This approach was augmented with a multi response optimisation approach incorporating a desirability function to determine the optimal solid loading and cellulase dosage. Nine paper sludge samples obtained from Nampak Tissue (Pty) Ltd. were evaluated in terms of ethanol production and those samples yielding the highest and lowest ethanol titres were selected for optimisation. This allowed the determination of a range of ethanol concentrations and yields, expressed as percentage of the theoretical maximum that could be expected on an industrial scale. The optimum solid loading was found to range between 20.79% and 21.75% (w/w), whereas the optimum cellulase dosage ranged between 14.23 and 15.0 FPU g^{-1} dry solids. Using the optimal solid loadings and cellulase dosages, a minimum ethanol concentration of 47.36 g l^{-1} (84.69% of

theoretical maximum) and a maximum ethanol concentration of 57.06 g l^{-1} (93.66% of theoretical maximum) were obtained for the range of samples tested.

3.1 Introduction

According to Seabra *et al.* (2010), 94% of the liquid transportation fuels are derived from oil. Recently there has been an increased focus on bio-fuels due to the impact of fossil fuel consumption on global warming, which requires a reduction in greenhouse gas emissions, the increased energy demand worldwide and the depletion of fossil fuel reserves (Dias *et al.*, 2011). Lignocellulosic biomass is a renewable resource ideal for energy production, as this sustainable feedstock can be converted to bioethanol by biological conversion.

First generation bioethanol is commercially established and is produced from feedstock such as corn and sugar cane however it is not sustainable as food crops are used as feedstock (Sims *et al*., 2010). Second generation ethanol is not commercially established but can be produced from sustainable (non-food crop) lignocellulosic biomass that include straw (Tomás-Pejó *et al*., 2008), sugarcane bagasse (da Silveira dos Santos *et al*., 2010), energy crops (Mishima *et al*., 2008) and various forestry and agriculture residues (Sims *et al*., 2010).

One of the main challenges of producing ethanol from lignocellulosic feedstock in general is that pre-treatment is required to increase the digestibility of cellulose and to maximise the sugar yield from hemi-cellulose (Cruz *et al*., 2011). However, most heat- and acid-based pre-treatment processes generate unwanted inhibitory by-products such as furans and phenolic compounds that negatively affects yeast

performance (Parawira and Tekere, 2011). Typically, such pre-treatment processes were carried out using steam explosion, dilute acid treatment, organosolv or sulphite pre-treatment (Zhu and Pan, 2010). Paper sludge, on the other hand, presents one major benefit in that no pre-treatment is required. This is due to the fact that significant disruption of the cellulosic crystalline structure occurs during the paper pulping process (Kang *et al*., 2010). As a result paper sludge is a viable feedstock for bioethanol production.

In the manufacturing of paper, pulp fibres are damaged and shortened, resulting in disposal of between 15 and 20% of the pulp feed stock as paper sludge during the manufacturing process (Jeffries and Scartman, 1999). The increasing population of South Africa will generate increasing amounts of paper waste in the future, making the conversion of waste a matter of increasing importance. Such conversion to ethanol, for example, would limit the amount of solid waste that needs to be disposed of (van Wyk, 2003), but could also serve as a valuable source of income through value addition. Paper sludge typically contain 50% or more carbohydrates with glucan and xylan as the main components (Lynd *et al*., 2001), making this material a suitable source for conversion to bioethanol.

The major drawback with second generation ethanol is that the cost of cellulase contributes significantly to the production cost of bioethanol and according to Klein-Marcuschamer *et al*. (2012) enzyme cost could be as high as \$ 1.47 gal^{-1} (R 3.28 l^{-1}) ethanol produced. For a SSF process to be economically viable at industrial conditions an ethanol concentration of 40 g l^{-1} is required to reduce product recovery cost (Fan, 2004). As a result it is critical to minimise cellulase dosage while optimising ethanol concentration and yield. Response surface methodology (RSM)

can be used to optimise cellulase dosage and paper sludge loading to obtain high ethanol concentrations and yields. RSM is a statistical technique comprising of a collection of mathematical and statistical techniques that can be used to model the response of a system influenced by several variables (Han *et al.*, 2011).

Response surface methodology was used to model and optimise key aspects of SSF, namely enzymatic hydrolysis (Liu *et al*., 2009), pre-treatment of sugarcane bagasse (Cruz *et al*., 2011) and the fermentation of sago starch (Ratnam *et al*., 2003). The conditions optimised in the RSM with regards to fermentation of sago starch were time and temperature. In another study, RSM was used to optimise time, pH and temperature for the SSF of kitchen waste (Wang *et al*., 2008). However, RSM has not been used to date to optimise ethanol concentration and ethanol yield in SSF with regards to solid loading and cellulase dosage for any lignocellulosic feedstock.

In recent times there has been an increased focus on the use of paper sludge as a feedstock for the production of second generation ethanol using SSF. Most of this research was conducted on sludge emanating from the Kraft pulping process (Fan *et al*., 2003; Fan and Lynd, 2006; Kang *et al*., 2010; Zhang and Lynd, 2010). There is a lack of information available on the SSF process utilising recycled paper sludge as feedstock. Given this lack in information pertaining to an often neglected renewable source of energy, this study focused on determining the composition, fermentability and optimum solid loading and cellulase dosage for producing ethanol from recycled paper sludge.

The composition of the paper sludge samples was determined by the method prescribed by NREL and the fermentability of the samples was determined by SSF in

100 ml fermentation bottles. Based on ethanol titres from these experiments, two samples yielding the lowest and highest ethanol concentration were selected for optimisation by RSM in 1.3 L BioFlo Modular Benchtop Fermenters. In this work, RSM was used to maximise ethanol production and yield by optimising paper sludge solids loading and cellulase dosage. Multi response optimisation using a desirability function approach was used to optimise the results obtained from the RSM. The desirability function approach is widely used in industry to optimise a system with multiple responses (StatSoft, Inc, 2011). The data obtained from the optimisation of SSF will be used as input for process modelling and economic evaluation of ethanol production from paper sludge (Chapter 4).

3.2 Materials and Methods

3.2.1 Yeast strains, paper sludge feedstock, and enzymes

Nine paper sludge samples were obtained from three paper mills at different time intervals. Nampak Tissue (Pty) Ltd. has paper mills located throughout South Africa, and produces both low and high grade tissue paper from various grades of recycled paper. Paper sludge is obtained from the production of both low and high grade tissue paper. The paper sludge samples obtained from Nampak Tissue (Pty) Ltd., South Africa was stored at -20 ℃. A wild type *Saccharomyces cerevisiae* strain MH1000 (van Zyl *et al*., 2011) and an engineered strain of *S. cerevisiae* D5A ATCC-200062 (NREL-D5A) with xylose utilising capabilities were stored in 1.5 ml aliquots as freezer stock at -80 ℃. Optiflow RC 2.0 (Genenc or, Finland), Spezyme CP (Danisco Genencor, Denmark) and Cellic CTec 1 (Novozymes, Denmark) cellulase preparations were used for enzymatic hydrolysis, with activities of 148, 60, 92 FPU ml^{-1} respectively. The activities were calculated using the filter paper method described in

Ghose, (1987). Most commercial cellulase enzymes do not have a high β-glucosidase activity, which is essential to convert cellobiose to glucose for fermentation to ethanol (Kádár *et al.,* 2004). As a result, the cellulase preparations were supplemented with 60 IU β-glucosidase g^{-1} dry solids. The β-Glucosidase activity of Novozym 188 (Novozymes, Denmark) was determined as 677 IU ml^{-1}.

Filter paper strips (Whatman No. 1) are used as substrate in the calculation of the cellulase activities. A short description of the method is given below (Ghose, 1987):

1. Add 1 ml of 0.05 M Na-Citrate solution at a pH of 4.8 to a test tube (25 ml).
2. Add 0.5 ml of enzyme diluted with citrate buffer.
3. Heat to 50 ℃ and add paper strip.
4. Incubate for 60 minutes at 50 ℃.
5. Add 3 ml DNS and boil for 5 minutes.
6. Add 20 ml distilled water and mix.
7. After pulp settles, measure against spectro zero at 540 nm.

The spectro zero was used to calibrate the spectrometer to zero absorbance. The spectro zero was prepared by the same procedure as described above but without enzyme addition. The glucose standards are prepared the same as the spectro zero but with the addition of glucose. To calculate the activity in FPU, a linear glucose standard curve was constructed and plotted against A_{540}. In the calculation of the β-glucosidase activity a similar method was followed but with cellobiose as substrate.

3.2.2 Ash removal from paper sludge

Due to the high ash content of the paper sludge samples tested, an ash removal protocol was developed to remove as much ash from the paper sludge as possible. This protocol was similar to the method described by Kang *et al.* (2011), where ash was removed from Kraft mill sludge. The aim of the ash removal protocol was to simulate an industrial wash cycle on laboratory scale with acceptable fiber loss. Water was added to the paper sludge to obtain a concentration of 20 g l^{-1} and disintegrated for 37 500 revolutions (British Pulp Evaluation Apparatus, Mavis Engineering, London) according to the method prescribed by Tappi (1995). The paper sludge slurry was washed over a 200 µm screen until the supernatant was clear and pressed to 35% (w/w) solids. The paper sludge obtained from this washing method has a similar ash content to sludge that will be available at Nampak in the near future, based on proposed minor process modifications (Chapter 4).

3.2.3 Simultaneous saccharification and fermentation in batch culture

The batch culture SSF experiments were carried out in 100 ml rubber-capped fermentation bottles. The inocula for SSF experiments using either *S. cerevisiae* strains MH 1000 or D5A, as indicated in the text, were grown in YPD containing (per litre; all from Merck South Africa): 10 g yeast extract, 20 g peptone and 20 g glucose at 37 ℃ for 18 h and 200 rpm. A low cost medium wa s used in all SSF experiments, consisting out of (per litre): 3 g corn steep liquor (Sigma-Aldrich, South Africa) and 2.5 mM $MgSO_4.7H_2O$ (Merck, South Africa), which provided the necessary nitrogen and vitamins required by the fermenting micro-organisms, with both components available on industrial scale (Kadam and Newman, 1997).

For fermentations, low cost medium and paper sludge were added to the fermentation bottles to obtain final paper sludge loadings of 20 g l^{-1} and autoclaved at 121 ℃ for 15 minutes. The fermentation bottles were inoculated with 5% (v/v) of a yeast culture grown on YPD for 18 h and 15 FPU g^{-1} cellulase, as indicated in the text, together with 60 IU β-Glucosidase (Novozym 188). The enzyme preparations were filter sterilised using 0.22 µm syringe filters and added aseptically. Due to the presence of ash ($CaCO_3$) no pH adjustments were made, as this component added to the buffering capacity of substrate. The fermentation bottles were incubated on an orbital shaker (MRC Orbital shaker TS600, United Scientific, South Africa) at 37 ℃ and 200 rpm for 168 h. All experiments were done in triplicate.

3.2.4 Simultaneous saccharification and fermentation in fed-batch culture

The fed-batch SSF experiments were performed in 500 ml baffled Erlenmeyer flasks with a final working mass of 250 g. The fed-batch experiments were done on mass basis as the high solid loadings used contributed significantly to the final volume. The same procedure was followed as for the batch SSF described above, with the only difference being that initial paper sludge loadings of 3% (w/w) were used. The fed-batch SSF experiments were performed at 37 ℃ and 150 rpm for 168 h. For the fed-batch component of the cultivations, 3% (w/w) aliquots of paper sludge was autoclaved at 121 ℃ for 15 minutes and added every 12 h to the Erlenmeyer flasks until the pre-determined final paper sludge concentration was reached, as indicated in text. All fed-batch SSF experiments were done in triplicate.

An experimental design was used to optimise the final ethanol concentration and ethanol yield, expressed as a percentage of the maximum theoretical ethanol yield.

All runs for the experimental design were completed in 1.3 L BioFlo 110 Modular Benchtop Fermenters (New Brunswick Scientific, United Kingdom) at a stirrer rate of 200 rpm. A final working mass of 500 g was used for all fed-batch bioreactor SSF experiments. The same cultivation procedure was used as described for the fed-batch SSF in 500 ml Erlenmeyer flasks. Optiflow RC 2.0 (Genencor, Finland), dosages indicated in text, together with 60 IU β-Glucosidase (Novozym 188, Novozymes, Denmark) was not sterilised prior to addition to the fermentation vessel, since feeding required opening the fermenter ports which exposed the culture to the atmosphere.

3.2.5 Experimental design

A popular RSM technique used in industry is a central composite design (CCD), due to its efficiency with respect to the number of experimental runs required for optimisation (StatSoft, Inc, 2011). A CCD can be made rotatable with the correct choice of axial spacing. If the CCD is rotatable, the standard deviation is constant at all points the same distance from the centre point (Montgomery and Runger, 2007). For a design to be rotatable, the axial spacing should be set as $(2^k)^{0.25}$, where k is the number of independent variables investigated. Whereas the factorial points of the CCD determine the main and interaction effects of the model, the axial points determine the quadratic terms of the model and the centre points determine the adequacy of the model (Donkoh *et al*., 2012).

The impact of cellulase dosage and paper sludge loading (independent variables) on final ethanol concentration and ethanol yield (dependent variables) were investigated using a CCD, which was designed using Statistica version 10 (StatSoft, Inc, 2011).

For the CCD to be rotatable, the axial spacing was calculated as 1.41421 with the design depicted in Table 3.1.

Table 3.1: Central Composite Design with alpha values for rotatability

Run	Cellulase Loading	Solid Loading
1	-1.00000	-1.00000
2	-1.00000	1.00000
3	1.00000	-1.00000
4	1.00000	1.00000
5	-1.41421	0.00000
6	1.41421	0.00000
7	0.00000	-1.41421
8	0.00000	1.41421
9 (C)	0.00000	0.00000
10 (C)	0.00000	0.00000
11 (C)	0.00000	0.00000

The relationship between the response of dependent variables obtained from the RSM models and the desirability of the response is known as the desirability function. Different dependent variables may have different response values and different levels of desirability (StatSoft, Inc, 2011). For each response the desirability function assigns a value between zero and one, with zero representing an undesirable response and one the most desirable response. The individual desirability's of the responses are combined to obtain the overall desirability (Myers *et al*., 2009). In SSF for example, high ethanol concentrations at high ethanol yields are desired. However, whereas a high solid loading results in high ethanol concentrations, this could result in lower ethanol yields, due to decreased enzyme efficiency (Zhang *et al*., 2009). The response desirability profiler function in the Statistica software package was therefore used, to optimise the SSF process for both high ethanol concentrations and high ethanol yields.

3.2.6 Analytical methods

The solid content of the paper sludge samples were determined gravimetrically by drying at 102 ℃. The ash content was determined by combustion at 575 ℃ ± 25 ℃ for three hours and the chemical composition of the paper sludge was determined using the standard laboratory analytical procedures for biomass analysis provided by the National Renewable Energy Laboratory (NREL, 2012). The summative analysis was used to determine the acid insoluble lignin, acid soluble lignin and sugar content of the paper sludge. To calculate the acid insoluble lignin contained in the paper sludge the following method was used (NREL, 2012):

1. Place a 0.3 ± 0.1g sample in a test tube.
2. Add 3 ± 0.01 ml H_2SO_4 and mix for 1 minute.
3. Hydrolyse for 2 hours in water bath at 30℃ ± 1° C.
4. Transfer to a glass bottle and dilute to 4% H_2SO_4 by adding 84 ± 0.04 ml water.
5. Autoclave at 121 ℃ ± 3℃ for 1 hour.
6. Decant 25 ml filtrate into container and store at 4℃ for the determination of sugars and acid soluble lignin
7. Wash filtered residue free from acid.
8. Dry crucible for 2 hours at 105 ℃ ± 3℃ and rec ord weight.
9. Ignite contents in crucible for 3 hours at 575 °C ± 25 ℃ and record weight of ash.

The sugar content of the paper sludge was determined by high performance liquid chromatography (HPLC) and the acid soluble lignin content in the paper sludge was determined by spectrophotometry at 205 nm. The sugar and ethanol concentrations obtained during the compositional analysis and fermentation were determined by

HPLC using a BioRad Aminex HPX-87H, a BioRad guard column and a RI detector. The column was operated at a temperature of 65℃ with a mobile phase of 5 mM H_2SO_4 at a flow rate of 0.6 mL/min.

3.2.7 Calculations

The ethanol yield from the combined hydrolysis-fermentation process, expressed as a percentage of the theoretical maximum, was calculated using the equation (NREL, 2012)

$$\% \, Yield = \frac{\text{EtOH}}{0.51 \, \text{f} \, [\text{Biomass}]} \, x \, 100 \qquad \text{Eq. 1}$$

Where EtOH is the final ethanol concentration in g l^{-1}, 0.51 is the maximum theoretical conversion of glucose to ethanol utilising a fermenting yeast, f is the glucose fraction contained in the biomass feedstock (g g^{-1}) and [Biomass] is the feedstock biomass loading in g l^{-1}.

Using this equation, a yield of 100% will be obtained if complete hydrolysis of paper sludge occurs combined with the theoretical maximum conversion of glucose to ethanol during fermentation, but in reality neither is possible. To determine the extent of hydrolysis and the actual fermentation yield, the chemical composition of the solid residues obtained from fermentation was determined at the end of this chapter.

Statistical significance was calculated using analysis of variance (ANOVA) using Statistica version 10 (StatSoft, Inc, 2011). Statistical significance was reported as ($p < 0.05$).

3.3 Results

The chemical composition of nine paper sludge samples received from Nampak Tissue (Pty) Ltd., South Africa are shown in Table 3.2.

Table 3.2: Composition of the paper sludge samples received, on a dry basis

	Ash (g/100g)	Glucose (g/100g)	Xylose (g/100g)	Lignin (g/100g)	Extractives (g/100g)	Σ Components (%)
Sample 1	61.61	23.09	3.41	5.32	4.44	97.87
Sample 2	61.18	25.04	4.20	4.11	3.82	98.35
Sample 3	60.31	25.28	4.22	5.09	2.88	97.78
Sample 4	54.59	31.11	3.15	6.21	4.41	99.47
Sample 5	62.49	23.23	4.47	3.23	3.65	97.07
Sample 6	56.29	29.30	4.74	4.06	2.76	97.15
Sample 7	65.50	21.97	4.56	2.36	4.11	98.50
Sample 8	58.65	24.49	4.83	5.63	5.74	99.34
Sample 9	60.55	25.12	3.45	4.94	3.83	97.89

Generally, the ash content of the nine paper sludge samples tested was very high and ranged from 56.29% to 65.50% (w/w). This high ash content would necessitate inflated equipment costs for a SSF process due to a requirement of larger volumes to accommodate the high ash loading, and was a consequence of the Nampak process currently not separating ash and fibre, but combining these into a single paper sludge stream. A minor process modification has been proposed (Chapter 4) that would allow for the separation of ash and fibre. The removal of ash from the paper sludge using an industrial wash cycle was simulated on laboratory scale. A mass balance on the washing process showed that less than 9% fibre was lost (data not shown). The composition of the various washed paper sludge samples was determined using the NREL method and can be seen in Table 3.3.

Table 3.3: Composition of the washed paper sludge samples on dry basis

Sample	Ash (g/100g)	Glucose (g/100g)	Xylose (g/100g)	Lignin (g/100g)	Extractives (g/100g)	Σ Components (%)
Sample 1	14.87	54.01	8.10	13.47	9.36	99.82
Sample 2	10.08	57.99	11.26	10.44	8.42	98.19
Sample 3	13.78	55.03	11.43	11.28	6.76	98.27
Sample 4	15.49	56.41	6.90	9.92	8.50	97.21
Sample 5	13.31	56.19	10.21	9.09	9.19	97.98
Sample 6	19.31	53.96	10.69	8.59	6.07	98.62
Sample 7	13.12	55.29	12.67	6.05	11.91	99.04
Sample 8	12.54	52.29	11.00	12.25	11.29	99.37
Sample 9	10.70	56.79	8.82	11.29	9.79	97.39

Washing the sludge samples resulted in a decrease in ash content from between 56.29% and 65.50% (w/w), to some samples reaching a minimum of 10.08% (w/w). Similar reduction in ash content was obtained by washing Kraft mill sludge where the ash content was lowered to 14% (Kang *et al.*, 2011). The glucose content of the washed paper sludge samples ranged between 52.29% and 57.99% (w/w), and were up to two-fold greater than the unwashed paper sludge. Similar increases in the xylose concentrations were evident; the increase in sugar content should result in higher ethanol concentrations and yields during SSF.

3.3.1 Simultaneous saccharification and fermentation in batch culture

3.3.1.1 Enzyme selection

Simultaneous saccharification and fermentation in batch culture using *S. cerevisiae* strain MH1000 was used to screen three different cellulase preparations (Optiflow RC 2.0, Cellic CTec 1 and Spezyme CP) at a dosage of 15 FPU g^{-1} dry solids, to ascertain which enzyme preparation would be most appropriate to digest the fibres in the paper sludge used in this study. This was achieved by exposing each of the nine samples to the three enzyme cocktails in SSF culture. The ethanol concentrations

recorded using this enzyme selection strategy is shown in Table 3.4. Furthermore, the influence of ash in the samples on fermentation and ethanol production was also evaluated by using both washed and unwashed samples for the SSF experiments. The total solid loading using washed paper sludge in batch SSF culture was 20 g l^{-1}. To obtain the same sugar content using the unwashed samples, the total solid loading had to be increased to 50 g l^{-1}, again accentuating the influence of the high ash content in this substrate.

The influence of ash on ethanol production was clearly evident since ethanol concentrations from washed samples were up to two-fold greater than when unwashed substrate was fermented, irrespective of the enzyme cocktail used (Table 3.4). Significantly greater ethanol concentrations ($p < 0.05$) from cultures with Optiflow RC 2.0 and Spezyme CP were obtained compared to cultures with Cellic CTec 1 irrespective of the paper sludge sample used. Ethanol concentrations using Optiflow RC 2.0 as hydrolysing enzyme were consistently greater than with the Spezyme CP preparation, although these differences were not statistically significant ($p > 0.05$). Based on this data, Optiflow RC 2.0 was selected as the enzyme cocktail of choice for further experimental work.

Table 3.4: Ethanol produced in SSF batch cultures with *S. cerevisiae* MH 1000 with washed and unwashed paper sludge as carbon source with Optiflow RC 2.0, Cellic CTec 1 and Spezyme CP as the digesting enzymes. Standard deviations of triplicate experiments are shown.

		Ethanol Concentration (g l^{-1})	
Sample	**Enzyme**	**Washed Sludge**	**Unwashed Sludge**
Sample 1	Optiflow	4.32 ± 0.43	1.97 ± 0.25
	Cellic CTec	2.70 ± 0.24	1.14 ± 0.22
	Spezyme	4.89 ± 0.40	2.12 ± 0.16
Sample 2	Optiflow	5.90 ± 0.44	2.81 ± 0.14
	Cellic CTec	3.41 ± 0.16	1.50 ± 0.28
	Spezyme	5.09 ± 0.23	2.49 ± 0.32
Sample 3	Optiflow	4.89 ± 0.56	2.17 ± 0.26
	Cellic CTec	2.69 ± 0.37	1.20 ± 0.17
	Spezyme	4.25 ± 0.23	2.22 ± 0.14
Sample 4	Optiflow	5.35 ± 0.51	2.52 ± 0.27
	Cellic CTec	3.77 ± 0.29	1.65 ± 0.23
	Spezyme	5.21 ± 0.33	2.58 ± 0.21
Sample 5	Optiflow	5.58 ± 0.58	2.64 ± 0.22
	Cellic CTec	3.46 ± 0.24	1.57 ± 0.31
	Spezyme	5.77 ± 0.28	2.71 ± 0.11
Sample 6	Optiflow	5.29 ± 0.46	2.29 ± 0.37
	Cellic CTec	2.94 ± 0.17	1.42 ± 0.31
	Spezyme	5.07 ± 0.33	2.07 ± 0.18
Sample 7	Optiflow	5.42 ± 0.57	2.32 ± 0.18
	Cellic CTec	4.19 ± 0.32	1.85 ± 0.25
	Spezyme	5.03 ± 0.22	2.24 ± 0.12
Sample 8	Optiflow	5.02 ± 0.55	2.55 ± 0.15
	Cellic CTec	2.51 ± 0.38	1.22 ± 0.19
	Spezyme	4.79 ± 0.30	2.41 ± 0.24
Sample 9	Optiflow	4.89 ± 0.43	2.27 ± 0.33
	Cellic CTec	2.81 ± 0.34	1.31 ± 0.10
	Spezyme	5.12 ± 0.54	2.13 ± 0.16

3.3.1.2 *Comparison of S. cerevisiae MH 1000 and S. cerevisiae D5A in simultaneous saccharification and fermentation in batch culture with paper sludge as feedstock*

Two strains of *S. cerevisiae*, i.e. MH1000 and D5A, were compared to determine the most appropriate organism for subsequent fermentation experiments. *S. cerevisiae* strain D5A was engineered to express xylose isomerase, imparting the ability to utilise xylose in addition to glucose as carbon source (Dr E. van Rensburg, 2012, personal communication). The *S. cerevisiae* strain MH1000 is a wild type strain without the ability to ferment xylose. Co-fermentation of two carbon substrates was shown to result in enhanced levels of ethanol (Marques *et al*., 2008), which would be of great benefit to an ethanol from sludge process. The batch SSF experiments were carried out in 100 ml fermentation bottles with Optiflow RC 2.0 and 20 g l^{-1} washed paper sludge at 37 ℃ for 168 h. The maximum ethanol concentrations are shown in Table 3.5 and a representative graph (Sample 1) highlighting the trend observed with all paper sludge samples shown in Figure 3.1.

Table 3.5: Ethanol production by *S. cerevisiae* strains MH1000 and D5A in anoxic SSF batch culture using 100 ml fermentation bottles. Standard deviations of triplicate experiments are shown.

	Ethanol Concentration (g l^{-1})	
Sample	**MH 1000**	**D5A**
Sample 1	4.32 ± 0.43	4.55 ± 0.54
Sample 2	5.90 ± 0.44	5.84 ± 0.27
Sample 3	4.89 ± 0.56	4.57 ± 0.20
Sample 4	5.35 ± 0.51	5.53 ± 0.26
Sample 5	5.58 ± 0.58	5.33 ± 0.42
Sample 6	5.29 ± 0.46	5.06 ± 0.30
Sample 7	5.42 ± 0.57	5.70 ± 0.54
Sample 8	5.02 ± 0.55	5.18 ± 0.20
Sample 9	4.89 ± 0.43	4.75 ± 0.53

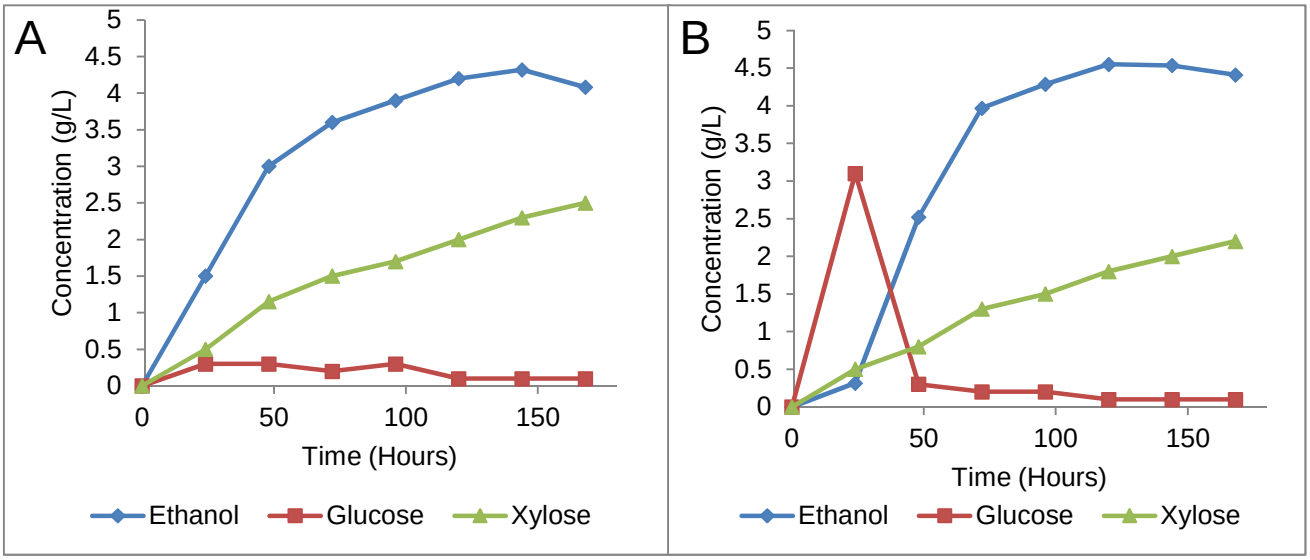

Figure 3.1: Representative graphs showing ethanol production from paper sludge and residual glucose and xylose concentrations in the culture with *S. cerevisiae* strains MH 1000 (A) and D5A (B) using Sample 1 as substrate.

Generally, there was no significant difference in the level of ethanol production between strains MH1000 and D5A using paper sludge as carbon source. This could be attributed to the fact that strain D5A did not utilise the available xylose which accumulated throughout the cultivation (Figure 3.1), in spite of the fact that this strain has been engineered to express the xylose isomerase enzyme. Furthermore, there appeared to be a distinct lag in the fermentation activity of strain D5A during the first 24 h of the cultivation. Preconditioning of the D5A strain in a medium containing xylose can possibly “activate” xylose utilisation (Zhang and Lynd, 2010).

With both strains the rate of ethanol production levelled off after 120 h (Figure 3.1) and similar maximum ethanol concentrations (Table 3.5) were produced. Given the similar maximum ethanol concentrations recorded for both strains and the initial lag in the performance of strain D5A, strain MH1000 was chosen as the preferred strain for further optimisation work even though it is a wild type strain.

Using washed paper sludge Sample 2 and *S. cerevisiae* strain MH 1000 a 1.37-fold increase in ethanol concentration was obtained compared to Sample 1 (Table 3.5). Therefore, washed paper sludge Samples 1 and 2 combined with Optiflow RC 2.0 and *S. cerevisiae* strain MH 1000 were selected for further optimisation work. The washed paper sludge Samples 1 and 2 were selected since it resulted in the lowest and the highest ethanol concentrations (Table 3.4 and 3.5), thus providing a representative range of data that can be expected on industrial scale.

3.3.2 Fed-batch simultaneous saccharification and fermentation

For SSF of any lignocellulosic substrate to be economically viable, it is critical to produce ethanol at concentrations in excess of 40 g l^{-1}. The distillation process at lower ethanol concentrations would be highly energy intensive, making such a process not viable (Fan *et al*., 2003). However, achieving these ethanol concentrations in SSF would require high solid loadings, which are often impractical at industrial scale due to the high viscosity associated with such high loadings. Fed-batch SSF cultivations provide a practical alternative in that fermentable solids can be fed incrementally to reach an overall high solid loading, while avoiding negative viscosity effects (Olofsson *et al*., 2008). To obtain a representative range of data that can be expected on industrial scale, Samples 1 and 2 from batch SSF experiments (Table 3.5) were chosen based on the greatest and lowest ethanol titres attained using *S. cerevisiae* strain MH1000. To accommodate large volumes of solids, the SSF experiments were carried out in 500ml Erlenmeyer flasks with a final working mass of 250 g.

A two-fold approach was followed to determine a basic range for solid loading and enzyme dosage in shake-flask cultures, for subsequent optimisation experiments using an RSM experimental design. In the first instance the solids loading was incrementally increased in the presence of excess enzyme (20 FPU g^{-1} Optiflow RC 2.0). Upon elucidation of the required solid loading to achieve the 40 g l^{-1} ethanol target, the enzyme dosage was incrementally decreased to determine the minimum enzyme dosage required to maintain the ethanol concentration at the desired level. The fed-batch experiments were completed in triplicate. The standard errors are not shown in the figure due to the small errors observed with all data points. Ethanol production profiles for Samples 1 and 2 are shown in Figure 3.2.

An increase in the solid loading resulted in a proportional increase in ethanol production, with 20% (w/w) solids giving the greatest ethanol concentration from both samples tested (Fig. 3.2). For both samples this proportional increase in ethanol concentration was similar, with a 1.5-fold increase between 10% and 15% (w/w) solids and a 1.3-fold increase between solid loadings of 15% and 20% (w/w). The nature of the sample also had a distinct effect on ethanol production where Sample 2 resulted in a more than 1.2-fold greater ethanol concentration of 53.05 g l^{-1} after 120 h compared to Sample 1. In the case of Sample 2, an ethanol concentration of 40.5 g l^{-1} was recorded at a solids loading of 15% (w/w). However, since the economic requirement for 40 g l^{-1} ethanol is but the threshold, further optimisation was carried out at a solids loading of 20% (w/w).

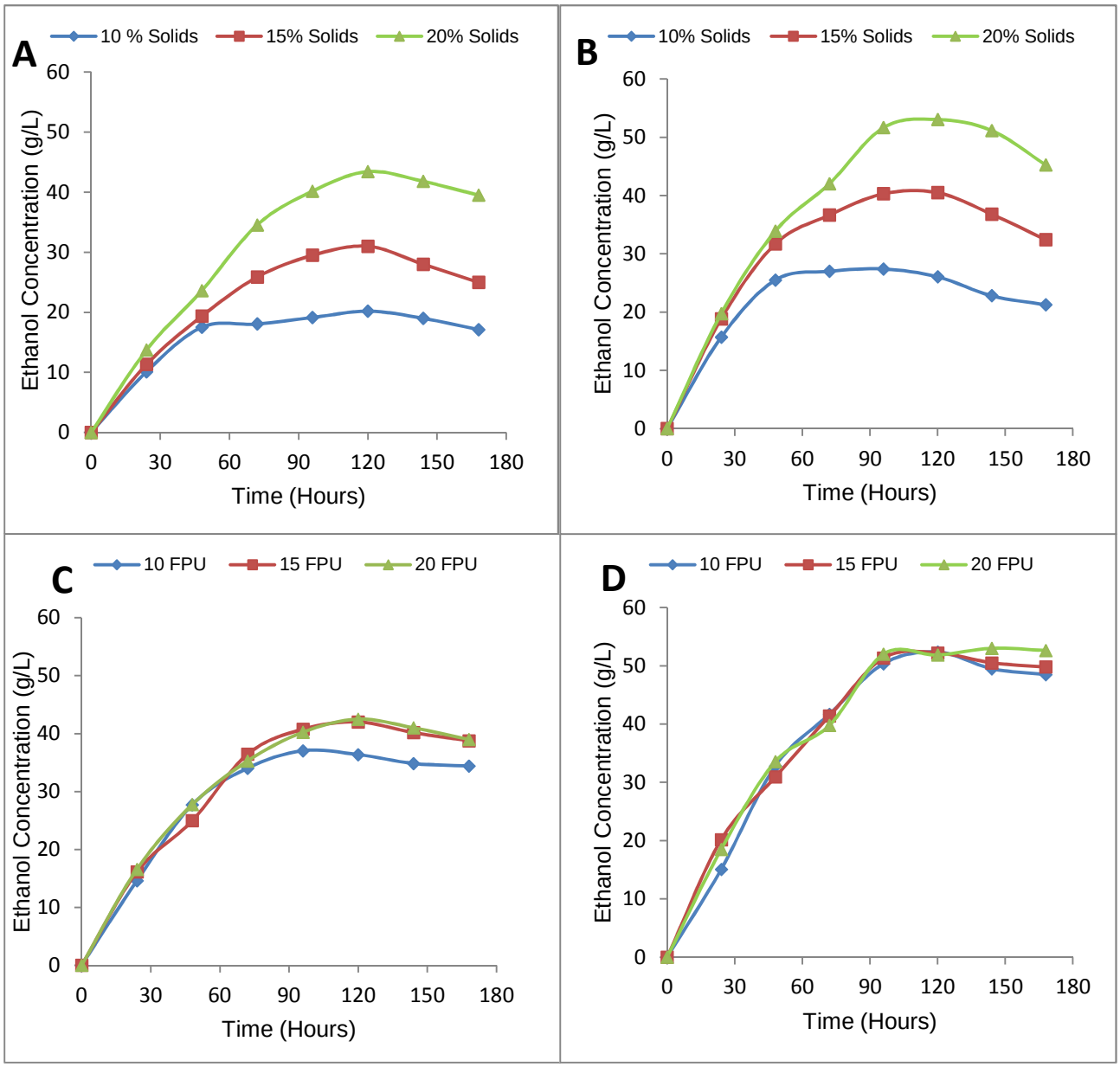

Figure 3.2: Ethanol production from paper sludge Sample 1 (A, C) and Sample 2 (B,D) in SSF batch culture using *S. cerevisiae* MH1000 where solid loading was incrementally increased (A, B) at a constant enzyme loading of 20 FPU g^{-1} and enzyme dosage was incrementally decreased (C, D) at a solids loading of 20% (w/w).

The superior digestibility of Sample 2 became evident with a decrease in enzyme dosage, since no significant difference was observed in the ethanol concentration when solids from this sample were fermented. On the other hand, based on the data

of Sample 1 an enzyme dosage of less than 15 FPU g^{-1} dry solids should be avoided, as evident from an almost 1.3-fold decrease in ethanol concentration after 120 h when an enzyme dosage of 10 FPU g^{-1} dry solids was used (Fig. 3.2).

3.3.3 Experimental design - central composite design

The aim of the CCD experimental design was to optimise the fed-batch SSF process in a bioreactor, by simultaneously varying both the solid loading and enzyme dosage, since a play-off is required between these two variables where working at high solid loadings might result in a decreased efficiency of the enzyme. The response variables for this work were ethanol concentration and yield, expressed as a percentage of the theoretical maximum. Based on the shake flask SSF results, a solid loading of 20% (w/w) and a cellulase dosage of 15 FPU g^{-1} Optiflow RC 2.0 was selected as the centre point for the CCD with paper sludge Sample 1 (Figure 3.2), whereas the centre point for paper sludge Sample 2 was based on a solids loading of 20% (w/w) and a cellulase dosage of 10 FPU g^{-1} Optiflow RC 2.0 (Figure 3.2). All experimental runs for the CCD were conducted in 1.3 L bioreactors due to the superior degree of control and mixing that this system provides compared to Erlenmeyer flasks.

The experimental design for the two CCDs, together with the dependent variables of final ethanol concentration and ethanol yield using Samples 1 and 2 are shown in Tables 3.6 and 3.7, respectively. The experimental designs were carried out in random order as depicted in these tables.

Table 3.6: Ethanol concentrations and yields from bioreactor cultures of S. *cerevisiae* strain MH1000 using an SSF fed-batch regimen at solid loadings and enzyme dosages as determined by CCD with Sample 1.

Run	Cellulase Loading (FPU/ g)	Solid Loading (g/100g)	Final Ethanol Concentration (g/L)	% of Theoretical Ethanol Yield
10 (C)	15.00	20.00	47.17	87.35
5	7.93	20.00	43.90	81.29
7	15.00	12.93	31.20	89.37
9 (C)	15.00	20.00	45.54	84.33
3	20.00	15.00	36.80	90.86
11 (C)	15.00	20.00	46.43	85.98
1	10.00	15.00	33.90	83.70
6	22.07	20.00	48.31	89.46
2	10.00	25.00	53.89	79.83
4	20.00	25.00	56.34	83.46
8	15.00	27.07	52.21	71.43

Table 3.7: Ethanol concentrations and yields from bioreactor cultures of S. *cerevisiae* strain MH1000 using an SSF fed-batch regimen at solid loadings and enzyme dosages as determined by CCD with Sample 2.

Run	Cellulase Loading (FPU/ g)	Solid Loading (g/100g)	Final Ethanol Concentration (g/L)	% of Theoretical Ethanol Yield
3	5.00	25.00	52.66	72.63
1	5.00	15.00	36.22	83.20
5	10.00	12.93	35.58	94.89
11 (C)	10.00	20.00	53.45	92.15
8	17.07	20.00	56.38	97.20
4	15.00	25.00	63.23	87.21
10 (C)	10.00	20.00	54.21	93.46
7	2.93	20.00	33.93	58.50
2	15.00	15.00	42.39	97.44
6	10.00	27.07	61.99	78.96
9 (C)	10.00	20.00	52.19	89.98

Ethanol concentrations between 31.20 g l^{-1} and 56.34 g l^{-1} which corresponded to ethanol yields that ranged from 71.43% to 90.86% of the theoretical maximum were recorded for Sample 1. Higher ethanol concentrations between 33.93 g l^{-1} and 63.23 g l^{-1} at yields between 72.63% and 97.44% were obtained for Sample 2. The range in ethanol concentrations and yields obtained from the CCD confirms the correct choice of independent variables used in this investigation.

3.3.3.1 Impact of solid loading and cellulase dosage on the final ethanol concentrations obtained in fed-batch simultaneous saccharification and fermentation

The data obtained from the experimental runs in Tables 3.6 and 3.7 were used to develop models to predict the final ethanol concentration using paper sludge Samples 1 and 2. The correlation between final ethanol concentration and solid loading and cellulase dosage for Samples 1 and 2 can be seen in Equations 2 and 3 respectively.

$$Z_1 = -24.2769 + 0.1464X + 0.0078X^2 + 5.0075Y - 0.0802Y^2 - 0.0045XY \quad \text{Eq. 2}$$

$$Z_1 = -31.3220 + 3.2527X - 0.1460X^2 + 4.3624Y - 0.0734Y^2 + 0.0440XY \quad \text{Eq. 3}$$

Where Z_1, X and Y represents the final ethanol concentration, the cellulase dosage and the solid loading respectively. Respective R^2 values of 0.973 and 0.968 were obtained for Samples 1 and 2, which indicated that the model exhibited a high degree of fit to the data obtained from the experimental work.

Surface plots of the quadratic models predicting the final ethanol concentration using paper sludge Samples 1 and 2 were developed to visually illustrate the impact of the solid loading and cellulase dosage on the final ethanol concentration. An analysis of variance (ANOVA) was performed on the quadratic models to determine the significance of the independent variables on the final ethanol concentration. The surface plots and results of the ANOVA are shown in Figure 3.3.

Generally, the ethanol concentration increased with an increase in paper sludge loading and cellulase dosage for both Samples 1 and 2. As seen in Figure 3.3, no substantial increase in ethanol concentration was obtained with solid loadings in excess of 22% (w/w) and cellulase dosages in excess of 15 FPU g^{-1} dry solids. From the Pareto charts of standardised effects it can be seen that solid loading is the major contributor to the ethanol concentrations obtained for both samples.

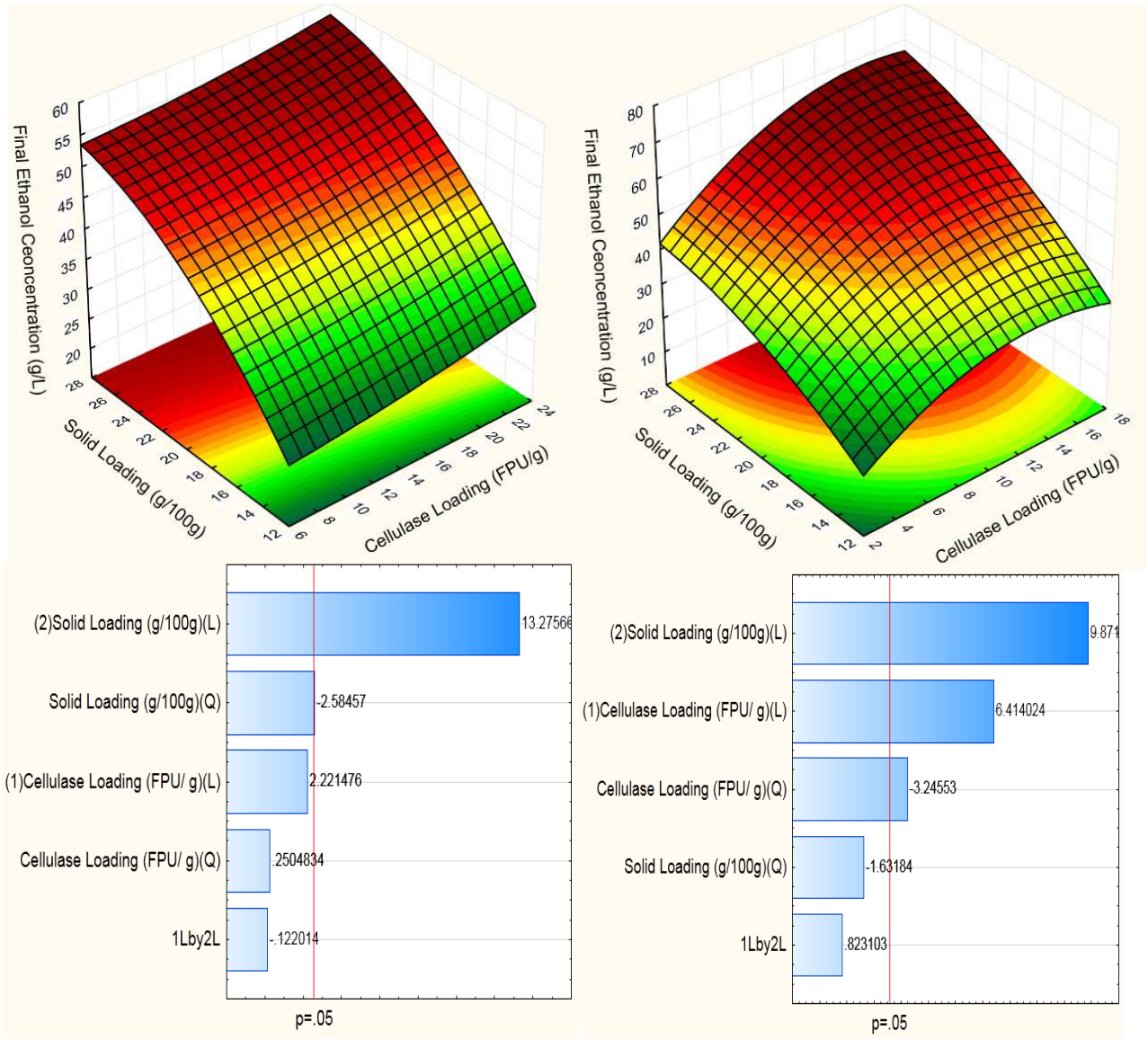

Figure 3.3: Surface plots of the quadratic models predicting the final ethanol concentration for Sample 1 and 2 (top left, top right) and Pareto charts of standardised effects for Sample 1 and 2 (bottom left, bottom right). The keys containing L and Q on the Pareto charts denote the main effects and the quadratic effects of the model respectively. The key 1Lby2L denotes the interaction effects of the model.

3.3.3.2 *Impact of solid loading and cellulase dosage on percentage of theoretical ethanol yield*

The data obtained from the experimental runs in Tables 3.6 and 3.7 were used to develop quadratic models to predict the percentage of theoretical ethanol yield obtained from paper sludge Samples 1 and 2. The correlation between ethanol yield and the solid loading and cellulase dosage for Samples 1 and 2 can be seen in Equations 4 and 5 respectively.

$$Z_2 = 48.9215 + 1.0974X + 0.00558X^2 + 3.3709Y - 0.0934Y^2 - 0.0353XY \quad \text{Eq. 4}$$

$$Z_2 = 39.6859 + 7.0644X - 0.2528X^2 + 1.7271Y - 0.0713Y^2 + 0.0039XY \quad \text{Eq. 5}$$

Where Z_2, X and Y represents the percentage of theoretical ethanol yield, the cellulase dosage and the solid loading respectively. R^2 values of 0.866 and 0.926 were obtained for Samples 1 and 2 respectively, which indicated that the model fitted the data obtained from the experimental runs.

Surface plots of the quadratic models predicting the percentage of theoretical ethanol yield using paper sludge Samples 1 and 2 were developed to visually illustrate the impact of the solid loading and cellulase dosage on the percentage of theoretical ethanol yield. An analysis of variance (ANOVA) was performed on the quadratic models to determine the significance of the independent variables on the percentage of theoretical ethanol yield. The surface plots and results of the ANOVA can be seen in Figure 3.4.

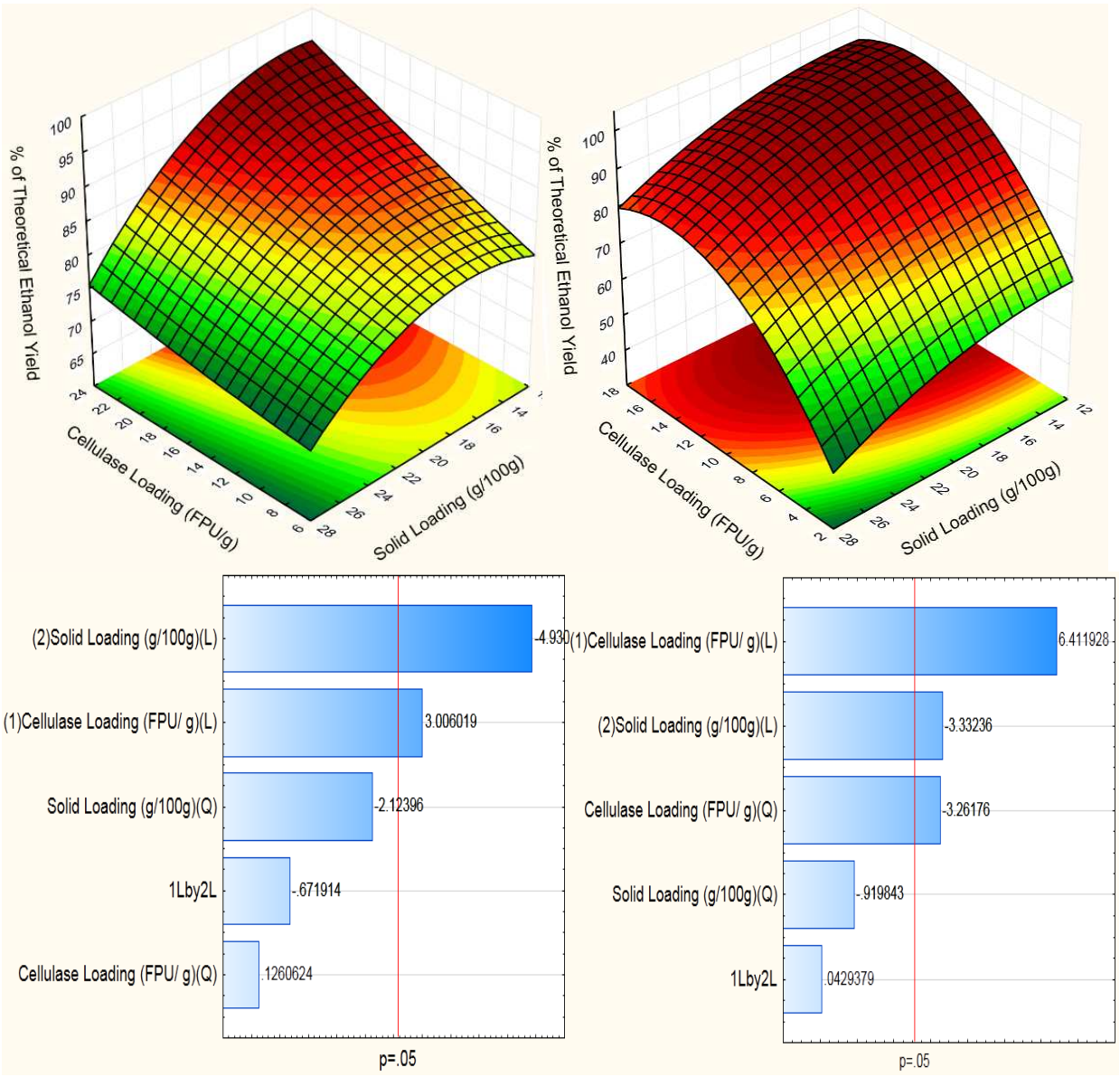

Figure 3.4: Surface plots of the quadratic models predicting the percentage of theoretical ethanol yield for Samples 1 and 2 (top left, top right) and Pareto charts of standardised effects for Samples 1 and 2 (bottom left, bottom right). The keys containing L and Q on the Pareto charts denote the main effects and the quadratic effects of the model respectively. The key 1Lby2L denotes the interaction effects of the model.

From Figure 3.4 it can be seen that ethanol yield increases with a decrease in paper sludge loading and an increase in cellulase dosage for both Samples 1 and 2. The

ethanol yield drops off at solid loadings in excess of 20% (w/w) and at cellulase dosages less than 10 FPU g^{-1}dry solids. From the Pareto charts of standardised effects it can be seen that the solid loading and cellulase dosage contributes significantly to ethanol yields obtained with paper sludge as feedstock, compared to Figure 3.3 where solid loading was the main contributor to final ethanol concentrations obtained.

As seen in Figure 3.4 high ethanol yields are obtained at low solid loadings and high cellulase dosages, whereas high final ethanol concentrations are obtained at high solid loadings (Figure 3.3), consequently there is an optimum solid loading where both ethanol yield and final ethanol concentration can be optimised.

3.3.3.3 Response desirability optimisation

Response desirability optimisation was used to optimise the solid loading and enzyme dosage to obtain high final ethanol concentrations combined with high ethanol yields, expressed as percentages of the theoretical maximum. For the final ethanol concentration a desirability input value of 0 was selected for all ethanol concentrations lower than 40 g l^{-1}, whereas a value of 1 was selected for the highest ethanol concentration obtained in the CCD for both Samples 1 and 2. As previously mentioned, an ethanol concentration of 40 g l^{-1} is regarded as the minimum ethanol concentration for the SSF process to be economically viable and thus served as the lower threshold for this design.

A desirability input value of 0 was selected for ethanol yields lower than 80% from the combined hydrolysis-fermentation process with a value of 1 for the highest yield

obtained in the CCD experimental design for both Samples 1 and 2. A minimum ethanol yield of 80% of the theoretical maximum was used since yields of 80% and greater were reported for SSF using paper sludge as feedstock (Ballesteros *et al*., 2002; Fan and Lynd, 2006; Kang *et al*., 2010).

The maximum Optiflow RC 2.0 dosage was limited to 15 FPU g^{-1} dry solids as enzyme cost is a major contributor to the overall ethanol production cost when using SSF (Fan, 2004). Furthermore, the data shown in Figure 3.2 confirmed that there was no additional benefit by using more than 15 FPU g^{-1}dry solids for SSF with paper sludge. The results from the response desirability optimisation can be seen in Figure 3.5.

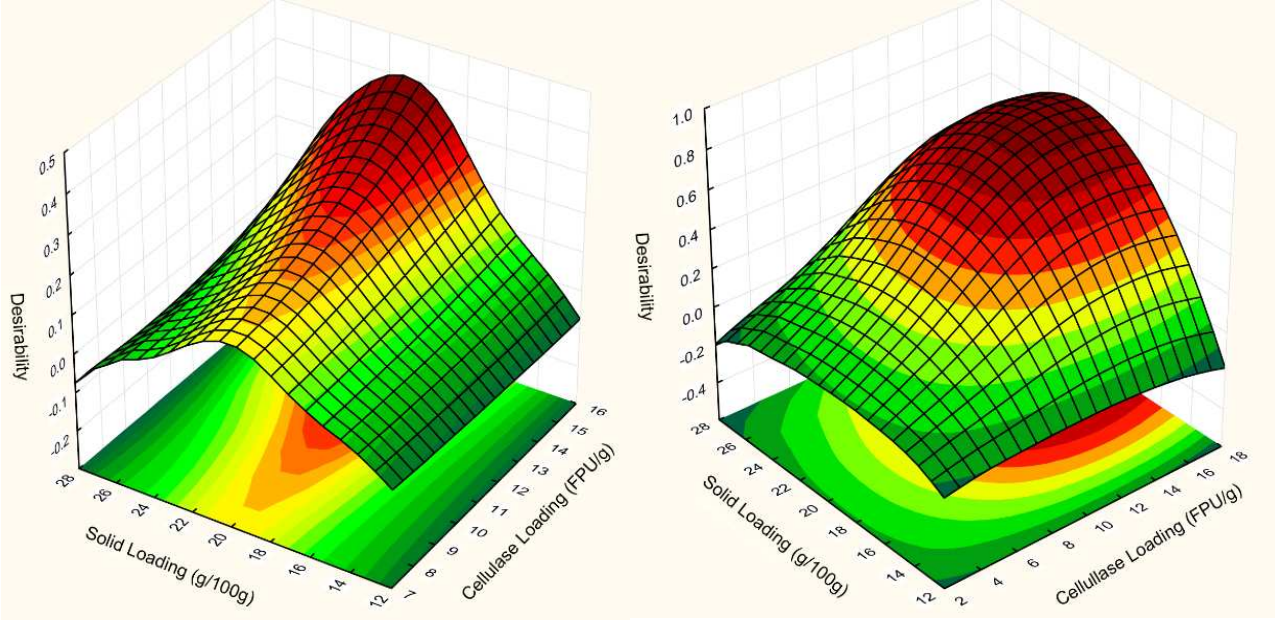

Figure 3.5: Multiple response optimisation with desirability functions using Sample 1 (Left) and Sample 2 (Right)

Based on the responses from the desirability optimisation analysis, the optimum cellulase dosage and solid loading for Sample 1 was determined as 15 FPU g^{-1} dry solids and 20.79% (w/w), respectively. The corresponding values for Sample 2 were

similar at 14.225 FPU g^{-1} dry solids and 21.754% (w/w). A clear optimum was obtained with Sample 2, whereas the optimum of Sample 1 is likely to be at an enzyme dosage in excess of 15 FPU g^{-1} dry solids. The difference in optima can be attributed to the fact that Sample 2 was more digestible, which resulted in higher ethanol titres and yields (Figures 3.3 and 3.4). This observation was also confirmed by the desirability scores obtained.

3.3.3.4 Model validation

To validate the models developed from the CCDs (Equations 2 to 5), the optimum cellulose dosages and paper sludge loadings obtained from the response desirability optimisation were used in fed batch SSF cultures using the 1.3 L BioFlo 110 Modular Benchtop Fermenter. The quadratic models from the CCDs predicted a final ethanol concentration of 47.72 g l^{-1} at an ethanol yield of 85.34% using Sample 1 as feed stock (Figures 3.3 and 3.4), whereas a final ethanol concentration of 57.31 g l^{-1} at an ethanol yield of 94.07% was predicted using Sample 2 (Figures 3.3 and 3.4). The optimum cellulase dosages and paper sludge loadings obtained from the response desirability optimisation were used for triplicate runs and can be seen in Figure 3.6.

As evident from these cultivations, the quadratic models were accurate in predicting the final ethanol concentration and yield for both Samples 1 and 2. A mean ethanol concentration of 47.37 g l^{-1} with a standard deviation of 1.28 g l^{-1} was obtained for Sample 1 and a mean ethanol concentration of 57.06 g l^{-1} with a standard deviation of 1.45 g l^{-1} was obtained for Sample 2. The models obtained from the two CCDs predicted the final ethanol concentration within 1% of the actual experimentally obtained values.

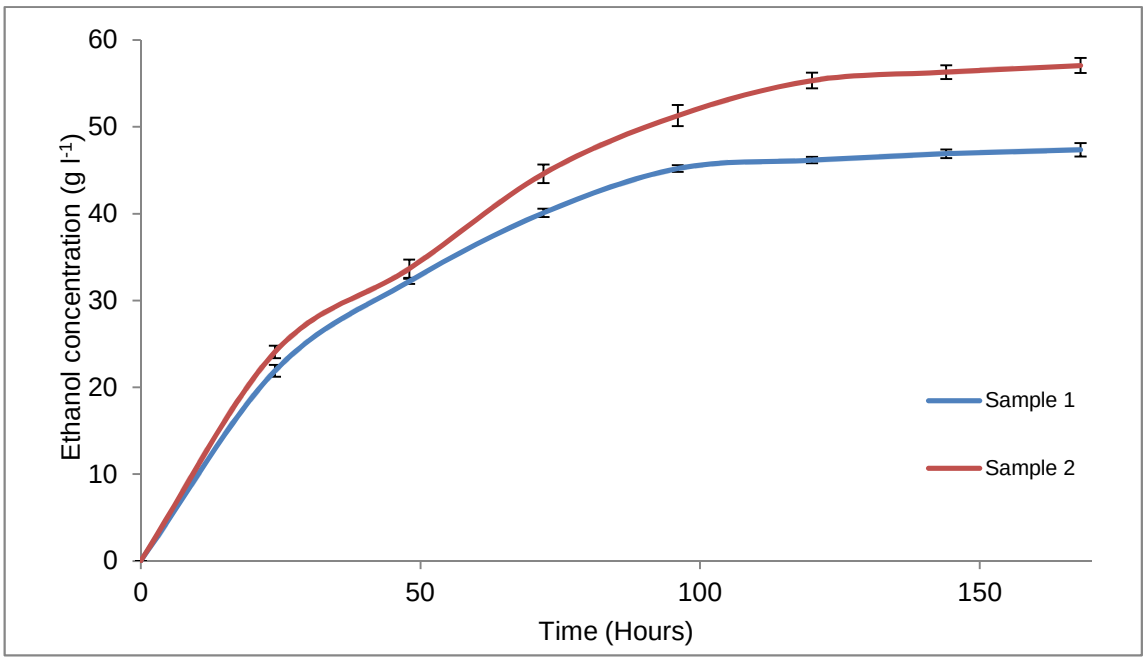

Figure 3.6: Triplicate Fed-batch SSF runs using the 1.3 L BioFlo 110 Modular Benchtop Fermenter using Samples 1 and 2 at optimum paper sludge loadings and Optiflow RC 2.0 dosages, suggested by multiple response optimisation. Error bars indicate the standard deviations obtained by the triplicate runs.

The chemical composition of the solid residues after fermentation (Figure 3.6) was determined using the standard Laboratory Analytical Procedures for biomass analysis (NREL, 2012). This was done to determine the overall ethanol yield based on the glucose consumed for Samples 1 and 2 respectively. The composition of the fermentation residues can be seen in Table 3.8. The impact of sampling and yeast growth during the fermentation was assumed to be negligible.

Table 3.8: The chemical composition of the fermentation residues

	Ash (g/100g)	**Glucose (g/100g)**	**Xylose (g/100g)**	**Lignin (g/100g)**	**Extractives (g/100g)**	**Σ Components (%)**
Sample 1	41.16	9.50	8.835	28.80	7.92	96.22
Sample 2	34.80	4.51	12.13	31.87	11.52	94.82

Sample 2 is more digestible than Sample 1 as there is less glucose contained in the fermentation residue. It should be noted that all the solubilised glucose were consumed by the yeast in the fermentation of both Samples 1 and 2. Although a significant portion of the xylose were hydrolysed by the enzymes it was not consumed by the yeast and was present in the supernatant but not in the solid residues. A mass balance was calculated over the ash content in the solid residues to determine the mass of glucose contained in the residues. The overall ethanol yield was determined on a basis of 1 kg fermentation broth and can be seen in Table 3.9.

Table 3.9: Overall ethanol yields obtained from SSF for Samples 1 and 2 at optimum cellulase dosage and solids loading as determined by multi response optimisation. The percentages of the theoretical maximum are shown in brackets.

	Sample 1	**Sample 2**
Paper sludge In (g)	208	217
Glucose In	112.34	125.83
Glucose Out	7.13	2.84
Glucose Hydrolysed	105.21 (93.65%)	122.99 (97.74%)
Xylose In	16.84	24.43
Xylose Out	6.63	7.62
Xylose Hydrolysed	10.21 (60.63%)	16.81 (68.80%)
Ethanol Produced (g/L)	47.38	57.06
Ethanol Yield (g/g $_{\text{GLUCOSE CONSUMED}}$)	**0.450 (88.23%)**	**0.464 (90.98%)**
Overall Ethanol Yield (kg/ton)	**228.00 (82.63%)**	**262.90 (88.92%)**

More glucose from Sample 2 was hydrolysed compared to Sample 1, which explains the higher ethanol concentrations and yields compared to Sample 1 (Figure 3.6), although similar ethanol yields were obtained based on glucose consumed. Overall ethanol yields of 262.90 kg per dry ton paper sludge were obtained with Sample 2 compared to the 228 kg per dry ton Sample 1 which can be attributed to the superior digestibility of Sample 2. Overall percentage yields of 82.63% and 88.92% were obtained for Samples 1 and 2 respectively, which is slightly less than the percentage yields of 85.34% and 94.07% predicted by the two CCDs for both Samples 1 and 2. The difference can be attributed to sugar contained in the yeast cells and the impact of sampling on the fermentation.

More than 60% of the available xylose in the paper sludge were hydrolysed by the enzymes but not consumed by the *S. Cerevisiae* strain MH 1000 as it is a wild type strain. Assuming a xylose utilising recombinant strain could convert the hydrolysed xylose to ethanol with a conversion 80% of what is theoretically possible, ethanol concentrations can be increased by up to 20 kg ton^{-1} paper sludge

3.4 Discussion

In recent times there has been an increased focus on the use of paper sludge as a feedstock for the production of second generation ethanol using SSF. Most of this research was conducted on sludge emanating from the Kraft pulping process (Fan *et al*., 2003; Fan and Lynd, 2006; Kang *et al*., 2010; Zhang and Lynd, 2010). The results presented in this study differed from published results as the paper sludge used in this study emanated from recycled fibre operations. This work, therefore, fills a distinct gap in the literature as evident from the distinct paucity in the literature

where fibre from recycling operations was used in SSF culture. In fact, only one paper could be found where recycled paper sludge was used as feedstock for the production of ethanol (Marques *et al*., 2008).

3.4.1 Ash content

The major challenge facing the production of ethanol from paper sludge emanating from recycle fibre operations is the high ash content of the material. As shown in Table 3.2, paper sludge from recycled fibre operations contains ash in excess of 55% (w/w). There is no literature available where the ash content of paper sludge was as high as found in this study. In the study of Fan *et al*. (2003) the average ash content of Kraft sludge was 23.2% (w/w), whereas the ash content of the recycled paper sludge used by Marques *et al.*, (2008) was 29.3 % (w/w).

Ash found in recycled paper sludge mostly originates from fillers used in the papermaking process and consist mostly out of calcium carbonate, clays and titanium dioxide (Biermann, 1993). More than 80% of recycled fibre plants produce corrugated material, paper board, chipboard and roofing material, where contaminant removal is not as important as for tissue and other bright grades of paper-based products (Biermann, 1993). Therefore, the high ash content found in the paper sludge received from Nampak Tissue (Pty) Ltd. could be attributed to the fact that this plant produces tissue paper where it is essential to remove all ash and contaminants to ensure final tissue paper of high quality, which explains the high ash content of the sludge effluent.

A major drawback of the ash content in the paper sludge is the buffering effect it has on the fermentation broth, which is usually close to neutrality. This pH is higher than the optimum for both the cellulase enzymes and the fermenting yeast (Kang *et al*., 2010), resulting in a decreased efficiency in both digestibility and fermentation performance. Furthermore, a process using sludge with high ash content as present in this study would result in high capital costs for the SSF process. This is due to equipment requirements, especially in terms of sizing, to accommodate the high ash content of the paper sludge. By removing the ash from the paper sludge, smaller equipment can be used, which in turn would result in a lower capital investment. A third disadvantage presented by the high ash content is that it proportionally decreases sugar content of the material on a dry mass basis, resulting in an average glucose content of only 25.4% (w/w) (Table 3.2). This value is considerably lower than the average sugar content of 42% (w/w) found in sludge from the Kraft process (Fan *et al*., 2003). The influence of ash on the work of Marques *et al*. (2008) was also evident from a proportionally lower glucose content of 34% (w/w), using sludge from a process that manufactured recycled fibre products.

In this study, washing of paper sludge was investigated to lower the ash content, thereby proportionally increasing the glucose concentration in the paper sludge in a manner similar to what can be achieved in an industrial recycling operation. As shown in Table 3.3, a wash step based on disintegration and washing over a 200 µm screen lowered the ash content to between 10.08% and 19.34% (w/w). This resulted in an increased glucose content that ranged between 52.29% and 57.99% (w/w), ultimately leading to higher ethanol concentrations per unit of paper sludge. These values are comparable to glucose concentrations obtained in the washing of Kraft mill sludge (Kang *et al.,* 2011). The improvement in ethanol yield was confirmed in

Table 3.4 where washed paper sludge samples produced ethanol titres that were up to 2-fold greater than that from unwashed paper sludge samples, even though an equivalent sugar loading for the unwashed sludge was used. The influence on process volume during this work was also quite evident since the use of an equivalent fibre loading required a 2.5-fold greater paper sludge loading, compared to the washed sludge. The data thus confirmed that the ash content in paper sludge reduces the fermentability of the paper sludge.

3.4.2 Variation in ethanol concentrations obtained with the nine paper sludge samples used in this study

Generally, there was a 9.8% (w/w) difference in glucose content between the various washed paper sludge samples (Table 3.3), whereas the final ethanol concentration in batch fermentations differed by up to 26.7% (Table 3.4). This difference could be attributed to the nature of the paper feedstock used in the paper mill.

During manufacture of lower grade tissue paper, larger fractions low quality paper feedstock such as magazines and newsprint are used compared to the manufacture of higher grade tissue paper where high quality feedstock such as office paper is used. Newsprint and magazines are produced from mechanical or chemi-mechanical (CTMP) pulp which is less extensively chemically treated compared to office paper which is produced from pulp emanating from Kraft (NaOH and Na_2S) or sulphite (H_2SO_3) pulping processes where the pulp is extensively chemically treated (Biermann, 1993). The paper sludge samples which yielded lower ethanol concentrations are obtained from the manufacturing of low quality tissue paper where the feedstock is less refined and not as extensively chemically treated compared to

high grade paper feedstock. The lower quality of the feedstock could thus be related to a lower degree of digestibility, due to less severe chemical pretreatment, which is a requirement for high digestibility.

It is important to obtain different samples of paper sludge at the same source. Although there may be no significant variation in composition, differences in fermentability were observed in this work. The variation in fermentability was attributed to variations in feedstock used at the paper mill. To apply SSF technology on industrial scale a range of paper sludge samples over a significant time period should be analysed, to obtain a range of fermentability data for the design of the SSF plant to handle a worst case feedstock, i.e. material that is most resistant to enzymatic degradation.

3.4.3 Cellulase preparations

In literature a variety of cellulase preparations were used for SSF with paper sludge as feedstock. In most of these studies Spezyme CP was the preferred cellulase preparation (Kang *et al*., 2010; Zhang *et al*., 2009; Zhang and Lynd, 2010). Other cellulase preparations used were Celluclast 1.5L (Marques *et al*., 2008), DP151 (Fan *et al*., 2003) and Novozym 342 (Peng and Chen, 2011). In most studies the cellulase preparation was supplemented with β-glucosidase (Novozym 188), since commercial cellulase enzymes are usually not rich in β-glucosidase enzymes, although it is essential to convert cellobiose to glucose for fermentation to ethanol (Kádár *et al*., 2004). Furthermore, cellobiose results in feedback inhibition on cellobiohydrolases, further stressing the requirement for inclusion of β-glucosidase in the hydrolysis-fermentation process (Zhao, 2004).

There is a lack of literature available where different cellulase preparations were compared using paper sludge as a feedstock, with limited information available where different cellulase preparations were compared to other sources of lignocellulosic feedstock. Three available cellulase preparations (Optiflow RC 2.0, Spezyme CP and Cellic CTec 1) were compared in SSF with paper sludge as substrate. Due to its popularity in the literature, Spezyme CP was used as benchmark in the present study allowing a comparison in the performance of Optiflow RC 2.0 and Cellic CTec 1. Other instances where the latter two preparations were used include an SSF process with sugarcane bagasse as feedstock with Optiflow RC 2.0 as cellulase preparation (Cruz *et al*., 2011), whereas Cellic Ctec1 was used as cellulase preparation for the enzymatic hydrolysis of waste paper as feedstock (Wang *et al*., 2012).

Ethanol concentrations achieved when using of Optiflow RC 2.0 and Spezyme CP in SSF using paper sludge emanating from Nampak Tissue Pty Ltd. was up to 2-fold greater than the ethanol concentrations obtained when using Cellic CTec 1 (Table 3.4). This data highlighted the importance of screening cellulase preparations to obtain the best possible SSF results. Although Optiflow RC 2.0 and Spezyme produced similar results, Optiflow RC 2.0 produced more ethanol than Spezyme CP for 6 of the 9 paper sludge samples used in this study (Table 3.4) and served as basis for the decision for using this cellulase preparation to conduct all optimisation work in fed-batch culture.

3.4.4 Simultaneous saccharification and fermentation process optimisation

An RSM technique widely used in industry is a CCD as it can be used to fit second order response surfaces (Montgomery and Runger, 2007). In this work, a CCD was used to optimise the final ethanol concentration and ethanol yield, expressed as a percentage of theoretical maximum through variation of the solid loading and cellulase enzyme dosage.

The final ethanol concentration increased with an increase in paper sludge loading and cellulase dosage (Figure 3.3) for both Samples 1 and 2, initially selected based on their performance in terms of ethanol titre in batch culture (Table 3.5). The increase in ethanol concentration could be attributed to the increase in sugars available for fermentation and can be directly related to the increase in solids concentration. However, Figure 3.4 clearly showed that although the ethanol concentration at high solid loadings increased, the higher loading resulted in a decreased ethanol yield. The drop in yield could be attributed to poor mixing (Olofsson *et al*., 2008), which impacts on the efficiency of the enzymes (Rosgaard *et al*., 2007). Furthermore, at high paper sludge loadings the ash content would increase to levels where the pH of the broth would increase above pH 5.0, even though washed solids were used. According to a model developed by South *et al*. (1995) ethanol concentrations in excess of 50 g l^{-1} are near the inhibitory threshold of S. *cerevisiae* at 37 ℃. Although the fermentations were carried out at 35 ℃ it is close to 37 ℃ and as a result ethanol inhibition could b e impacting yeast performance.

Multiple response optimisation incorporating desirability functions was used to optimise SSF with regards to ethanol concentration and ethanol yield, which revealed that the optimum solid loading and cellulase dosage for both Samples 1 and 2 were almost identical. Therefore an optimum solid loading of 21% (w/w) and an Optiflow RC 2.0 dosage of 14.5 FPU g^{-1} dry solids can be used for an SSF process utilising paper sludge from Nampak Tissue (Pty) Ltd. as feedstock regardless the nature of the paper sludge used. At the optimum solid loading and cellulase dosage ethanol concentrations and ethanol yields in excess of 47.0 g l^{-1} and 85.0% can be expected regardless the paper sludge produced at Nampak Tissue (Pty) Ltd.

Ethanol yields of 0.450 and 0.464 g/g glucose consumed (Table 3.9) were obtained for Samples 1 and 2 respectively at the optimal solid loading and enzyme dosage as determined by multi response optimisation, which were similar to the ethanol yield of 0.466 g/g glucose consumed obtained by Fan *et al*. (2003). Although similar ethanol yields were obtained based on glucose consumed, higher ethanol concentrations and ethanol yields based on the theoretical maximum were obtained (Figure 3.6). The high ethanol concentrations obtained in this study can be attributed to the composition of the washed sludge as washing resulted in glucan concentrations that ranged between 52.0% (w/w) and 57.9% (w/w), which is higher than the glucan contained in Kraft mill sludge used by Fan *et al*. (2003).

3.4.5 Micro-organism used for fermentation

It is recommended that a recombinant yeast strain be used in SSF with paper sludge as a feedstock as xylose contained in the paper sludge could be used as a secondary carbon source (Table 3.9), which could result in higher ethanol

concentrations and yields. Recombinant yeast strains are widely used in SSF of lignocellulose to produce ethanol and specifically for the fermentation of paper sludge (Kang *et al*., 2010). A recombinant *Escherichia coli* ATCC-55124 (KO11) was used by Kang *et al*. (2010), whereas *Pichia stipitis* CBS 5773 was used by Marques *et al*. (2008). *S. cerevisiae* RWB222 and *Zymomonas mobilis* 8b were used by Zhang and Lynd (2010). The use of a recombinant yeast strain may result in a 20% increase on ethanol titre compared to a non-recombinant strain, as it co-utilises xylose as a carbon source (Kang *et al*., 2010). In this study, a yeast strain *S. cerevisiae* D5A engineered to express xylose isomerase, thus inferring a capability to the yeast to utilise xylose as carbon source, was used but data revealed that the D5A strain did not utilise the available xylose and resulted in glucose accumulation during the first 24 h of the cultivation (Table 3.5). The engineered D5A strain requires pre-conditioning in a medium containing xylose to “activate” xylose utilisation (Zhang and Lynd, 2012).

3.5 Conclusions

Paper sludge has an advantage compared to other lignocellulosic feedstock as no pre-treatment is required prior to hydrolysis-fermentation for conversion to bio-ethanol. By washing paper sludge received from recycled fibre operations the ash content could be decreased to lower than 20% (w/w), which results in a higher level of fermentability compared to unwashed paper sludge. Paper sludge is an excellent feedstock for use in SSF to produce ethanol as final ethanol concentrations in excess of 47.37 g l^{-1} and yields in excess of 85% of the theoretical maximum were obtained.

It is important to obtain different samples of paper sludge from the same source at different time intervals. Although there may be no significant variation in composition, differences in fermentability were observed in this work. To apply SSF technology on industrial scale a range of paper sludge samples should be analysed to obtain a range of fermentability data to design a SSF plant to handle a worst case feedstock, i.e. material that is most resistant to enzymatic degradation. Presently, no literature is available where cellulase preparations were compared using paper sludge as a feedstock. From this study it can be concluded that screening of different paper sludge samples and matching cellulase enzymes is critical to optimise SSF.

The results obtained with Samples 1 and 2 confirmed that a CCD is an effective RSM method that can be used to model SSF. Furthermore, multi-response optimisation incorporating a desirability function approach was successfully used to optimise the response obtained during SSF. However, it is important to use the correct range of independent variables when using RSM to obtain an optimal response. Using multi-response optimisation, the optimal solid loading for samples 1 and 2 were within 5% of each other and similar results were obtained with the cellulase dosage. It can be concluded that a paper sludge loading of approximately 21% (w/w) and a cellulase dosage of 14.5 FPU g^{-1} be used irrespective of the paper sludge sample to obtain optimal ethanol concentrations and ethanol yields.

It is recommended that a recombinant strain utilising both glucose and xylose as carbon source be used as it would improve ethanol concentrations and yields obtained in this study. Assuming a xylose utilising recombinant strain could convert the hydrolysed xylose to ethanol with a conversion 80% of what is theoretically possible, ethanol yield can be increased by up to 20 kg ton^{-1} paper sludge.

4. Economic evaluation of paper sludge as feedstock for ethanol production from simultaneous saccharification and fermentation

Abstract

Whereas fuel for transport and energy are mainly fossil–derived, there has recently been an increased focus on bio-fuels due to the environmental impact of fossil fuels, an increased energy demand worldwide concomitant with depletion of fossil fuel reserves. Paper sludge produced by paper mills are high in lignocellulose and represents a largely untapped feedstock for ethanol production. In this study, an economic assessment was conducted to ascertain whether ethanol production from paper sludge using simultaneous saccharification and fermentation (SSF) is economically viable. Three scenarios were investigated. In the first scenario revenue was calculated from the ethanol sales linked to the basic fuel price, whereas in the second and third scenarios, LPG consumption at the paper mill was respectively replaced with anhydrous and 95% ethanol produced from the sludge. In all cases, paper sludge feed rates of 15, 30 and 50 t d^{-1} were used. The first scenario yielded higher IRR values compared to scenarios two and three. The SSF process is economically viable at a paper sludge feed rate of 50 t d^{-1} as an IRR value of 30% were obtained at an enzyme cost of $ 0.90 gal^{-1} (R 2.01 $litre^{-1}$). These findings were supported by a sensitivity analysis and a Monte Carlo analysis. An additional environmental benefit of a greenhouse gas reduction of 42.5% was obtained for the production of ethanol from paper sludge.

4.1 Introduction

According to Seabra *et al.* (2010), the vast majority of liquid transportation fuels are derived from fossil-based oil. Furthermore, the impact of global warming as a result of the utilisation of fossil oil reserves led to the need to curb greenhouse gas emissions (Dias *et al.*, 2011). Lignocellulosic biomass is a renewable and sustainable resource, ideal for energy production and can be converted to liquid, gaseous and solid fuels by thermochemical and biological conversion.

Recent developments in the lignocellulosic waste to biofuels field illustrated that a broad range of waste materials can be converted to biofuels such as bioethanol (van Wyk, 2003). The present study focuses on the production of ethanol by the fermentation of paper sludge, obtained from the manufacturing of tissue paper where recycled paper is used as feedstock. Since the manufacturing of paper results in the damaging and shortening of pulp fibres, between 15 – 20% of the pulp feed stock is removed as paper sludge for disposal (Jeffries and Scartman, 1999). The high carbohydrate content of paper sludge enables it to be biologically converted to ethanol with an additional benefit of negative feedstock cost associated with the use of paper sludge (Kang *et al.*, 2010).

The cells in lignocellulosic biomass consist out an amalgamation of lignin, hemicellulose and cellulose. The lignin and hemicellulose encloses the cellulose fibres and prevent cellulase enzymes from hydrolysing the cellulose to its monomeric form which is required for fermentation to ethanol (Cruz *et al.*, 2011). Lignocellulose requires a pre-treatment step to increase the digestibility of cellulose and is usually carried out using steam explosion, acid treatment, or a combination thereof (Fan, 2004), which

contributes up to 30% of the total production cost of the bioethanol process. However, pre-treatment is not required to enhance the amenability of paper sludge for enzymatic hydrolysis due to disruption of the lignocellulosic structure during paper manufacturing (Kang *et al*., 2010).

Ethanol can be produced from paper sludge by separate hydrolysis and fermentation (SHF) or simultaneous saccharification and fermentation (SSF) (Marques *et al*., 2008). The major disadvantage of SHF resides in the feedback inhibition of cellulase enzymes by accumulated glucose, which results in low ethanol yields. On the other hand, in SSF the fermenting micro-organism converts glucose to ethanol as soon as it is hydrolysed eliminating glucose inhibition and resulting in higher ethanol yields (Lin and Tanaka, 2005).

The data obtained in Chapter 3 revealed that ethanol concentrations and yields in excess of 47.0 g l^{-1} and 84.5% of the theoretical maximum were obtained from the fermentation of paper sludge. The next step is to do an economic assessment to determine if investing in the SSF process with paper sludge as a feed stock is an economically viable option.

For an investor or organisation to consider an investment in a new project or the expansion of current operations, the economic viability of the project should be proven beyond reasonable doubt. Such economic viability can be determined by using economic modelling techniques, based on financial statements where resulting cash flows indicates whether or not proceeds will sustain the investment with a reasonable rate of return. Key economic indicators such as the internal rate of return

(IRR), net present value (NPV), payback period and discounted payback period are often used as a measure of economic viability (Perry and Green, 2008).

However, economic analyses that use deterministic estimates in calculating key economic indicators neglect uncertainty and risk in the model. The use of probability distributions quantifies the possibility of economic success and risk of failure (Amigun *et al*., 2011). A probabilistic method such as the Monte Carlo analysis provides a powerful tool used to model the uncertainty in the input by accounting for the uncertainties characterised by probability distributions. The response from the Monte Carlo analysis results in a probability distribution of key economic indicators (Petersen, 2012), thus providing an assessment of the probability of success.

To date, there is no information in literature available where a Monte Carlo analysis was used to estimate the economic viability of the production of ethanol from paper sludge. In fact, such analyses are quite scarce for lignocellulosic material in general, especially in the South Africa setting. In one instance, such an analysis was used to model the risk of producing ethanol from wheat in South Africa. For a bioethanol plant with a capacity of 103 million m^3, a probability of 97% was obtained to achieve a positive NPV at a discount rate of 25% (Richardson *et al.*, 2007) assuming a project life of 25 years. However, although the tool was highly effective, wheat might not be a suitable substrate for ethanol production due to its possible competition with staple food reserves.

The aim of this chapter is four-fold, namely (1) to model the SSF process *in silico* to obtain the necessary mass and energy balances required for the estimation of the capital investment, (2) to assess key economic indicators forthcoming from the

resultant discounted cash flow, (3) to assess the degree of uncertainty associated with these outputs and (4) to determine the greenhouse gas (GHG) reduction from the production of ethanol from paper sludge. Consequently, the chapter was divided into four main sections. In the first section the SSF process was modelled using Aspen Plus® to obtain the necessary mass and energy balances required to develop equipment specifications for the process. The optimal solid loading (21% w/w) and cellulase dosage (14.5 FPU g^{-1}) obtained in Chapter 3 was used as input for the model. The required capital investment for the SSF process was calculated from equipment costs obtained from Logichem, a prominent chemical engineering contractor specialising in ethanol plants (http://logichem.com, 2012).

In the second section of this chapter a discounted cash flow was used to evaluate three scenarios, where the first scenario incorporated the sales of ethanol, based on the basic fuel price. The second and third scenarios were based on the replacement of liquefied petroleum gas (LPG) at recycled fibre paper mills with anhydrous and 95% (w/w) ethanol respectively. LPG is used at recycled fibre paper mills for heat generation which is used to dry tissue paper on the paper machine.

A Monte Carlo analysis was used in the third part of the chapter to assess the degree of uncertainty associated with the input variables used in the discounted cash flow sheet, and in the fourth part of the chapter the GHG reductions from the production of ethanol from paper sludge was compared to a scenario where the paper sludge is landfilled.

4.2 Process Design, Modelling and Capital investment

4.2.1 Assumptions

The core assumption was that the bioethanol plant be built on-site at the paper recycling mill to reduce the distance the paper sludge feedstock would have to be transported, which would automatically result in a substantial saving in terms of transport costs. The utilities and process water required for the bioethanol plant was assumed to be supplied by the paper mill. An added advantage of this approach is that the waste water from the bioethanol plant be integrated into the existing waste water treatment works of the paper mill, thus obviating water treatment and associated costs.

4.2.2 Methodology

The first step required in the development of a paper sludge to ethanol process model was to develop a process flow diagram (PFD). A novel PFD was developed from first principle fundamentals. The equipment presented in the PFD was then used to develop a model in Aspen Plus® to obtain the necessary mass and energy balances required to size the equipment in the SSF process. Process designs were developed for paper sludge feed rates of 15, 30 and 50 t d^{-1} as most paper mills in South Africa produce between 15 and 50 dry tons paper sludge a day. Cost estimates for the equipment required in the process design were obtained from Logichem. Cost estimates were obtained from Logichem due to the variance of estimates found in literature and Aspen Icarus® (Section 4.2.4). The total capital investment was calculated using installation factors supplied by Sinnott (2005).

After the determination of equipment costs for the SSF process at paper sludge feed rates of 15 and 50 t d^{-1}, the economy of scale equation was used to determine equipment costs for the SSF process at 30 t d^{-1}. The economy of scale equation (Seider *et al*., 2004) can be stated as:

$$\frac{Cost\ 2}{Cost\ 1} = \left(\frac{Capacity\ 2}{Capacity\ 1}\right)^{m} \qquad \text{Eq. 1}$$

where Cost 1 is the known equipment cost at a known paper sludge feed rate (Capacity 1) and Cost 2 is the equipment cost at a desired paper sludge feed rate (Capacity 2). The exponent (m) is the economy of scale exponent which can generally be assumed to be 0.6, but can be calculated if equipment costs at different paper sludge feed rates are known.

4.2.3 Results and Discussion

4.2.3.1 Proposed ash removal system at the paper mill of Nampak Tissue (Pty) Ltd. Bellville.

Data from Chapter 3 revealed that the original paper sludge samples contained between 56.29% and 65.50% (w/w) ash. The high ash content resulted in low ethanol yields and thus required an additional wash step for ash removal. This is a crucial requirement since the high ash content of the paper sludge would result in increased equipment costs for the SSF process, as larger equipment will be required to accommodate larger process volumes as a result of the high ash content. For the SSF process to be implemented on industrial scale an ash removal system should, therefore, be incorporated.

A schematic representation of the sludge operations at the paper mill of Nampak Tissue (Pty) Ltd. located in Bellville, South Africa can be seen in Figure 4.1. Waste streams from the dissolved air flotation and waste water clarifiers are pumped to sludge tank 3. Waste streams from the KROFTA flotation system and de-inking sections of the paper mill are pumped to sludge tank 1 where it is combined with the slurry from sludge tank 3. The waste slurry is pumped to centrifuges 1 and 2 for dewatering. The paper sludge emanating from the centrifuges are conveyed to the waste skips for disposal.

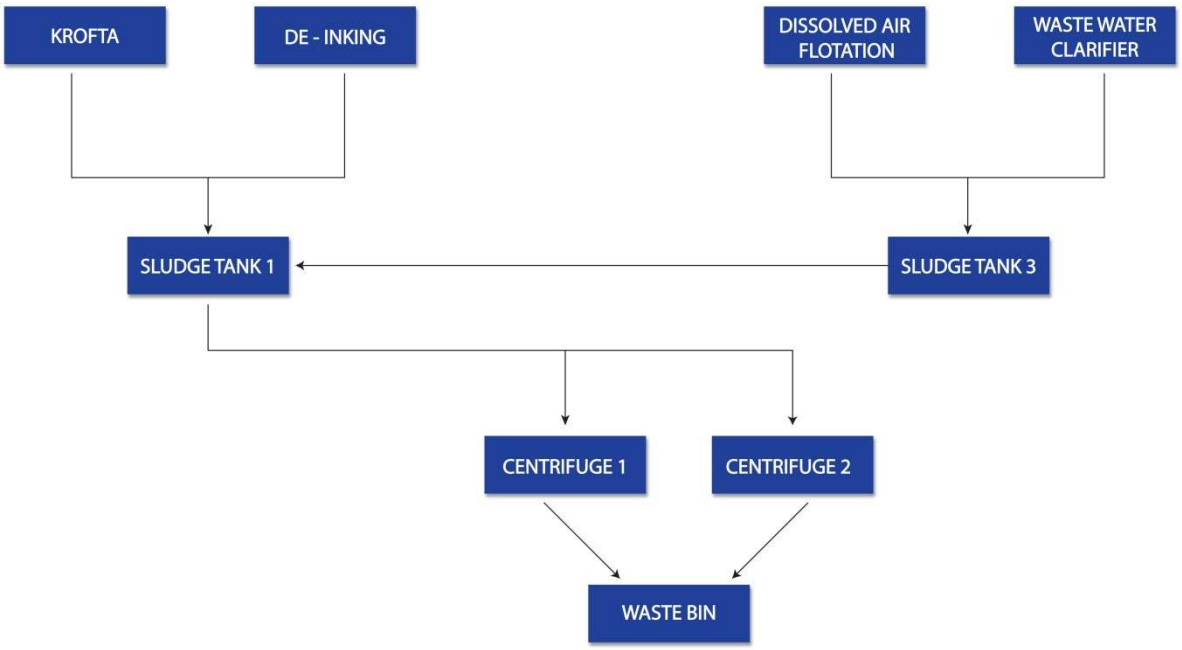

Figure 4.1: Schematic representing the current sludge operations at the paper mill

The waste stream from the KROFTA flotation system contributes the majority of the ash contained in the paper sludge (Nampak Tissue, 2012). It is proposed that an additional sludge storage tank (Sludge Tank 4) and centrifuge (Centrifuge 3) be installed and to reroute the waste stream from the KROFTA flotation system to the proposed sludge tank and centrifuge. The KROFTA waste material is thus partitioned away from the rest of the waste, but would require removal to landfill. A schematic

representation of the proposed alterations to the sludge system at the existing paper mill of Nampak Tissue (Pty) Ltd. can be seen in Figure 4.2.

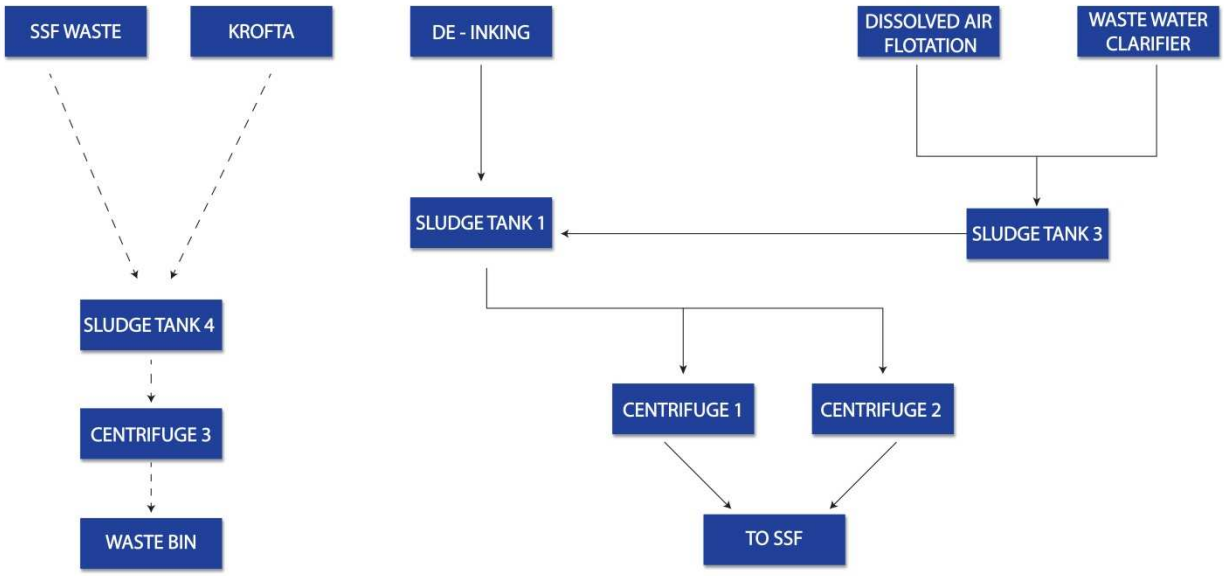

Figure 4.2: Schematic representing the proposed alterations the paper mill

From the Figure 4.2 it can be seen that the washed sludge used in SSF is obtained from the current sludge system with the KROFTA waste stream rerouted to the proposed sludge tank and centrifuge. The waste emanating from SSF will be combined with the waste stream from the KROFTA system and dewatered in the proposed centrifuge. The sludge from the centrifuge is conveyed to a waste skip for disposal. The proposed changes will result in paper sludge with substantially less ash and save on cost as no washing cycle is required to remove ash from the paper sludge.

The paper mill at Nampak Tissue (Pty) Ltd. Bellville produces 22 dry tons paper sludge a day. The KROFTA flotation system at the paper mill is designed to remove 10.46 kg solids a minute (15.06 t d^{-1}), but is running at an efficiency of 50% due to

technical difficulties (Nampak, 2012). Even at an efficiency of 50%, the installation of the proposed equipment allows for the removal of 7.53 tons of ash from the paper sludge a day. This would result in 15.47 tons of washed paper sludge a day. The equipment costs for the proposed alterations to the existing sludge system were based on washed paper sludge feed rates of 15, 30 and 50 t d^{-1} and calculated from Seider *et al*. (2004) and shown in Table 4.1. The equipment costs for the proposed alterations were included in the economic model (Chapter 4.3)

Table 4.1: Equipment cost estimation for the proposed alterations.

Items	**Paper sludge feed rate**		
	15 t/d	**30 t/d**	**50 t/d**
Decanter centrifuge	R 565 000	R 856 000	R 1 163 000
Sludge tank	R 40 000	R 60 000	R 81 500

The process for the production of ethanol from paper sludge using SSF can be broadly divided into three process areas namely: sterilisation, SSF and product recovery, which is discussed in detail below. The three process areas were modelled in Aspen Plus® to obtain the necessary mass and energy balances required to develop equipment specifications to acquire equipment cost estimates. The PFDs developed for the SSF process can be seen in Appendix A.

4.2.3.2 Paper sludge sterilisation

Sterilisation is a crucial first step in this process design since contamination during fermentation could result in decreased ethanol yields (de Souza *et al*., 2007), due to competition for liberated carbon with the fermentation yeast. Typical contaminants include the lactic acid bacteria such as *Lactobacillus. fermentum, L. salivariu,* and

L. casei (Skinner and Leathers, 2004). A schematic representation for the sterilisation section of the bioethanol plant can be seen in Figure 4.3.

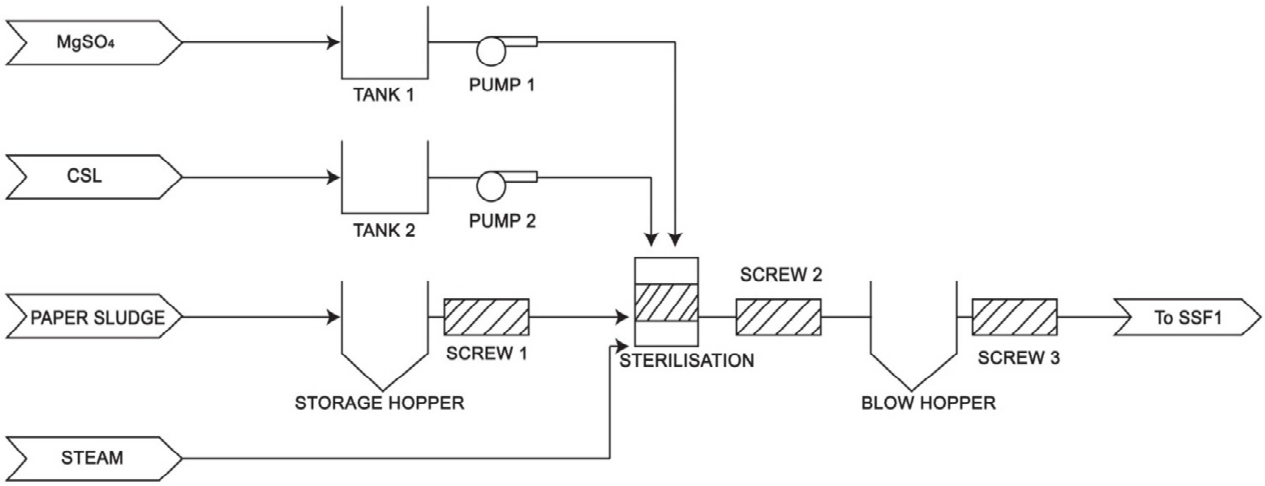

Figure 4.3: Schematic representation showing a batch-operated sterilisation process incorporating paper sludge and nutrient feeds.

Paper sludge, corn steep liquor (CSL) and $MgSO_4$ are conveyed to a sterilisation reactor. This reactor operates in batch mode with direct steam injection once the desired filling volume was reached, with the sterilisation process carried out at a designated temperature of 121 ℃ for 30 minutes.

Paper sludge with a solid content of 40% (w/w) received from the paper mill is stored in a storage hopper. The most practical method to convey the material from the hopper is by using a screw feeder since the high solid content of the sludge material precludes the use of a pump. Before onset of the sterilisation cycle, the CSL and $MgSO_4$ which are stored in separate storage tanks are simultaneously pumped into the sterilisation reactor.

The sterilisation reactor is operated in a batch mode, which is the preferred option instead of a continuously operated system. The primary reasons include simplicity in maintenance and a decreased likelihood of “sealing” problems. According to Logichem (personal communication), a continuous steriliser would be a combination of a screw feeder and a steam steriliser. It is suspected that difficulties may arise in maintaining the “seal” of a continuous sterilisation system, which could cause difficulties in maintaining the required steam pressure, especially over a prolonged usage period. By contrast, a batch steriliser is a simpler design without moving parts and due to a lower maintenance cost and greater longevity would result in an overall decrease in operational cost and capital expenditure.

Steam is injected into the contents of the reactor to raise and then maintain the temperature of the sterilisation vessel at 121 ℃. The paper sludge is sterilised for 30 minutes to ensure that all the paper sludge reaches the sterilisation temperature. Shorter sterilisation times could lead to cold spots in the paper sludge which is not sterilised. Sterilised paper sludge is fed by either a screw conveyor or under pressure from steam to a blow hopper. The use of steam will facilitate passage of the dense material through the feed line in a blowing action and will also result in decreased equipment and maintenance costs at this crucial step. From the blow hopper, which is specifically designed to accommodate the hot sterilised slurry, the material is fed by screw feeder to the first fermenter.

4.2.3.3 Continuous simultaneous saccharification and fermentation

In contrast to the sterilisation process which is operated in batch, the remainder of the process incorporating fermentation and distillation is operated on a continuous

basis, with a schematic representation of the fermentation process depicted in Fig. 4.4.

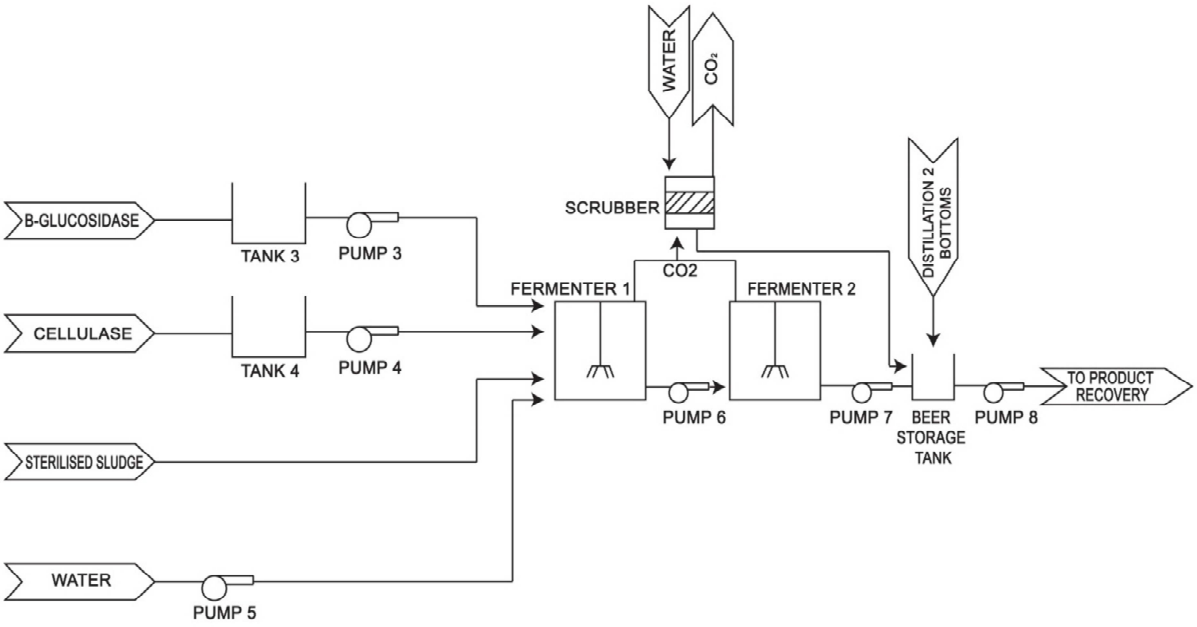

Figure 4.4: Schematic depicting the SSF section of the bioethanol plant

Paper sludge and the nutrients obtained from the blow hopper are fed to the first fermenter with process water to lower the solid content from 29% to 21% (w/w), which is the optimum solid loading to maximise ethanol production as well as ethanol yield. Cellulase and β-glucosidase addition is controlled with metering pumps to ensure precise dosage of these costly components. The CSL and $MgSO_4$ added to paper sludge supplies the necessary nutrients and nitrogen for the growth of yeast in the fermenters. Dry industrial yeast (*S. cerevisiae*) is added twice a year to replenish the yeast in the fermenters and is therefore not shown in Figure 4.4, nor does it present a significant operational cost. During shut down for maintenance the fermenters will be cleaned and sterilised with ethanol and as a result it will be necessary to add yeast to start the SSF process.

The heat contained in the paper sludge conveyed from the blow hopper is used to maintain the temperature in the first fermenter at 35 ℃. Both fermenters are fitted with external heating or cooling via two small heat exchangers to maintain the temperature in the fermenters. Both fermenters are designed to have a residence time of 60 hours each. From the experimental work in Chapter 3 it can be seen that there is no benefit in running fermentations longer than 120 hours since little ethanol is produced at longer fermentation times.

The basis for using two continuous fermenters in series in this design is based on the process design of Shoa (2007), who found that low conversions of paper sludge to ethanol were obtained during continuous SSF using a single fermenter. The poor conversion was attributed to a proportion of undigested paper sludge particles exiting the reactor, thus prohibiting full digestion of the sugar polymers and resulting in lower ethanol yields. The introduction of a second fermentation step in the process, therefore, decreases the probability of unconverted particles exiting fermentation, maximising conversion to ethanol.

An additional advantage of using two fermenters in series is that less mixing energy is required when compared to the use of a single fermenter. At the start of the fermentation the viscosity of the fermentation slurry is high but the viscosity is reduced rapidly during enzymatic hydrolysis. Whereas the slurry in a single fermenter would result in a high viscosity, the use of multiple fermenters would imply that only a fraction of the total fermentation volume is at high viscosity (Shoa, 2007).

The fermenters are operated at a meter pressure of 0.1 bar to prevent atmospheric air or contaminants from entering the fermenter vessel during the fermentation. The

pressure is maintained by the CO_2 produced during the fermentation. The CO_2 produced in the fermenters are purged to the scrubber to recover the ethanol in the vapour stream, while the flow rate of the exit gas is controlled to maintain the pressure in the reactor vessel.

The beer storage tank adds controllability to the system from a process control viewpoint as it absorbs fluctuations in the SSF process prior to distillation. It is important to ensure that there is no fluctuation in the distillation feed rate since tight control of the column is essential to ensure product quality.

4.2.3.4 Ethanol recovery

The ethanol contained in the beer is recovered via two distillation columns in series and purified to 95% (w/w) ethanol. Molecular sieves are used as the final stage of purification to obtain anhydrous ethanol. The ethanol recovery process is depicted in Figure 4.5. Beer from the beer storage tank is pumped to the feed heat exchanger where it is heated to 80 ℃ before entering the first distillation column. The process was designed in such a manner that heat contained in the bottoms of the first distillation column can be recovered and used to heat the feed to the column, thus making the process more energy efficient. The cooled distillation bottoms are pumped to the waste water treatment plant of the paper mill. The distillate from the first distillation column is condensed and contains 35% (w/w) ethanol, which is subsequently fed to the second distillation column, where ethanol is purified to 95% (w/w). The bottoms of the second distillation column contain 5% (w/w) ethanol and are pumped to the beer storage tank in the SSF section for ethanol recovery. The

ethanol product obtained from the second distillation column is sent to the molecular sieves where the water is adsorbed to produce anhydrous ethanol.

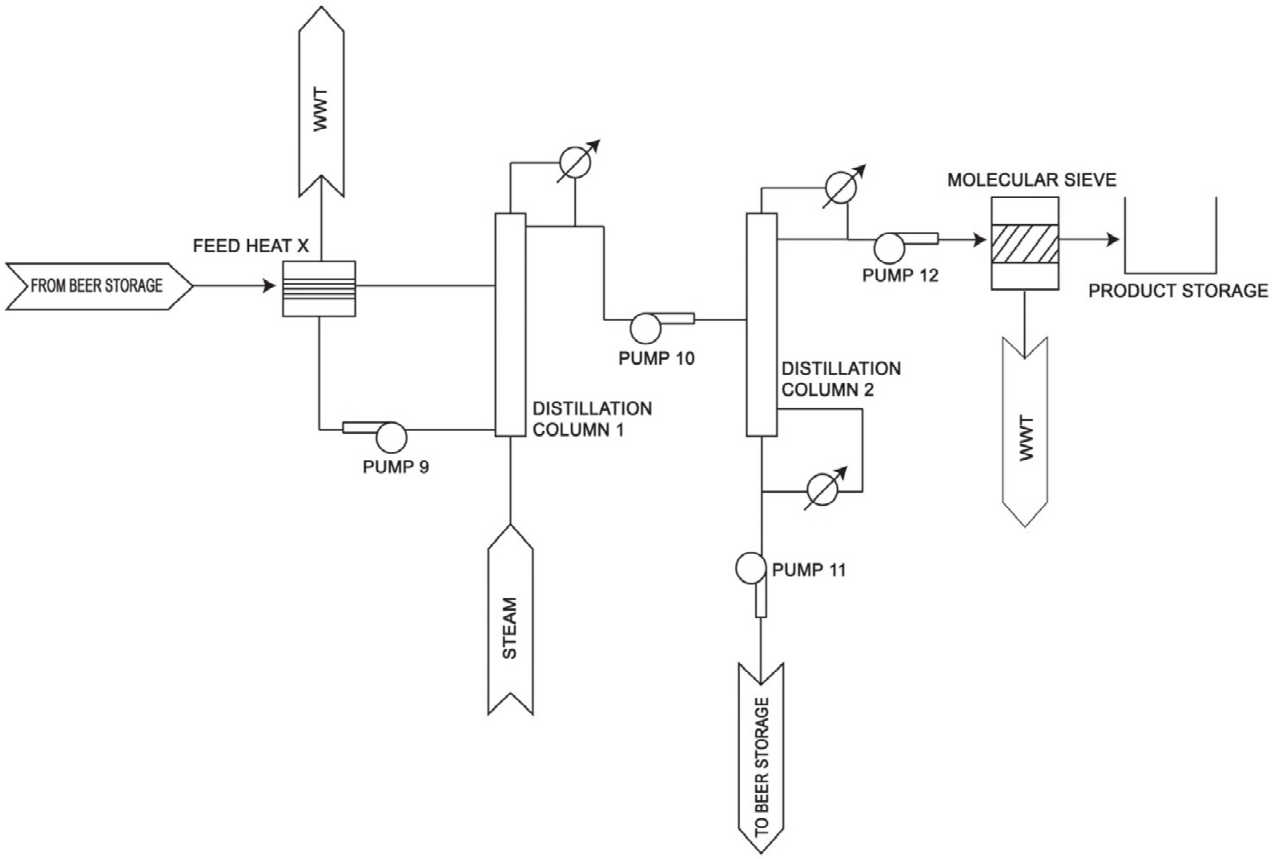

Figure 4.5: Schematic showing the ethanol recovery area of the bioethanol plant

The first distillation column contains 40 sieve plates calculated using Aspen Plus® at a plate efficiency of 65% with the feed entering the column on plate 15. According to this design, the distillation column will recover 99% (w/w) of the ethanol in the beer feed with a final distillate concentration of 35% (w/w) ethanol. The first distillation column operates with direct steam injection. Although the ash content is significantly lowered during ash removal, the paper sludge used in the SSF contains a significant portion of ash. The ash contained in the beer would result in substantial fouling if a reboiler was used. This in turn would increase maintenance costs and could result in substantial down-time of the plant, as well as inefficient ethanol recoveries once

fouling has reached a critical level. A second function of the first distillation column is to fulfil a sterilisation function. The beer which enters the distillation column may contain genetically modified organisms which need to be destroyed prior to waste water treatment.

The second distillation column purifies the ethanol product to 95% (w/w), which is near the azeotrope of the ethanol/ water mixture. The second distillation column contains 35 sieve plates with the feed entering the column on plate 20. Heat is provided to the second distillation column via a reboiler. A reboiler can be used in the second distillation column as all the ash has been removed in the bottoms of the first column.

The vapour ethanol product obtained from the second distillation column is sent to the molecular sieves where the water is adsorbed to produce anhydrous ethanol. Molecular sieves work on the principle of adsorption where zeolite in the molecular sieves has an affinity for the water molecules in the vapour feed. Following this adsorption step, an ethanol concentration of 99.9% (w/w) can be obtained (Dias, *et al*., 2011).

4.2.4 Capital Investment

Aspen Icarus® was used to determine the capital investment for the process models developed and can be seen in Table 4.2. For comparison, capital investments for a similar SSF process developed by Fan (2004) are shown.

Table 4.2: Capital investment estimates obtained from Aspen Icarus® and Fan (2004).

Method	Paper sludge feed rate		
	15 t/d	**30 t/d**	**50 t/d**
Aspen Icarus®	R 28.0 mil	R 31.3 mil	R 35.6 mil
Fan (2004)	R 37.2 mil	R 67.8 mil	R 84.7 mil

As seen in Table 4.2 there is a significant difference between the estimates obtained using Aspen Icarus® and Fan (2004), especially at high paper sludge feed rates. A possible explanation for the low capital investments obtained at high paper sludge feed rates when using Aspen Icarus® is that the range of equipment sizes required is to narrow as Aspen Icarus® sources estimates from a database of known costs for existing equipment. To obtain cost estimates from Logichem, the equipment sizing was calculated by hand from the mass and energy balances obtained from Aspen Plus®. The detailed equipment specifications used for equipment cost estimates at paper sludge feed rates of 15 and 50 t d^{-1} can be seen in Appendix B with the equipment cost estimates obtained from Logichem shown in Appendix C.

Using the equipment cost estimates obtained from Logichem, the exponent of the economy of scale equation (Equation 1) was calculated as 0.532, by solving the economy of scale equation using the mean cost estimate obtained for each flow rate. Using this information, the equipment purchase costs obtained for the 15 t d^{-1} bioethanol plant and the calculated exponent were used to calculate equipment purchase costs for an SSF plant with a sludge feed rate of 30 t d^{-1}.

The total capital investment for the SSF process at paper sludge feed rates of 15, 30 and 50 t d^{-1} was then calculated from the equipment purchase costs (including the equipment required for ash removal (Table 4.1)). The equipment purchase cost,

installed equipment cost and fixed capital investment for paper sludge feed rates of 15, 30 and 50 t d^{-1} are shown in Table 4.3.

Table 4.3: The equipment purchase cost, installed cost and fixed capital investment

Items	Paper sludge feed rate		
	15 t/d	30 t/d	50 t/d
Equipment Purchase Cost	R 10 377 000	R 15 036 000	R 19 776 000
Installed Equipment Cost	R 23 348 000	R 33 830 000	R 44 495 000
Fixed Capital Investment	R 32 688 000	R 74 363 000	R 62 293 000

From the table it can be seen that the fixed capital investment for the SSF process at a paper sludge feed rate of 50 t d^{-1} is almost double that of the SSF process with paper sludge feed rate of 15 t d^{-1}, but the feed rate is 3.33-fold higher compared to 15 t d^{-1}. Therefore the SSF process will be more profitable at higher paper sludge feed rates due to the economy of scale.

4.3 Economic Modelling

An assessment of the economic viability of a project as described herein requires a deeper investigation of key financial indicators, primarily forthcoming from cash flow forecasts. Furthermore, to compensate for the time value of money, a discounted cash flow analysis is required. Key economic indicators, such as the internal rate of return (IRR), net present value (NPV), payback period and discounted payback period were used as a measure of economic viability.

The IRR can be defined as the discount rate that results in a NPV value of zero, implying that it is the maximum possible rate of return on an investment (Seider *et al*., 2004). Therefore, from an investment point of view the IRR should always be greater

than the required rate of return for a project. The NPV can be defined as the sum of all the discounted cash flows over the life of a project at a fixed discount rate (Amigun *et al*., 2011).

Normally, the payback period, in this study without using discounted values, is the time required for the cumulative cash flow to equal the total investment. When evaluating chemical plants a non-discounted payback period of four years and longer are usually not acceptable, with a payback period of less than three years preferred (Seider *et al*., 2004).

4.3.1 Scenarios

Revenue for the SSF process can be obtained from the sales of ethanol linked to the basic fuel price. However, given the relative infancy of bio-ethanol production in South Africa, contrasting to the situation in Brazil for example (Richardson *et al*., 2007), an investigation into an alternative use for ethanol was warranted. A possible viable alternative could be found by replacing LPG used at the paper mill of Nampak Tissue (Pty) Ltd. in Bellville, South Africa. The main use of LPG at this plant is to power burners to generate heat for drying tissue paper on the paper machine. Three scenarios were investigated. In the first scenario revenue is calculated from ethanol sales. In scenarios 2 and 3 LPG at the paper mill is replaced by anhydrous and 95% ethanol respectively.

4.3.2 Methodology

4.3.2.1 Revenue scenario 1

The annual revenue was calculated from the sales of ethanol as well as from the paper sludge disposal cost saved. The cost saved from paper sludge disposal is seen as revenue, and can be regarded as an opportunity cost due to the decreased levels of sludge residue that requires disposal as a result of conversion to ethanol in SSF.

According to the Department of Minerals and Energy (2007), the bioethanol price should be determined from the basic fuel price using the energy equivalent of petrol. Bioethanol producers are also exempt from fuel tax. The basic fuel price for petrol is R 7.08 per litre (October 2012) and a fuel tax of R 1.98 per litre is levied on petrol (Sasol, 2012). According to Leibbrandt (2010), the energy in a unit of ethanol is equivalent to 63% of the energy in a unit of petrol. Therefore, based on this 63% energy equivalent, the ethanol sales price would be R 4.46 per litre, which combined with the fuel tax exemption of R 1.98 per litre, amounts to a total selling price of R 6.44 per litre of ethanol.

Paper sludge disposal costs saved consist out of two parts, namely landfill charges of R 705 per dry ton and transport costs of R 174 per dry ton (Nampak Tissue, 2011). The fermentation of paper sludge reduces disposal cost by 35.1%. The disposal cost reduction was calculated on 22 t d^{-1} unwashed paper sludge, which equates to 15 t d^{-1} washed sludge. The washed sludge contains 55% glucose of which more than 93.6% is hydrolysed (Chapter 3).

Average ethanol yields of 245 kg t^{-1} paper sludge were obtained in the results presented in Chapter 3, using a paper sludge loading of 21% (w/w). This data was based on the use of the yeast *S. cerevisiae* strain MH1000, which is a wild-type industrial strain that only use glucose as a source of carbon. However, paper sludge contains approximately 10% xylose from which 65% is hydrolysed to monomeric form (Chapter 3). Therefore, with the use of a yeast strain genetically engineered to co-utilise xylose, the ethanol yield can be increased to 266 kg t^{-1} assuming an 80% theoretical xylose to ethanol yield.

4.3.2.2 Revenue scenario 2

In this scenario, revenue was calculated as the savings from not having an expenditure on LPG. In other words, the saving on this operating cost was regarded as a source of revenue. Similar to scenario 1, the disposal cost saved on sludge disposal was included into the revenue amount. The current cost of LPG is R 13.00 per kg (Nampak, 2012). The LPG replacement ethanol price was calculated on an energy equivalent basis, where LPG has an energy content of 46.1 MJ kg^{-1} and ethanol 26.9 MJ kg^{-1} (Perry and Green, 2008). The energy equivalent price for ethanol was calculated as R 7.58 kg^{-1} or R 5.99 $litre^{-1}$. An additional benefit of replacing LPG with ethanol is that no tax is payable since this process occurs internally and the revenue does not feature on the earnings before interest and tax (EBIT) line in the income statement.

4.3.2.3 Revenue scenario 3

Revenue was calculated as the savings from not having an expenditure on LPG similar to scenario 2 including disposal cost saved. In this scenario the use of 95% (w/w) ethanol is considered for the replacement of LPG which obviates the need for molecular sieves to produce anhydrous ethanol resulting in lower capital investments. The heating value of 95% (w/w) ethanol is 25.46 MJ kg^{-1} (Perry and Green, 2008) resulting in an energy equivalent price of R 5.69 $litre^{-1}$.

4.3.2.4 Fixed and operating costs

Costs associated with the production of ethanol can be broken down into six categories, namely costs for enzymes and nutrients, utilities and process water, and labour and maintenance. It is also important to note that these costs will remain the same, irrespective of the scenarios described above, as the scenarios only influences the value of the ethanol product. A summary of the costs associated with the production of ethanol can be seen in Table 4.4.

Table 4.4: Summary of the costs associated with the production of ethanol

Item	Cost	Source
Enzyme	R 2.01 litre^{-1}	See below
CSL	R 1921 ton^{-1}	(Petersen, 2012)
$MgSO_4$	R 4676 ton^{-1}	(Fan, 2004)
Process Water	R 9.18 m^{-3}	(Nampak Tissue, 2011)
Steam	R 230 ton^{-1}	(Nampak Tissue, 2011)
Electricity	R 0.40 kW^{-1} h^{-1}	(Nampak Tissue, 2011)
Labour	R100 per Operator per	Estimate
Maintenance	4% of Total depreciable	(Seider *et al*., 2004)

Enzyme cost estimates found in literature varied considerably and a summary of enzyme costs found in literature can be seen in Table 4.5.

Table 4.5: Summary of the enzyme costs found in literature

Enzyme Cost	Source
\$ 0.10 gal^{-1} (R 0.22 l^{-1})	(Aden and Foust, 2009)
\$ 0.30 gal^{-1} (R 0.66 l^{-1})	(Lynd *et al*., 2008)
\$ 0.40 gal^{-1} (R 0.88 l^{-1})	(Kazi *et al*., 2010)
\$ 0.68 gal^{-1} (R 1.50 l^{-1})	(Klein-Marcuschamer *et al*., 2012)
\$ 1.47 gal^{-1} (R 3.28 l^{-1})	(Klein-Marcuschamer *et al*., 2012)

The maximum enzyme cost of \$ 1.47 gal^{-1} (R 3.28 litre^{-1}) obtained by Klein-Marcuschamer *et al*., (2012) was based on 46% of the theoretical conversion of total sugars to ethanol using corn stover as feed stock at an enzyme dosage of 10 FPU g^{-1} cellulose. In this study the optimum cellulase dosage was found to be 14.5 FPU g^{-1} dry solids which correspond to an enzyme dosage of 7.98 FPU g^{-1} cellulose. At a 95% theoretical conversion of all the cellulose present, the enzyme costs can be lowered to \$ 1.13 gal^{-1} (R 2.52 litre^{-1}) (Klein-Marcuschamer *et al*., 2012). Since

conversions of up to 95% were obtained using paper sludge as a feed stock (Chapter 3) a more realistic maximum enzyme cost estimate would be \$ 1.13 gal^{-1} (R 2.52 $litre^{-1}$), but the \$ 1.13 gal^{-1} estimate is based on an enzyme cost of 10 FPU g^{-1} cellulose, when converted to the optimum cellulase loading used in this study a maximum enzyme cost of \$ 0.90 gal^{-1} (R 2.01 $litre^{-1}$) is obtained.

The costs of process water, steam and electricity were obtained from (Nampak Tissue, 2011). Maintenance for a chemical plant can be estimated as 4% of the total depreciable capital (Seider *et al*., 2004). As the SSF process is most likely to be a minor extension to the existing paper mill, only one additional operator per shift would be required. It is estimated that operators earn approximately R 100 per hour.

4.3.2.5 Additional information

A discount rate of 10% was suggested by Nampak Tissue (2011), which is 1.5% higher than the prime lending rate of 8.5% reported by the South African Reserve Bank in November 2012. For comparison, the use of 40% equity financing was also considered, possibly from an investor, who would typically require a 25% rate of return (Richardson *et al*., 2007). This would result in a debt/equity ratio of 1.5 which results in a weighted average cost of capital (WACC) of 16%. The WACC was used as a second discount rate to discount the cash flows.

In South Africa, machinery and equipment used in agriculture or for the production of biofuels can be depreciated at 50% in the first year, 30% in the second year and 20% in the third year (Deloitte, 2011). This is an acceptable method to assist in the rapid recovery of the initial capital investment since depreciation can be deducted from tax.

A salvage value of 20% of the original equipment purchase price was assumed at the end of the project life.

The corporate tax rate in South Africa of 28% was used for calculations in the discounted cash flow sheet (Deloitte, 2011). In scenario 1 only the revenue from the sales of ethanol was used in tax calculations as savings on disposal cost is not an actual income, although the total revenue consisted out of ethanol sales and disposal cost saved. No tax is payable in scenarios 2 and 3 where LPG is replaced with ethanol since no income is generated.

A conservative plant life of 15 years was used in the calculations and inflation was ignored in the discounted cash flow sheet due to the uncertainty of the future value of inflation. It can also be argued that inflation on revenue and expenses cancel out (Seider *et al*., 2004). Since the SSF plant is likely to be a small extension to the paper mill, it is assumed that the plant is constructed and commissioned in one year. The working capital was calculated at 5% of the fixed capital investment, as suggested by Sinnott (2005).

Once the complete capital investment, revenue streams and annual costs were calculated, the information was be compiled in a discounted cash flow sheet to determine the present values of future cash flows to evaluate whether an investment would yield an IRR in excess of that required by from an investment point of view. A summary of the capital investment, costs and revenue used in the discounted cash flow sheet are shown in Table 4.6.

Table 4.6: Input used for the development of the discounted cash flow sheet

Items	Paper sludge feed rate		
	15 t d^{-1}	**30 t d^{-1}**	**50 t d^{-1}**
Fixed Capital	R 32 688 000	R 47 363 000	R 62 293 000
Working Capital	R 1 634 000	R 2 368 000	R 3 114 000
Annual Revenue			
Scenario 1: Ethanol Sales	R 11 887 000	R 23 774 000	R 39 623 000
Scenario 2: LPG Replacement	R 11 056 000	R 22 112 000	R 36 853 000
Scenario 3: LPG Replacement	R 10 502 000	R 21 004 000	R 35 006 000
Disposal Costs Saved	R 2 245 000	R 4 491 000	R 7 486 000
Annual Cost			
Enzyme	R 3 371 000	R 6 744 000	R 11 241 000
CSL	R 31 552	R 63 104	R 105 173
$MgSO_4$	R 64 002	R 128 004	R 213 340
Process Water	R 273 000	R 547 500	R 912 500
Steam	R 3 022 200	R 6 044 400	R 10 074 000
Electricity	R 318 864	R 637 728	R 1 062 880
Labour	R 876 000	R 876 000	R 876 000
Maintenance	R 1 307 000	R 1 894 000	R 2 491 000
Salvage	R 2 075 000	R 3 007 000	R 3 955 000

To standardise the different costs associated with the operation of a sludge-fed ethanol plant, values were expressed in terms of R $litre^{-1}$, i.e. per unit of ethanol produced. This method provides insight into the costs relative to the sales price of a unit of ethanol. Production prices lower than the sales price of ethanol would imply an increase in the profit margin.

To determine the cost of producing ethanol in Rand $litre^{-1}$, the capital investment had to be annualised. To annualise the capital investment it was assumed that 100% of the capital investment will be financed using debt. The annualised capital cost was determined by using the following equation (Seider *et al*., 2004)

$$Annualised\ Capital\ Cost = P\left(\frac{i(1+i)^n}{(1+i)^n - 1}\right)$$

Where P is the present value of the capital investment, *i* is the interest rate and *n* is the number of payments. An interest rate of 10% was used in the annualising of the capital cost. The saving on disposals cost was factored into the cost of producing ethanol as a negative feedstock cost.

4.3.3 Results and Discussion

A discounted cash flow sheet was developed for the SSF operation at paper sludge feed rates of 15, 30 and 50 t d^{-1}, with discounted cash flow sheets at these feed rates for scenario 1 shown in Appendix D. From these cash flows, the internal rate of return (IRR), net present value (NPV), payback period and ethanol production cost were calculated, with the output from this analysis presented in Table 4.7

The economic viability for the production of bioethanol from paper sludge clearly improved at higher paper sludge feed rates, which illustrated that this process would benefit from economies of scale. This benefit can be attributed to the capital investment that proportionally decreases relative to a greater magnitude of material throughput and resulting ethanol sales. A payback period of less than three years was obtained with scenario 1 at a paper sludge feed rate of 50 t d^{-1}.

Table 4.7: Key economic indicators from discounted cash flow analysis of a bio-ethanol plant based on three different paper sludge feed rates.

Feed Rate	Capital Investment	IRR	NPV (10 %)	NPV (16 %)	Payback	Production Cost
15 t/d						
Scenario 1	R 34.3 mil	11%	R 2.5 mil	-R6.5 mil	7.0 Years	R 6.49 litre^{-1}
Scenario 2	R 34.3 mil	8%	-R 3.2 mil	-R11.1 mil	9.0 Years	R 6.49 litre^{-1}
Scenario 3	R 27.1 mil	10%	R 0.5 mil	-R6.1 mil	7.5 Years	R 5.84 litre^{-1}
30 t/d						
Scenario 1	R 49.7 mil	22%	R 31.2 mil	R 11.6 mil	4.0 Years	R 5.35 litre^{-1}
Scenario 2	R 49.7 mil	18%	R 22.5 mil	R 4.4 mil	5.0 Years	R 5.35 litre^{-1}
Scenario 3	R 39.5 mil	21%	R 26.1 mil	R 9.5 mil	4.5 Years	R 4.89 litre^{-1}
50 t/d						
Scenario 1	R 65.4 mil	30%	R 73.2 mil	R 39.4 mil	3.0 Years	R 4.76 litre^{-1}
Scenario 2	R 65.4 mil	26%	R 63.5 mil	R 30.7 mil	3.5 Years	R 4.76 litre^{-1}
Scenario 3	R53.6 mil	30%	R 63.6 mil	R 33.6 mil	3.2 Years	R 3.44 litre^{-1}

*Ethanol selling price scenario 1 – R 6.44 litre^{-1} (Ethanol sales)

*Ethanol selling price scenario 2 – R 5.99 litre^{-1} (LPG equivalent)

*Ethanol selling price scenario 3 – R 5.69 litre^{-1} (LPG equivalent- 95% ethanol)

Positive NPV values were obtained for all the scenarios at a discount rate of 10% regardless of the paper sludge feed rate, except for scenario 2 at a paper sludge feed rate of 15 t d^{-1}. Positive NPV values were obtained for all scenarios at paper sludge feed rates of 30 t d^{-1} and higher at a discount rate of 16%. Higher NPV values were obtained at higher paper sludge feed rates which can be attributed to economies of scale. Significantly higher NPV values were obtained for scenario 1 compared to scenarios 2 and 3.

Most investors require IRR values in excess of 25% to invest in a project (Richardson *et al*., 2007). IRR values greater than 25% were recorded at a paper sludge feed rate of 50 t d^{-1}, irrespective of the scenario. This feed rate appeared to be the threshold

for this process since the IRR was markedly lower at lower feed rates, especially at 15 t d^{-1}. At a feed rate of 50 t d^{-1}, the IRR values for scenarios 1 and 3 were the same at 30%. However, at this feed rate, the NPV of the cash flows for scenario 1 were greater than that of scenarios 2 and 3, whereas scenario 1 also resulted in a shorter payback period. This trend was also evident at lower feed rates, implying that sales of ethanol would be more profitable than replacing LPG gas with ethanol. Consequently, scenario 1 was selected for the sensitivity analysis and the Monte Carlo analysis in the following sections.

4.3.4 Sensitivity Analysis

To gain a greater level of insight into the economic viability of an SSF process producing ethanol, a sensitivity analysis was performed to determine the effect of fluctuations in key variables, such as capital and enzyme costs. Usually a sensitivity analysis would also include utilities but actual costs for utilities were obtained from Nampak Tissue (2011).

4.3.4.1 Influence of capital investment

The capital investment presented a distinct source of variation, since this value is a function of the equipment purchase price, which varied up to 14% in the estimates obtained from Logichem (Appendix C). To evaluate the impact of capital investment on the economic viability of the SSF process utilising paper sludge as a feedstock, the minimum, mean and maximum equipment costs obtained was used to generate a range estimate of capital costs. The resulting range of total fixed capital investments

that formed the basis of the sensitivity analysis are shown in Table 4.8, with the effect of these variations on the IRR shown in Figure 4.6.

Table 4.8: Total fixed capital investments investigated in the sensitivity analysis

Paper sludge feed rate	Fixed Capital Investment		
	Minimum	Mean	Maximum
15 t d^{-1}	R 28.3 mil	R 32.7 mil	R 37.0 mil
30 t d^{-1}	R 41.0 mil	R 47.4 mil	R 53.7 mil
50 t d^{-1}	R 52.9 mil	R 62.3 mil	R 71.6 mil

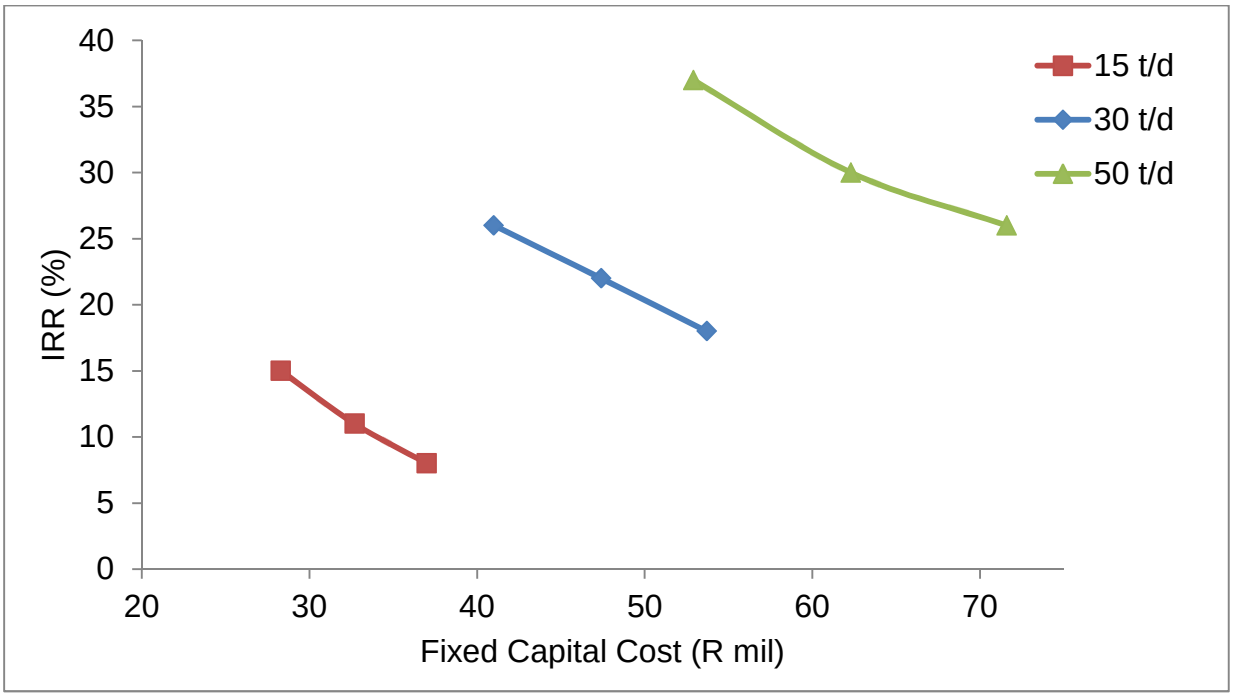

Figure 4.6: The impact of fixed capital investment on the IRR at various paper sludge feed rates for the production of ethanol to replace LPG.

An increase in capital investment resulted in a decrease in the IRR, irrespective of the feed rate of the plant. As seen in the Figure 4.6 IRR values higher than 25% were obtained at a paper sludge feed rate of 50 t d^{-1} regardless capital cost, which points to economic viability for the production of ethanol from paper sludge at that specific paper sludge feed rate.

From the ethanol production cost it can be seen that capital investment is a major contributor to the overall production costs of ethanol. The disposal costs saved is seen as a negative feedstock cost necessitating the use of a negative y-axis in the plot shown in Figure 4.7. At higher paper sludge feed rates, lower ethanol production costs were obtained which can be attributed to the benefit of economies of scale.

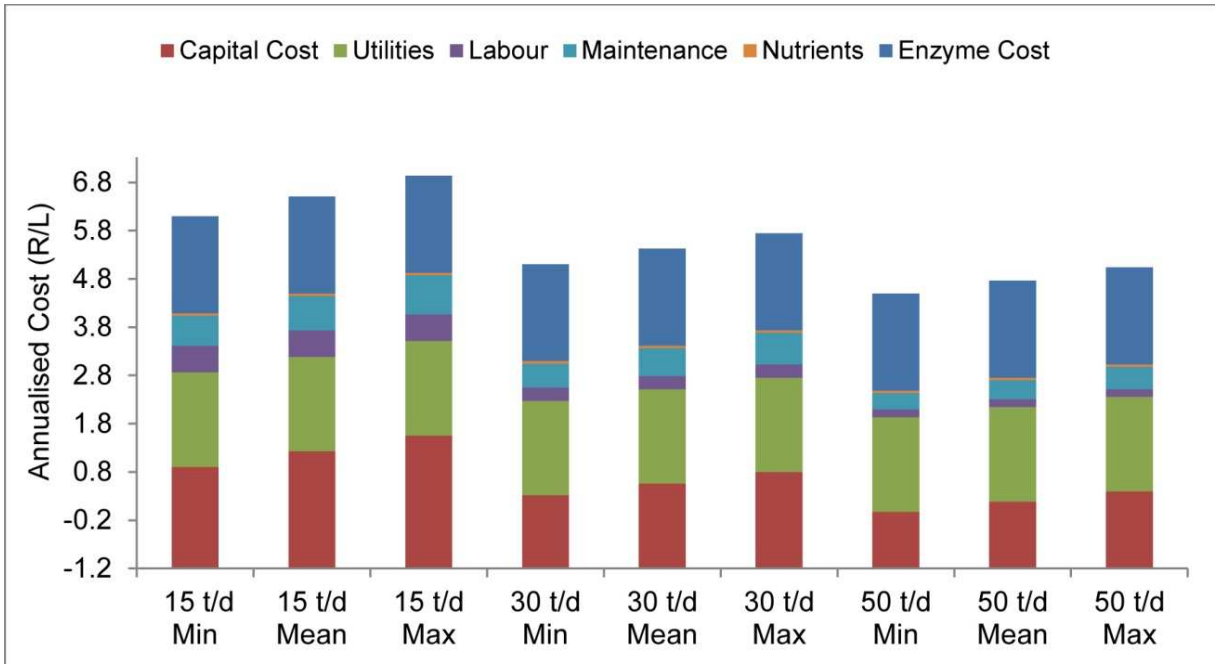

Figure 4.7: The impact of fixed capital cost on the annualised ethanol production cost

4.3.4.2 Influence of enzyme cost

As shown in Table 4.5, the enzyme cost estimates in the literature varied considerably from as low as \$ 0.20 gal^{-1} (R 0.45 $litre^{-1}$) to as high as \$ 1.47 gal^{-1} (R 3.28 $litre^{-1}$). The impact of enzyme cost on the IRR of the SSF process is shown in Figure 4.8. A minimum enzyme cost for the sensitivity analysis was assumed as \$ 0.00 gal^{-1}, which can theoretically be obtained using consolidated bio-processing

(CBP) since no additional enzyme is required. In a CBP process the fermenting micro-organism produces the enzyme, performs the hydrolysis and ferments the hydrolysed sugar to ethanol in one step (Gírio *et al*., 2010). Although a full CBP process is not yet a reality, this future possibility was considered as bottom of the range for this analysis. An enzyme cost of \$ 1.47 gal^{-1} (R 3.28 $litre^{-1}$) was used as the maximum in the sensitivity analysis.

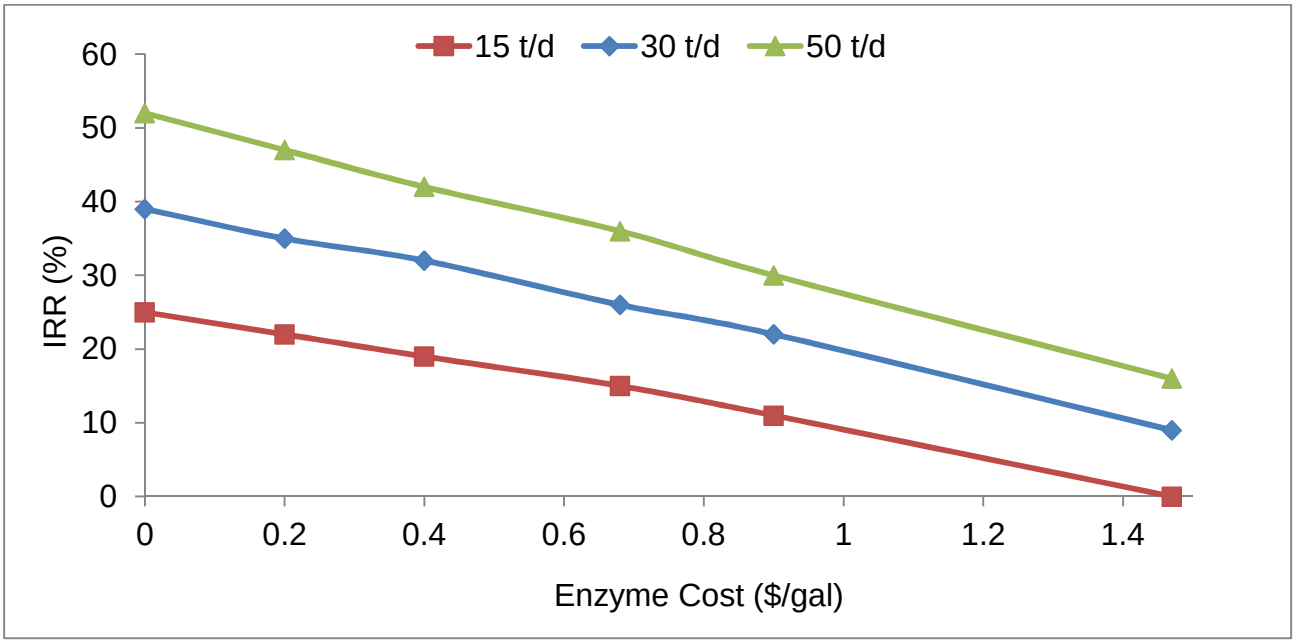

Figure 4.8: The impact of enzyme cost on the IRR at various paper sludge feed rates

It is an established fact that enzyme cost has a substantial effect on the economic viability of lignocellulose-based process (Klein-Marcuschamer *et al*., 2012), and this fact was substantiated by the results presented in Figure 4.8. At the top end of the enzyme cost range at paper sludge feed rates of 15, 30 and 50 t d^{-1}, IRR values of 0%, 9% and 16% were obtained, respectively, compared to 25%, 39% and 52% if a feasible CBP organism can be developed, i.e. at the bottom of the enzyme cost range.

The maximum enzyme cost of $ 1.47 gal^{-1} (R 3.28 $litre^{-1}$) obtained by Klein-Marcuschamer *et al*., (2012) was based on 46 % of the theoretical conversion of total sugars to ethanol. At a 95% theoretical conversion of the cellulose available to the fermenting organism, a more realistic maximum enzyme cost estimate of $ 0.90 gal^{-1} (R 2.01 $litre^{-1}$) was obtained. At a more realistic maximum enzyme cost estimate of $ 0.90 gal^{-1} (R 2.01 $litre^{-1}$), respective IRR values of 11%, 22% and 30% were obtained at the three different feed rates. As a result, the SSF process is only economically viable at a paper sludge feed rate of 50 t d^{-1}, using the more realistic enzyme cost estimate of $ 0.90 gal^{-1} (R 2.01 $litre^{-1}$).

From the breakdown of cost components making up the total ethanol production cost (Figure 4.9 below) it is clearly evident that enzyme cost contributed substantially to the production cost of ethanol and, therefore, had the most severe impact on the economic viability of the process. The saving on disposal costs was regarded as a negative feedstock cost, thus necessitating a negative axis on the graph in Figure 4.9.

In conclusion, as evident from Figures 4.8 and 4.9, enzyme cost had the most dramatic impact on the SSF process, especially at low paper sludge feed rates. Therefore, given the combined impact of relatively high capital costs and enzyme costs, an SSF process at a paper sludge feed rate of 15 t d^{-1} would not economically viable. Furthermore, the SSF process would only be economically viable if the enzyme cost could be reduced to below $ 0.70 gal^{-1} (R 1.56 $litre^{-1}$) at a paper sludge feed rate of 30 t d^{-1}. On the other hand, at a paper sludge feed rate of 50 t d^{-1} the SSF process would be economically viable at all enzyme costs below $ 1.20 gal^{-1}

(R 2.68 litre^{-1}), implying that this is the enzyme cost threshold for a process using recycled paper sludge as feedstock.

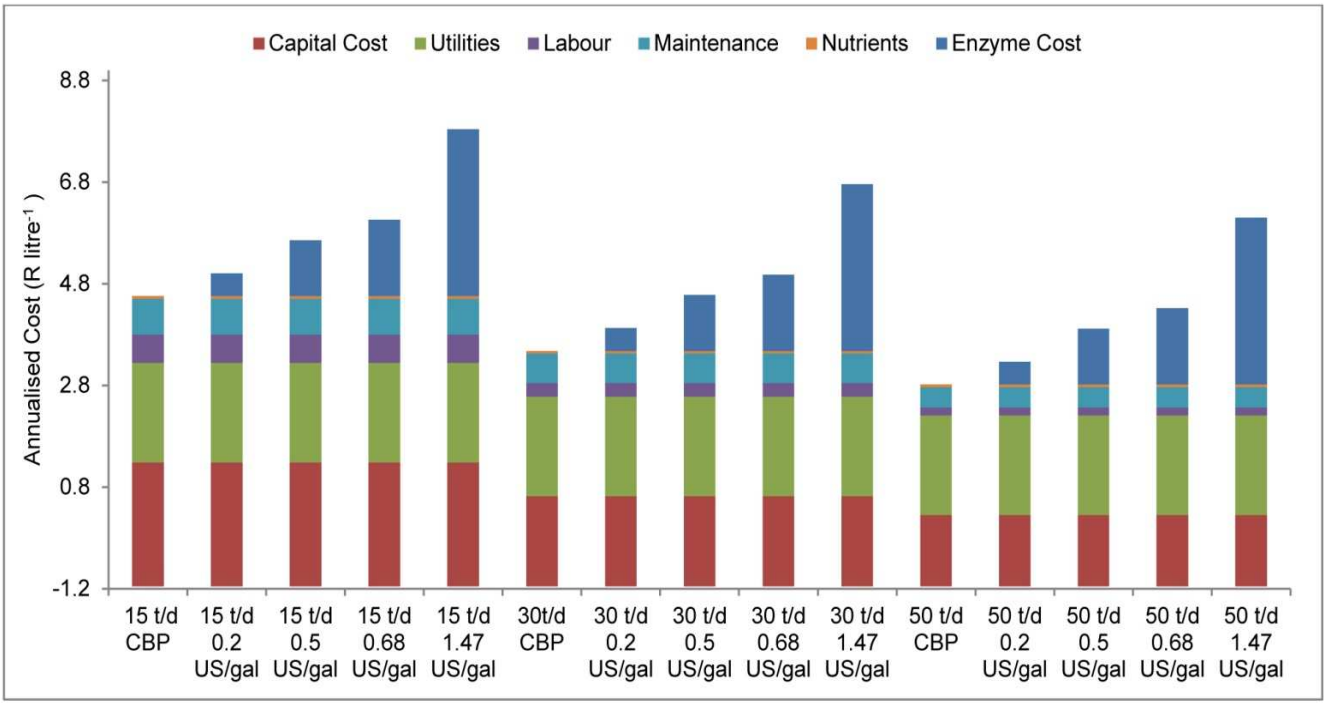

Figure 4.9: The impact of enzyme cost on the ethanol production cost

4.3.5 Monte Carlo simulation

Economic analyses that use fixed input estimates in calculating key economic indicators neglect uncertainty and risk in the model. The use of probability distributions instead of point estimates for input costs quantifies the possibility of economic success or risk of failure (Amigun *et al*., 2011). A probabilistic method such as the Monte Carlo analysis can be used to model the uncertainty in the input by accounting for the uncertainties characterised by a probability distribution. The response from the Monte Carlo analysis, therefore, results in a probability distribution of key economic indicators (Petersen, 2012).

According to Moonery (1997), the procedure used in Monte Carlo simulations can be summarised as:

1) Generation of the input fields as probability distributions.
2) Generation of random inputs from probability distributions.
3) Determine and store outcomes generated from economic model
4) Repeat steps 2 and 3
5) Determine outcome distribution

4.3.5.1 Methodology

The Monte Carlo analysis was performed using the RiskSim add-in for MS Excel (Microsoft Corporation, Redmond, WA, USA). RiskSim provides probability distribution functions from random number generators using the random number generator function inherent in the Excel software. In other words, Risksim integrates into the existing random number generators of Excel, instead of running own algorithms to generate random numbers, as is more common in modern off-the-shelf simulation software packages. Multiple iterations of random inputs would then result in outputs of key dependent variables in the form of probability distributions, where the key output variables used in the present study was the IRR forthcoming from the cash flow sheet. In turn, the probability distributions can be used to assess the probability that the key output variable, such as the IRR would be greater than a certain threshold, such as the required return mandated by an investor.

The input variables that were identified to have the greatest magnitude of impact, thus representing the greatest degree of uncertainty, on the economic outcome of the

process developed in this study were the capital investment, enzyme cost and the ethanol yield obtained from paper sludge. A summary of the input variables and distribution type used to model the uncertainty in these input variables are shown in Table 4.9.

Table 4.9: Input variables and distribution used in the Monte Carlo Analysis

Input Variable	Minimum	Mean/Estimate	Maximum	Distribution
Capital Cost				Normal
15 t d^{-1}	R 28.3 mil	R 32.7 mil	R 37.0 mil	
30 t d^{-1}	R 41.0 mil	R 47.4 mil	R 53.7 mil	
50 t d^{-1}	R 52.9 mil	R 62.3 mil	R 71.6 mil	
Enzyme Cost	\$ 0.20 gal^{-1}	-	\$1.47 gal^{-1}	Uniform
Ethanol Yield	248 kg ton^{-1}	266 kg ton^{-1}	284 kg ton^{-1}	Normal

To introduce randomness into the capital investment, the range of the values at the different feed rates, presented in Table 4.8, were assumed to be normally distributed. The mean and standard deviation of the capital investments required for the normal distribution were calculated from the range of capital investments obtained at the various paper sludge feed rates.

Due to the variation in enzyme cost estimations in literature (Table 4.5), it was assumed that the enzyme cost will most likely have a uniform distribution, which implies that there is an equal probability that a value will occur within a specified range. Enzyme costs ranging from \$ 0.20 gal^{-1} (R 0.44 $litre^{-1}$) to \$ 1.47 gal^{-1} (R 3.28 $litre^{-1}$) were used for the Monte Carlo analysis.

Variation in composition of the paper sludge feed stock could lead to variations in the ethanol yield (kg ton^{-1}), as was shown in Chapter 3. According to Montgomery and Runger (2007), most experimental data can be modelled using a normal probability

distribution, which also seemed appropriate to use in this case. The mean and standard deviation required for the normal distribution was calculated from the range of ethanol yields investigated, with ethanol yields of 248 kg ton^{-1}and 284 kg ton^{-1} used in the Monte Carlo analysis.

4.3.5.2 Results and Discussion

Mean IRR values, with standard deviations shown brackets, of 13% (7%), 23% (8%) and 32% (9%) were obtained at paper sludge feed rates of 15, 30 and 50 t d^{-1}, respectively. This data confirmed the earlier observations as discussed in section 4.3.4 that economies of scale is a crucial requirement for the successful operation of this plant. More importantly, taking into consideration the uncertainty in input costs, the data suggested that a sludge feed rate of 50 t d^{-1} is the minimum threshold at which a process of this nature can be operated. Although the mean IRR was greater than the required return, the standard deviation was approximately 28% of the mean, implying a relatively high probability that the required return of 25% might not be met.

The above observations were better explained using cumulative probability distributions of the IRR for paper sludge at feed rates of 15, 30 and 50 t d^{-1} (Figure 4.10). At the highest feed rate, there was a 26% probability that the IRR would be below 25%. Although this probability value was comparatively low compared to the lower feed rates, this value would still represent a substantial degree of risk inherent in the project. It would thus depend on the risk appetite of the investor whether a 26% probability of not meeting the required return would be acceptable. At the lower feed rates, it was evident that no investor would consider this project. At a feed rate of 30 t d^{-1}, the probability of not meeting a required rate of return of 25% was almost 70%,

whereas at the lowest feed rate, the probability of the IRR being greater than 25% was only 5%. The data thus clearly points to feed rates in excess of 50 t d^{-1} for the process to be viable.

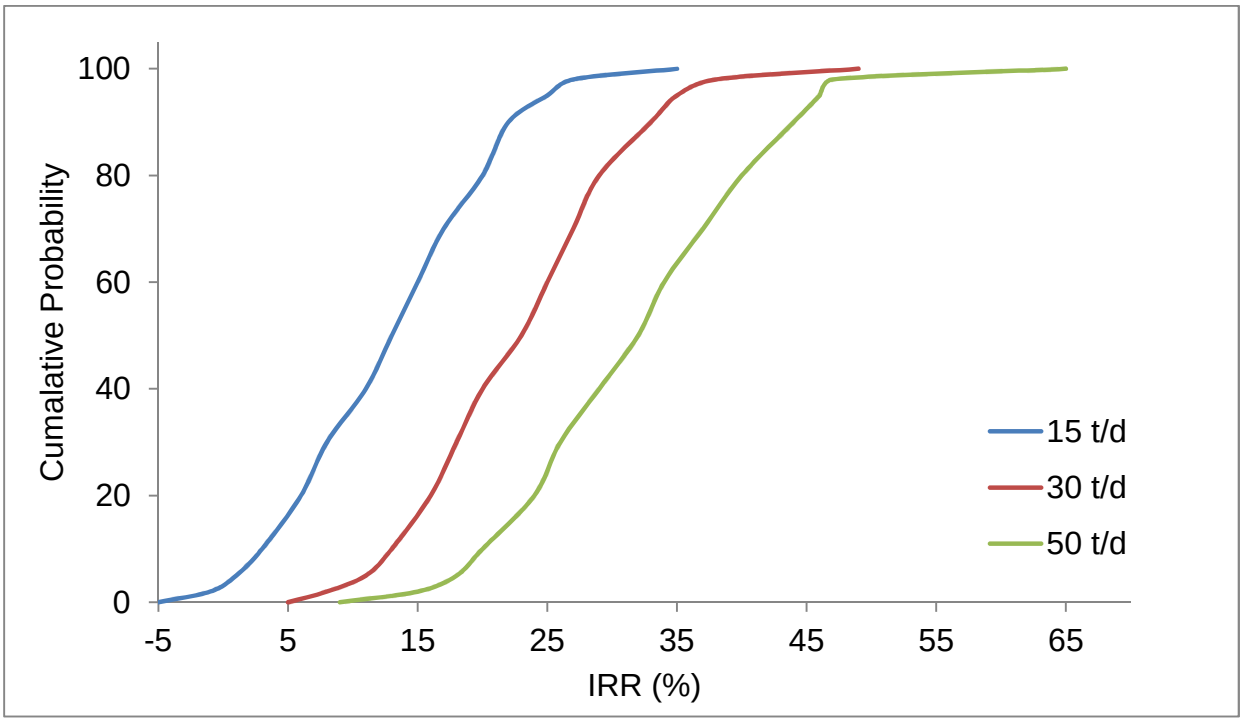

Figure 10: The cumulative probability vs. IRR plot at various paper sludge loadings

4.4 Greenhouse gas reduction

Reduction in greenhouse gas (GHG) emissions and biofuels go hand in hand since one of the main drivers for biofuels is the alleviation of the environmental impact brought about by our dependence on fossil derived fuels (Börjesson, 2009). Greenhouse gasses such as carbon dioxide, methane, nitrous oxide and ozone absorbs and emits radiation from the sun which results in the greenhouse effect. Governments in the European Union defined a minimum reduction in GHG of 35% for biofuels to eligible for public incentives (Gnansounou *et al*., 2009).

The GHGs obtained from the production of ethanol using paper sludge as feedstock was compared to the base scenario where the paper sludge is landfilled. The carbon dioxide emissions from the disposal of paper sludge were calculated from the carbon dioxide emissions obtained from transport and from the methane and carbon dioxide emissions emanating from paper sludge landfilled. One methane unit was assumed to be equivalent of 21 carbon dioxide units (Wang *et al*., 2007). The GHG emission calculations were based on an unwashed paper sludge rate of 22 dry tonnes per day, which equates to 15 dry tonnes washed sludge. The carbon content of lignocellulosic biomass (non-ash) is 50% (Biermann, 1993), which was used to calculate the CO_2 and methane emissions.

Two scenarios were investigated. In the first scenario GHG emissions were calculated from the disposal of the paper sludge and in the second scenario GHG emissions were calculated for the SSF process were the cellulose fraction is fermented to bioethanol. The results of the GHG emission calculations for the disposal of paper sludge can be seen in Table 4.10.

Table 4.10: GHG emissions for the disposal of paper sludge

	Input	**$tCO_{2\ eq}$ Emissions/year**
Moisture Content	55%	
Truck Load	20 tonnes	
Trips	3	
Distance Travelled	240 km	
CO_2 Emission Factor	870 g km^{-1} (ICFPA, 2005)	
$CO_{2\ eq}$ Emissions from Transport		**76**
Volatile Solids (dry weight)	40%	
Methane produced	0.34 m^3 kg^{-1} (Lin *et al*., 2009)	
Mass Methane Produced	1974 kg d^{-1}	
$CO_{2\ eq}$ emissions methane		**15 136**
Carbon Remaining	3 413 kg d^{-1}	
CO_2 Produced		**4 567**
Total $CO_{2\ eq}$/year		**19 779**

Methane emissions from paper sludge in landfill are the major contributor to the total GHG emissions, whereas the contribution of carbon dioxide emissions from the transportation of the paper sludge is negligible when compared to landfilling. As it is possible to produce 1974 kg methane a day utilising paper sludge as a feedstock, it is recommended that an economic model of a biogas process be developed for comparison to the bioethanol process.

The carbon dioxide emissions from the production of ethanol from paper sludge were calculated from the carbon dioxide released in the fermentation, the utilisation of steam and electricity and the disposal of the waste remaining after fermentation including the ash from ash removal. The results of the GHG emission calculations for the production of ethanol from paper sludge can be seen in Table 4.11.

From the table it can be seen that there is a 42.5% reduction in GHG emissions when using paper sludge to produce ethanol. The major contributor to the GHG emissions in the SSF process is the landfilling of the fermentation waste, but it is considerably less compared to the base scenario where all the paper sludge is landfilled as the majority of the volatile solids (cellulose) are converted to ethanol in SSF.

Table 4.11: GHG emissions for the production of ethanol from paper sludge

	Input	**$tCO_{2\,eq}$ Emissions/year**
$CO_{2\,eq}$ emissions from fermentation		**1 401**
Steam requirement	1400 kg d^{-1}	
Boiler efficiency	80%	
Coal requirement	3930 kg d^{-1}	
$CO_{2\,eq}$ emissions from steam		**3 927**
Electricity Requirement	110 kW	
Emission factor (Eskom, 2012)	0.962 kg $CO_{2\,eq}$ kW^{-1} h^{-1}	
$CO_{2\,eq}$ emissions from electricity		**978**
Waste remaining after fermentation	14 tonnes	
$CO_{2\,eq}$ emissions from transport		**51**
Volatile solids (dry weight)	19%	
Methane produced	596 kg d^{-1}	
$CO_{2\,eq}$ emissions methane		**4 568**
Carbon Remaining	734 kg d^{-1}	
$CO_{2\,eq}$ emissions from landfill		**982**
GHG emissions saved from LPG	0.014 kg MJ^{-1} (Toyota, 2004)	
GHG emissions saved		**-543**
$tCO_{2\,eq}$/year		**11 364**

4.5 Utilisation of Fermentation Residues

In the economic model it is assumed that the residue obtained from the fermentation of paper sludge is disposed of in a landfill. The utilisation of the fermentation residues could further reduce the amount of waste that need to be disposed of. The residues after fermentation contain approximately 38% ash, 30% lignin, 9.5% extractives with the remainder consisting out of xylose and glucose (Chapter 3). One possible use for the fermentation residues is to burn the residues in a boiler to produce process steam. The heat content of the fermentation residues (excluding ash) was assumed to be 22.5 MJ kg^{-1} (NREL, 2000). The heating value of the fermentation residues were calculated on a basis of 1 kg residue with a moisture content of 55% and can be seen in Table 4.12.

Table 4.12: Heating value of lignin rich fermentation residue

	Fraction	**Heating Value (MJ/kg)**
Moisture Content	55.0%	0.00
Ash	17.1%	0.00
Residue	27.9%	6.28
Heat required to evaporate water content		-1.38
Heating value of fermentation residues		**4.90**

The heating value of the fermentation residues was calculated as 4.90 MJ/kg which is only 16.5% of the heating value of coal which is normally used as fuel in a boiler to generate steam. The low heating value of the fermentation residue is attributed to the high moisture and ash content of the fermentation residues and as a result the fermentation residue is not a viable boiler feedstock.

4.6 Conclusions

In this chapter a techno economic model for the SSF process was developed. Three scenarios for the use of ethanol produced from paper sludge were investigated. In the first scenario, revenue was calculated from the sale of ethanol for consumption as transport fuel and the sales price was linked to the basic fuel price. In the second and third scenarios investigated, ethanol was used to replace LPG usage at the paper mill. The ethanol used was either anhydrous or of 95% purity for scenarios 2 and 3, respectively. Paper sludge feed rates of 15, 30 and 50 t d^{-1} were used for generation of all three scenarios. The production of ethanol from paper sludge for sales (scenario 1) resulted in higher IRR and NPV values, as well as shorter payback periods, compared to replacement of LPG at the paper mill (scenarios 2 and 3).

IRR values in excess of 25% and were obtained for all scenarios (Table 4.7) at a paper sludge feed rate of 50 t d^{-1} and an enzyme cost of $ 0.90 gal^{-1} (R 2.01 $litre^{-1}$). No scenario yielded IRR values in excess of 25% at lower paper sludge feed rates.

A sensitivity analysis performed on the total capital investment and enzyme cost also revealed that an SSF process is only economically viable at a paper sludge feed rate of 50 t d^{-1}, irrespective of the variation in capital investment. For the SSF process to be economically viable (IRR values in excess of 25%) the enzyme costs must be lower than $ 0.70 gal^{-1} (R 1.56 $litre^{-1}$) and $ 1.20 gal^{-1} (R 2.68 $litre^{-1}$) for paper sludge feed rates of 30 and 50 t d^{-1} respectively. The SSF process at a paper sludge feed rate of 15 t d^{-1} was not economically viable even assuming a zero enzyme cost.

A Monte Carlo simulation was used to model the impact of variation of the capital investment, enzyme cost and ethanol yield from the paper sludge feed stock on the economic viability of the SSF process. The SSF process is economically viable at a paper sludge feed rate of 50 t d^{-1} as a mean IRR value of 32% were obtained with a probability of 26% to obtain an IRR value lower than 25%. The SSF process at lower paper sludge loadings is not economically viable as probabilities of 70% and 95% were obtained to achieve IRR values lower than 25% for paper sludge feed rates of 30 and 15 t d^{-1}.

The production of ethanol from paper sludge using SSF results in a 42.5% reduction in GHG's when compared to the landfilling of paper sludge. The production of ethanol from paper sludge has distinct environmental benefits when compared to disposal, such as a reduction in methane from landfill.

From this study it can be concluded that paper sludge is viable feedstock for ethanol production for the sales of ethanol at paper sludge feed rates in excess of 50 t d^{-1} with the added environmental benefit of reducing GHG emissions by 42.5%.

5. Conclusions and Recommendations

The aim of this study was to determine the composition, fermentability and optimum operating conditions for producing ethanol from paper sludge. The information obtained from the experimental work was used to develop a model of the process in Aspen Plus®. The mass and energy balances obtained in the Aspen Plus® model was used to develop equipment specifications which was used to source equipment cost data. A techno – economic model was developed from the equipment cost data to assess the economic viability of the SSF process utilising paper sludge as feedstock.

Paper sludge has an advantage compared to other lignocellulosic feedstock as no pre-treatment of this material is required. By washing paper sludge received from recycled fibre operations the ash content could be decreased to lower than 20%, which results in a higher level of fermentability compared to unwashed paper sludge. Washed paper sludge is an excellent feedstock for use in SSF to produce ethanol as final ethanol concentrations in excess of 47.37 $g.l^{-1}$ and yields in excess of 85% of the theoretical maximum were obtained.

It can be concluded from RSM in conjunction with multi-response optimisation that a paper sludge loading of approximately 21% (w/w) and a cellulase dosage of 14.5 FPU g^{-1} be used to obtain optimal ethanol concentrations and percentage ethanol yields irrespective of the washed paper sludge used. It is recommended that a recombinant strain utilising both glucose and xylose as carbon source be used as it would improve the ethanol concentrations and yields obtained in this study. Assuming a xylose utilising recombinant strain could convert the hydrolysed xylose to

ethanol with a conversion 80% of what is theoretically possible, ethanol concentrations can be increased up to 20 kg ton^{-1} paper sludge.

A techno-economic model for the SSF process was developed to assess if the production of ethanol from paper sludge is economically viable when using SSF. Three scenarios for the use of ethanol produced from paper sludge were investigated. In the first scenario, revenue was calculated from the sale of ethanol for consumption as transport fuel and the sales price was linked to the basic fuel price. In the second and third scenarios investigated, ethanol was used to replace LPG usage at the paper mill. The ethanol used was either anhydrous or of 95% purity for scenarios 2 and 3, respectively. Paper sludge feed rates of 15, 30 and 50 t d^{-1} were used for generation of all three scenarios. The production of ethanol from paper sludge for sales (scenario 1) resulted in higher IRR and NPV values, as well as shorter payback periods, compared to replacement of LPG at the paper mill (scenarios 2 and 3).

A sensitivity analysis performed on the total capital investment and enzyme cost revealed that an SSF process is only economically viable at a paper sludge feed rate of 50 t d^{-1}, irrespective of the variation in capital investment. For the SSF process to be economically viable the enzyme costs must be lower than $ 0.70 gal^{-1} (R 1.56 litre^{-1}) and $ 1.20 gal^{-1} (R 2.68 litre^{-1}) for paper sludge feed rates of 30 and 50 t d^{-1} respectively. The SSF process at a paper sludge feed rate of 15 t d^{-1} was not economically viable even assuming a zero enzyme cost.

A Monte Carlo simulation revealed that the SSF process is economically viable at a paper sludge feed rate of 50 t d^{-1} as a mean IRR value of 32% were obtained with a

probability of 26% to obtain an IRR value lower than 25%. The SSF process at lower paper sludge loadings is not economically viable as probabilities of 70% and 95% were obtained to achieve IRR values lower than 25% for paper sludge feed rates of 30 t d^{-1} and 15 t d^{-1}.

The production of ethanol from paper sludge using SSF results in a 42.5% reduction in GHG's when compared to the landfilling of paper sludge. The production of ethanol from paper sludge has distinct environmental benefits when compared to disposal, such as a reduction in methane from landfill.

From this study it can be concluded that paper sludge is an excellent feedstock for ethanol production for the sales of ethanol at a paper sludge feed rate in excess of 50 t d^{-1} with the added environmental benefit of reducing GHG emissions by 42.5%.

From the GHG calculations it can be seen that it is possible to produce methane utilising paper sludge as a feedstock and as a result it is recommended that an economic model of a biogas process be developed for comparison to the bioethanol process.

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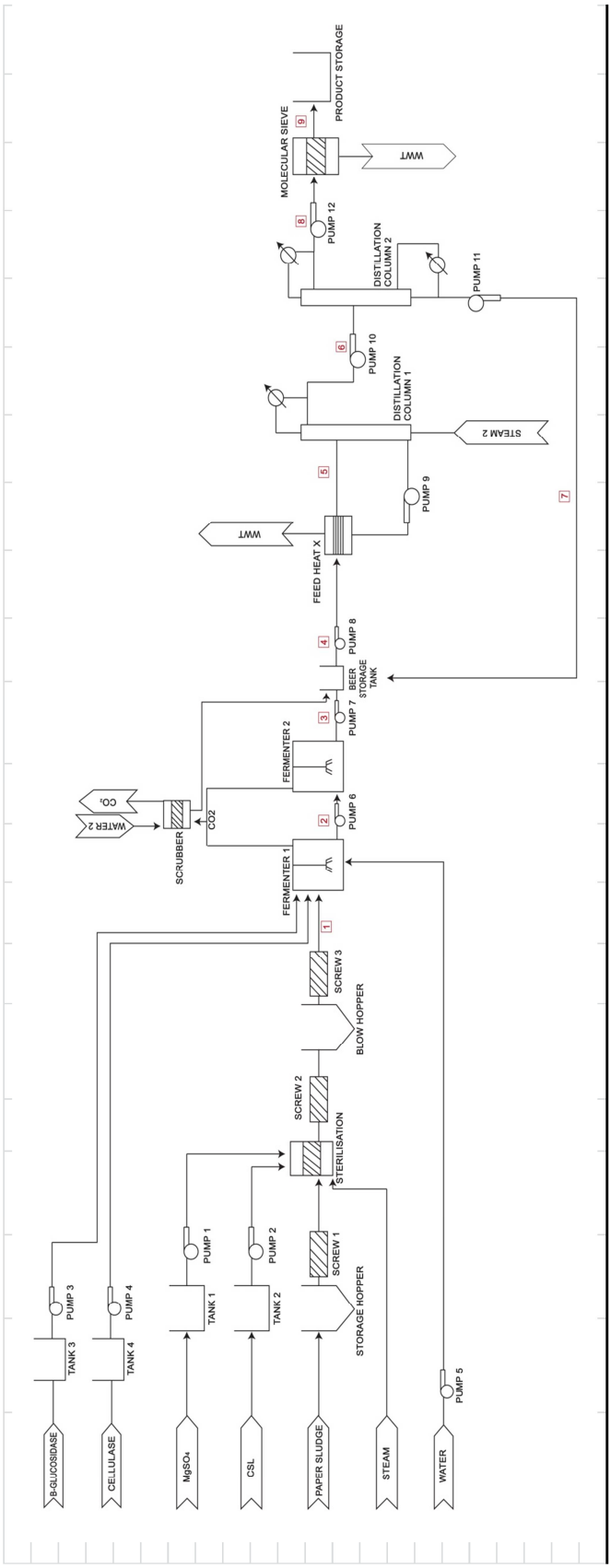
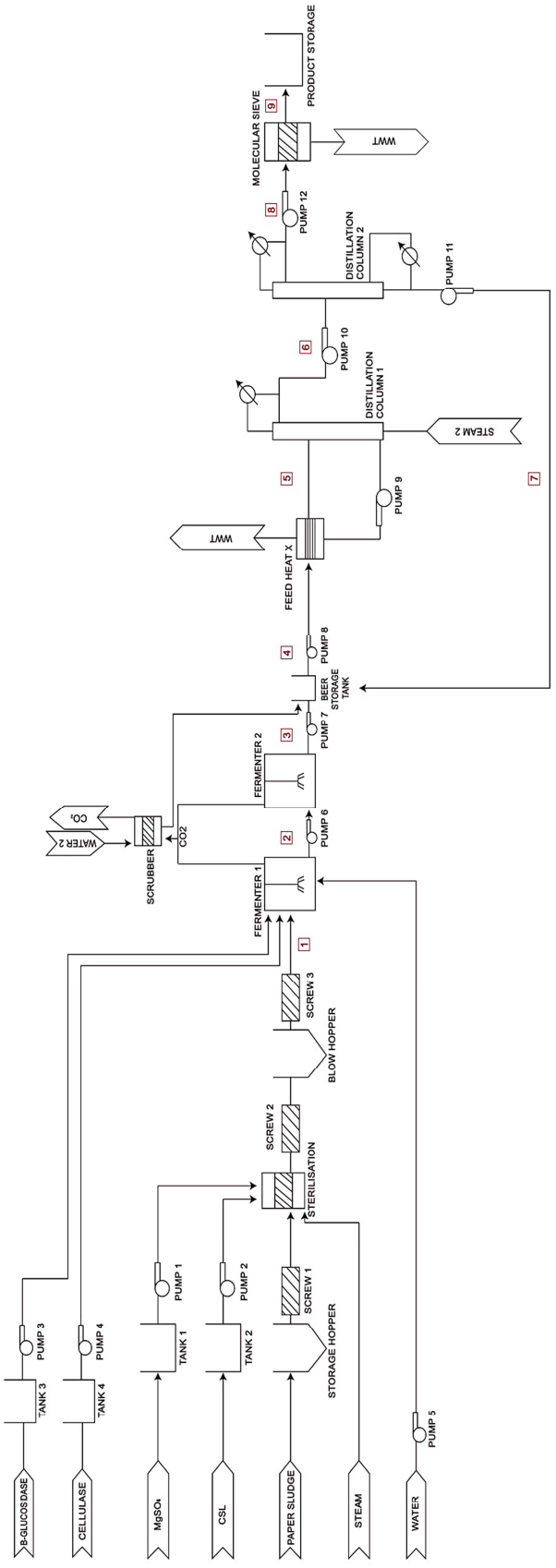
Appendix A

A.1 Process Flow Diagram – 15 t d^{-1}



	β-glucosidase	Cellulase	$MgSO_4$	CSL	Paper Sludge	Steam	Water	Water 2	CO_2	WWT	Steam 2	Stream 1	Stream 2	Stream 3	Stream 4	Stream 5	Stream 6	Stream 7	Stream 8	Stream 9
Temp (°C)	25	25	25	25	25	158.8	25	25	25	45	158.8	121	35	35	35	80	85	95	78	130
Pressure (bar)	0	0	0	0	0	5	0	0	0	0	5	0	0	0	0	0	0	0	0	5
% Solids	0	0	0	0	40%	0	0	0	0	8	0	29	15	10	9	9	0	0	0	0
Mass Flow (kg/h)																				
Water	0.0	0.0	0.0	0.0	936.0	400.0	880.0	10.0	0.0	2847.0	640.0	1336.0	2216.0	2216.0	2532.8	2532.8	324.8	315.8	8.0	0.0
Ash	0.0	0.0	0.0	0.0	93.6	0.0	0.0	0.0	0.0	93.6	0.0	93.6	93.6	93.6	93.6	93.6	0.0	0.0	0.0	0.0
Glucose	0.0	0.0	0.0	0.0	343.0	0.0	0.0	0.0	0.0	16.2	0.0	343.0	118.6	16.2	16.2	16.2	0.0	0.0	0.0	0.0
Xylose	0.0	0.0	0.0	0.0	62.4	0.0	0.0	0.0	0.0	21.8	0.0	62.4	38.0	21.8	21.8	21.8	0.0	0.0	0.0	0.0
Lignin	0.0	0.0	0.0	0.0	62.4	0.0	0.0	0.0	0.0	62.4	0.0	62.4	62.4	62.4	62.4	62.4	0.0	0.0	0.0	0.0
Extractives	0.0	0.0	0.0	0.0	62.4	0.0	0.0	0.0	0.0	62.4	0.0	62.4	62.4	62.4	62.4	62.4	0.0	0.0	0.0	0.0
Ethanol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	0.0	0.0	97.0	162.0	175.2	175.2	175.2	13.2	162.0	162.0
Enzyme	56.0	56.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	112.6	0.0	0.0	112.6	112.6	112.6	112.6	0.0	0.0	0.0	0.0
Nutrients	0.0	0.0	15.0	10.0	0.0	0.0	0.0	0.0	0.0	25.0	0.0	25.0	25.0	25.0	25.0	25.0	0.0	0.0	0.0	0.0
CO_2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	252.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TOTAL	**56.0**	**56.6**	**15.0**	**10.0**	**1560.0**	**400.0**	**880.0**	**10.0**	**252.0**	**3242.0**	**640.0**	**1984.8**	**2825.6**	**2772.0**	**3102.0**	**3102.0**	**500.0**	**330.0**	**170.0**	**162.0**

A.2 Process Flow Diagram – 50 t d^{-1}



	β-glucosidase	Cellulase	MgSO4	CSL	Paper Sludge	Steam	Water	Water 2	CO2	WWT	Steam 2	Stream 1	Stream 2	Stream 3	Stream 4	Stream 5	Stream 6	Stream 7	Stream 8	Stream 9
Temp (°C)	25	25	25	25	25	158.8	25	25	25	45	158.8	121	35	35	35	80	85	95	78	130
Pressure (bar)	0	0	0	0	0	5	0	0	0	0	5	0	0	0	0	0	0	0	0	5
% Solids	0	0	0	0	40%	0	0	0	0	8	0	29	15	10	9	9	0	0	0	0
Mass Flow (kg/h)																				
Water	0.0	0.0	0.0	0.0	3120.0	1333.3	2933.3	33.3	0.0	9490.0	2133.3	4453.3	7386.7	7386.7	8442.7	8442.7	1082.7	1056.0	26.7	0.0
Ash	0.0	0.0	0.0	0.0	312.0	0.0	0.0	0.0	0.0	312.0	0.0	312.0	312.0	312.0	312.0	312.0	0.0	0.0	0.0	0.0
Glucose	0.0	0.0	0.0	0.0	1143.3	0.0	0.0	0.0	0.0	54.0	0.0	1143.3	395.3	54.0	54.0	54.0	0.0	0.0	0.0	0.0
Xylose	0.0	0.0	0.0	0.0	208.0	0.0	0.0	0.0	0.0	72.7	0.0	208.0	126.7	72.7	72.7	72.7	0.0	0.0	0.0	0.0
Lignin	0.0	0.0	0.0	0.0	208.0	0.0	0.0	0.0	0.0	208.0	0.0	208.0	208.0	208.0	208.0	208.0	0.0	0.0	0.0	0.0
Extractives	0.0	0.0	0.0	0.0	208.0	0.0	0.0	0.0	0.0	208.0	0.0	208.0	208.0	208.0	208.0	208.0	0.0	0.0	0.0	0.0
Ethanol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.0	0.0	0.0	323.3	540.0	584.0	584.0	584.0	44.0	540.0	540.0
Enzyme	186.7	188.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	375.3	0.0	0.0	375.3	375.3	375.3	375.3	0.0	0.0	0.0	0.0
Nutrients	0.0	0.0	50.0	33.3	0.0	0.0	0.0	0.0	0.0	83.3	0.0	83.3	83.3	83.3	83.3	83.3	0.0	0.0	0.0	0.0
CO2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	840.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TOTAL	186.7	188.7	50.0	33.3	5200.0	1333.3	2933.3	33.3	840.0	10806.7	2133.3	6616.0	9418.7	9240.0	10340.0	10340.0	1666.7	1100.0	566.7	540.0

Appendix B

Equipment Required	Material	Equipment Information (15 t/d)	Equipment Information (50 t/d)
SLUDGE STERILISATION			
Storage Hopper	**Stainless Steel**	**Equipment Specification** 20 m3 Mass Flow Max angle 30° Min outlet Diameter: 0.3 m	**Equipment Specification** 66 m3 Mass Flow Max angle 30° Min outlet Diameter: 0.3 m
Screw Feeder 1	**Stainless Steel**	**Equipment Specification** Length 5m Diameter 0.3m 2 kW Drive	**Equipment Specification** Length 5m Diameter 0.3m 6 kW Drive
Tank 1	**Poly-Ethylene**	**Equipment Specification** 2 m3	**Equipment Specification** 6 m3
Pump 1		**Equipment Specification** Metering Pump 0.003 m3/h	**Equipment Specification** Metering Pump 0.01 m3/h
Tank 2	**Poly-Ethylene**	**Equipment Specification** 2 m3	**Equipment Specification** 6 m3
Pump 2		**Equipment Specification** Metering Pump 0.003 m3/h	**Equipment Specification** Metering Pump 0.01 m3/h
Sterilisation Reactor	**Stainless Steel**	**Equipment Specification** Volume of 3 m3 5 Bar operating pressure - Steam Injection 121 ℃ Insulation Required	**Equipment Specification** Volume of 10 m3 4 Bar operating pressure - Steam Injection 121 ℃ Insulation Required

Screw Feeder 2	**Stainless Steel**	**Equipment Specification** Length 5m Diameter 0.3m 2 kW Drive	**Equipment Specification** Length 5m Diameter 0.3m 6 kW Drive
Blow Hopper	**Stainless Steel**	**Equipment Specification** Volume of 4 m3 Insulation Required Mass Flow Max angle 30° Min outlet Diameter: 0.3 m	**Equipment Specification** Volume of 13 m3 Insulation Required Mass Flow Max angle 30° Min outlet Diameter: 0.3 m
Screw Feeder 3 (SCREW3)	**Stainless Steel**	**Equipment Specification** Length 5m Diameter 0.3m 2 kW Drive	**Equipment Specification** Length 5m Diameter 0.3m 6 kW Drive
SIMULTANEOUS SACHARIFICATION AND FERMENTATION			
Tank 3	**Poly-Ethylene**	**Equipment Specification** 2 m3	**Equipment Specification** 6 m3
Pump3		**Equipment Specification** Metering Pump 0.006 m3/h	**Equipment Specification** Metering Pump 0.02 m3/h
TANK 4	**Poly-Ethylene**	**Equipment Specification** 2 m3	**Equipment Specification** 6 m3
PUMP 4		**Equipment Specification** Metering Pump 0.007 m3/h	**Equipment Specification** Metering Pump 0.024 m3/h

PUMP 5	**Stainless Steel**	**Equipment Specification** 30 m Head 0.3 kW Required (30 % eff.) 15 % Solids Viscosity - 500 cP 0.9 m3/h	**Equipment Specification** 30 m Head 1 kW Required (30 % eff.) 15 % Solids Viscosity - 500 cP 3 m3/h
Fermenter 1	**Stainless Steel**	**Equipment Specification** Closed Top Flat Bottom 130 m3	Closed Top **Equipment Specification** 430 m3
PUMP 6	**Stainless Steel**	**Equipment Specification** 30 m Head 0.8 kW Required (30 % eff.) 15 % Solids Viscosity - 500 cP 2.8 m3/h	**Equipment Specification** 30 m Head 3.8 kW Required (30 % eff.) 15 % Solids Viscosity - 500 cP 9.5 m3/h
Agitator Fermenter 1	**Carbon Steel**	**Equipment Specification** 25 kW Motor Viscosity - 500 cP 15 % Solids	**Equipment Specification** 100 kW Motor Viscosity - 500 cP 15 % Solids
Heat Exchanger Fermenter 1	**Stainless Steel**	To maintain SSF Tank 1 at 37 ℃ Service Fluid - 45 ℃ Water	To maintain SSF Tan k 1 at 37 ℃ Service Fluid - 45 ℃ W ater
Scrubber	**Carbon Steel**	**Equipment Specification** Recover ethanol from CO2 purge Purify CO2 160 kg/h vapour (10kg/h Ethanol)	**Equipment Specification** Recover ethanol from CO2 purge Purify CO2 530 kg/h vapour (35kg/h Ethanol)
Fermenter 2	**Stainless Steel**	Closed Top Flat Bottom **Equipment Specification** 130 m3	Closed Top **Equipment Specification** 430 m3

Pump 7	**Stainless Steel**	**Equipment Specification** 30 m Head 0.7 kW Required (30 % eff.) 10 % Solids Viscosity - 100 cP 2.8 m3/h	**Equipment Specification** 30 m Head 3.2 kW Required (30 % eff.) 10 % Solids Viscosity - 100 cP 9.4 m3/h
Agitator Fermenter 2	**Carbon Steel**	**Equipment Specification** 25 kW Motor Viscosity - 100 cP 10 % Solids	**Equipment Specification** 100 kW Motor Viscosity - 100 cP 10 % Solids
Heat Exchanger Fermenter 2	**Stainless Steel**	To maintain SSF Tank 2 at 37 ℃ Service Fluid - 45 ℃ Water	To maintain SSF Tan k 2 at 37 ℃ Service Fluid - 45 ℃ W ater
Beer Storage (Incl. Agitator)	**Stainless Steel**	**Equipment Specification** 30 m3 Agitator Power - 5 kW Viscosity - 100 cP	**Equipment Specification** 100 m3 Agitator Power -20 kW Viscosity - 100 cP
Pump 8	**Stainless Steel**	**Equipment Specification** 40 m Head 0.9 kW Required (30 % eff.) 10 % Solids Viscosity - 100 cP 3.1 m3/h	**Equipment Specification** 40 m Head 3.2 kW Required (30 % eff.) 10 % Solids Viscosity - 100 cP 10 m3/h
PRODUCT RECOVERY			
Feed Heat Exchanger	**Stainless Steel**	**Equipment Specification** Area Required 9.5 m2 Overall Coefficient 700 W/m2.K Duty 130 kW PROCESS FLUID 10 % Solids 3.1 m3/h Heat from 37 to 80 ℃ Viscosity - 100 cP SERVICE FLUID 9.5 % Solids 3.2 m3/h Cool from 100 to 45 ℃ Viscosity - 100 cP	**Equipment Specification** Area Required 32 m2 Overall Coefficient 700 W/m2.K Duty 433 kW PROCESS FLUID 10 % Solids 10m3/h Heat from 37 to 80 ℃ Viscosity - 100 cP SERVICE FLUID 10 % Solids 11 m3/h Cool from 100 to 45 ℃ Viscosity - 100 cP

Pump 9	**Stainless Steel**	**Equipment Specification** 40 m Head 0.5 kW Required (30 % eff.) Viscosity - 100 cP 10 % Solids	**Equipment Specification** 40 m Head 1.8 kW Required (30 % eff.) Viscosity - 100 cP 10 % Solids
Distillation Column 1	**Stainless Steel**	**Equipment Specification** Diameter 0.52 m Atmospheric Pressure 40 Sieve Plates Feed Stage on 15 Hole Diameter 15 mm Active Area 10 % Weir length 378 mm Weir height 38 mm Tray Spacing 225 mm Reflux Ratio of 2	**Equipment Specification** Diameter 1.4 m Atmospheric Pressure 40 Sieve Plates Feed Stage on 15 Hole Diameter 15 mm Active Area 10 % Weir length 1 022 mm Weir height 38 mm Tray Spacing 225 mm Reflux Ratio of 2
Condenser 1	**Stainless Steel**	**Equipment Specification** Area Required 10 m2 Overall Coefficient 800 W/m2.K Duty Required -470 kW Process Vapour 1200 kg/h 35 % Ethanol (Mass Based) 65 % Water (Mass Based) Service Fluid Process water at 30 ℃	**Equipment Specification** Area Required 53 m2 Overall Coefficient 800 W/m2.K Duty Required -2 565 kW Process Vapour 4 000 kg/h 30 % Ethanol (Mass Based) 70 % Water (Mass Based) Service Fluid Process water at 30 ℃
Pump 10	**Stainless Steel**	**Equipment Specification** 40 m Required head 0.7 kW Required (30 % eff.) 1200 kg/h 1.1 m3/h	**Equipment Specification** 40 m Required head 4.0 kW Required (30 % eff.) 4 000 kg/h 3.5 m3/h
Distillation Column 2	**Stainless Steel**	**Equipment Specification** Diameter 0.32 m Atmospheric Pressure 35 Sieve Plates Feed Stage on 18 Hole Diameter 5 mm Active Area 10% Weir length 230 mm Weir height 38 mm	**Equipment Specification** Diameter 0.65 m Atmospheric Pressure 35 Sieve Plates Feed Stage on 18 Hole Diameter 5 mm Active Area 10% Weir length 475 mm Weir height 38 mm

		Tray Spacing 225 mm Reflux Ratio of 2	Tray Spacing 225 mm Reflux Ratio of 2
Pump 11	**Stainless Steel**	**Equipment Specification** 20 m Required Head 301 kg/h 0.3 m3/h	**Equipment Specification** 20 m Required Head 1 000 kg/h 1.0 m3/h
Condenser 2 (DIST-2)	**Stainless Steel**	**Equipment Specification** Area Required 7 m2 Overall Coefficient 1100 W/m2.K Duty – 174 kW Process Vapour 640 kg/h 95 % Ethanol (Mass Based) 5 % Water (Mass Based) Service Fluid Process water at 30 ℃	**Equipment Specification** Area Required 24 m2 Overall Coefficient 1100 W/m2.K Duty – 580 kW Process Vapour 2130 kg/h 95 % Ethanol (Mass Based) 5 % Water (Mass Based) Service Fluid Process water at 30 ℃
Pump 12	**Stainless Steel**	**Equipment Specification** 50 m Required Head 0.5 kW Required (30 % eff.)	**Equipment Specification** 50 m Required Head 1.8 kW Required (30 % eff.)
Reboiler (DIST-2)	**Stainless Steel**	**Equipment Specification** Required Surface Area 10 m2 Overall Coefficient 800 W/m2.K 237 kW Heating Required Process Fluid 520 kg/h 99 % Water Service Fluid Steam @ 5 bar Approx. 150 ℃	**Equipment Specification** Required Surface Area 33 m2 Overall Coefficient 800 W/m2.K 790 kW Heating Required Process Fluid 1 731 kg/h 99 % Water Service Fluid Steam @ 5 bar Approx. 150 ℃
Pressure Swing Adsorption with Molecular Sieves		**Equipment Specification** Feed of 95 % Ethanol At 170 kg/h To produce 99.9 % Ethanol at 162 kg/h	**Equipment Specification** Feed of 95 % Ethanol At 650 kg/h To produce 99.9 % Ethanol at 616 kg/h
Product Storage	**Carbon Steel**	**Equipment Specification** 20 m3	**Equipment Specification** 60 m3

Appendix C

C.1 Equipment cost estimations for the sterilisation section

Equipment Required	Cost Estimate (15 t d^{-1})	Cost Estimate (50 t d^{-1})
Storage Hopper	R 150 000 - R 200 000	R 300 000 - R 400 000
Screw Feeder 1	R 50 000 - R 70 000	R 100 000 - R 150 000
Tank 1	R 2 000 - R 4 000	R 3 000 - R 5 000
Pump 1	R 40 000 - R 60 000	R 40 000 - R 60 000
Tank 2	R 2 000 - R 4 000	R 3 000 - R 5 000
Pump 2	R 40 000 - R 60 000	R 40 000 - R 60 000
Sterilisation Reactor	R 400 000 - R 500 000	R 800 000 - R 900 000
Screw Feeder 2	R 50 000 - R 70 000	R 100 000 - R 150 000
Blow Hopper	R 75 000 - R 100 000	R 150 000 - R 200 000
Screw Feeder 3	R 50 000 - R 70 000	R 100 000 - R 150 000
TOTAL	**R 859 000 - R 1 138 000**	**R 1 636 000 - R 2 080 000**

C.2 Equipment cost estimations for the SSF section

Equipment Required	Cost Estimate (15 t d^{-1})	Cost Estimate (50 t d^{-1})
Tank 3	R 2 000 - R 4 000	R 3 000 - R 5 000
Pump 3	R 40 000 - R 60 000	R 40 000 - R 60 000
Tank 4	R 2 000 - R 4 000	R 3 000 - R 5 000
Pump 4	R 40 000 - R 60 000	R 40 000 - R 60 000
Pump 5	R 50 000 - R 70 000	R 80 000 - R 120 000
Fermenter 1	R 1 000 000 - R 1 250 000	R 2 000 000 - R 3 000 000
Pump 6	R 50 000 - R 70 000	R 80 000 - R 120 000
Agitator (Fermenter 1)	Included in SSF Fermenter 1 Cost	Included in SSF Fermenter 1 Cost
External Heating (HeatX + Pump)	Included in SSF Fermenter 1 Cost	Included in SSF Fermenter 1 Cost
Scrubber	R 70 000 - R 100 000	R 150 000 - R 200 000
Fermenter 2	R 1 000 000 - R 1 250 000	R 2 000 000 - R 3 000 000
Pump 7	R 50 000 - R 70 000	R 80 000 - R 120 000
Agitator (Fermenter 2)	Included in SSF Fermenter 2 Cost	Included in SSF Fermenter 2 Cost
External Heating (HeatX + Pump)	Included in SSF Fermenter 2 Cost	Included in SSF Fermenter 2 Cost
Beer Storage Tank	R 150 000 - R 200 000	R 300 000 - R 400 000
Pump 8	R 50 000 - R 70 000	R 80 000 - R 120 000
TOTAL	**R 2 454 000 - R 3 158 000**	**R 4 776 000 - R 7 090 000**

C.3 Equipment cost estimations for the ethanol recovery section

Equipment Required	Cost Estimate (15 t d^{-1})	Cost Estimate (50 t d^{-1})
Feed Heat Exchanger	R 200 000 - R 250 000	R 400 000 - R 600 000
Distillation Column 1	R 1 500 000 - R 2 000 000	R 2 500 000 - R 3 500 000
Condenser 1	R 250 000 - R 300 000	R 500 000 - R 600 000
Pump 10	R 30 000 - R 40 000	R 50 000 - R 70 000
Pump 9	R 50 000 - R 70 000	R 50 000 - R 70 000
Distillation Column 2	R 1 000 000 - R 1 500 000	R 2 000 000 - R 3 000 000
Reboiler (DIST-2)	R 200 000 - R 250 000	R 400 000 - R 500 000
Pump 11	R 40 000 - R 60 000	R 50 000 - R 70 000
Pump 12	R 40 000 - R 60 000	R 50 000 - R 70 000
Pressure Swing Adsorption with Molecular Sieves (MOLSIEVE)	R 1 500 000 - R 2 000 000	R 2 500 000 - R 3 000 000
Product Storage (PRODUCT)	R 75 000 - R 100 000	R 150 000 - R 250 000
TOTAL	**R 5 075 000 - R6 860000**	**R 9 150 000 - R 12 330 000**

Appendix D

D.1 Discounted Cash Flow Sheet - 15 t d^{-1}

Year	Fixed Capital	Working Capital	Production Costs	Revenue	Depreciation	Tax (28 %)	Tax Actual	Net Income	Salvage	Cash Flow	Cum Cash Flow	NPV
0	-R 32 687 550.00	R 1 634 377.50								-R 31 053 172.50	-R 31 053 172.50	-R 31 053 172.50
1			-R 9 734 613.16	R 14 132 909.64	R 16 343 775.00	-R 3 973 570.58	R 0.00	R 4 398 296.48		R 4 398 296.48	-R 26 654 876.02	-R 27 054 721.15
2			-R 9 734 613.16	R 14 132 909.64	R 9 806 265.00	-R 2 143 067.78	R 0.00	R 4 398 296.48		R 4 398 296.48	-R 22 256 579.53	-R 23 419 765.38
3			-R 9 734 613.16	R 14 132 909.64	R 6 537 510.00	-R 1 227 816.38	R 0.00	R 4 398 296.48		R 4 398 296.48	-R 17 858 283.05	-R 20 115 260.13
4			-R 9 734 613.16	R 14 132 909.64	R 0.00	R 602 686.42	R 0.00	R 4 398 296.48		R 4 398 296.48	-R 13 459 986.57	-R 17 111 164.46
5			-R 9 734 613.16	R 14 132 909.64	R 0.00	R 602 686.42	R 0.00	R 4 398 296.48		R 4 398 296.48	-R 9 061 690.09	-R 14 380 168.38
6			-R 9 734 613.16	R 14 132 909.64	R 0.00	R 602 686.42	R 0.00	R 4 398 296.48		R 4 398 296.48	-R 4 663 393.60	-R 11 897 444.68
7			-R 9 734 613.16	R 14 132 909.64	R 0.00	R 602 686.42	R 0.00	R 4 398 296.48		R 4 398 296.48	-R 265 097.12	-R 9 640 423.14
8			-R 9 734 613.16	R 14 132 909.64	R 0.00	R 602 686.42	R 0.00	R 4 398 296.48		R 4 398 296.48	R 4 133 199.36	-R 7 588 585.37
9			-R 9 734 613.16	R 14 132 909.64	R 0.00	R 602 686.42	R 0.00	R 4 398 296.48		R 4 398 296.48	R 8 531 495.85	-R 5 723 278.30
10			-R 9 734 613.16	R 14 132 909.64	R 0.00	R 602 686.42	R 0.00	R 4 398 296.48		R 4 398 296.48	R 12 929 792.33	-R 4 027 544.61
11			-R 9 734 613.16	R 14 132 909.64	R 0.00	R 602 686.42	R 0.00	R 4 398 296.48		R 4 398 296.48	R 17 328 088.81	-R 2 485 968.52
12			-R 9 734 613.16	R 14 132 909.64	R 0.00	R 602 686.42	R 0.00	R 4 398 296.48		R 4 398 296.48	R 21 726 385.29	-R 1 084 535.72
13			-R 9 734 613.16	R 14 132 909.64	R 0.00	R 602 686.42	R 0.00	R 4 398 296.48		R 4 398 296.48	R 26 124 681.78	R 189 494.10
14			-R 9 734 613.16	R 14 132 909.64	R 0.00	R 602 686.42	R 0.00	R 4 398 296.48		R 4 398 296.48	R 30 522 978.26	R 1 347 703.03
15		-R 1 634 377.50	-R 9 734 613.16	R 14 132 909.64	R 0.00	R 602 686.42	R 0.00	R 4 398 296.48	R 2 075 400.00	R 4 839 318.98	R 35 362 297.24	R 2 506 197.52

Discount Rate 10%

IRR 11%

D.2 Discounted Cash Flow Sheet – 30 t d^{-1}

Year	Fixed Capital	Working Capital	Production Costs	Revenue	Depreciation	Tax (28 %)	Tax Actual	Net Income	Salvage	Cash Flow	Cum Cash Flow	NPV
0	-R 47 363 400.00	R 2 368 170.00								-R 44 995 230.00	-R 44 995 230.00	-R 44 995 230.00
1			-R 17 741 358.31	R 28 265 819.28	R 23 681 700.00	-R 4 941 700.13	R 0.00	R 10 524 460.97		R 10 524 460.97	-R 34 470 769.03	-R 35 922 418.82
2			-R 17 741 358.31	R 28 265 819.28	R 14 209 020.00	-R 2 289 349.73	R 0.00	R 10 524 460.97		R 10 524 460.97	-R 23 946 308.07	-R 28 101 029.88
3			-R 17 741 358.31	R 28 265 819.28	R 9 472 680.00	-R 963 174.53	R 0.00	R 10 524 460.97		R 10 524 460.97	-R 13 421 847.10	-R 21 358 453.20
4			-R 17 741 358.31	R 28 265 819.28	R 0.00	R 1 689 175.87	R 0.00	R 10 524 460.97		R 10 524 460.97	-R 2 897 386.14	-R 15 545 887.10
5			-R 17 741 358.31	R 28 265 819.28	R 0.00	R 1 689 175.87	R 0.00	R 10 524 460.97		R 10 524 460.97	R 7 627 074.83	-R 10 535 054.25
6			-R 17 741 358.31	R 28 265 819.28	R 0.00	R 1 689 175.87	R 0.00	R 10 524 460.97		R 10 524 460.97	R 18 151 535.79	-R 6 215 370.76
7			-R 17 741 358.31	R 28 265 819.28	R 0.00	R 1 689 175.87	R 0.00	R 10 524 460.97		R 10 524 460.97	R 28 675 996.76	-R 2 491 505.69
8			-R 17 741 358.31	R 28 265 819.28	R 0.00	R 1 689 175.87	R 251 654.00	R 10 272 806.97		R 10 272 806.97	R 38 948 803.73	R 641 961.95
9			-R 17 741 358.31	R 28 265 819.28	R 0.00	R 1 689 175.87	R 1 689 175.87	R 8 835 285.10		R 8 835 285.10	R 47 784 088.82	R 2 965 226.49
10			-R 17 741 358.31	R 28 265 819.28	R 0.00	R 1 689 175.87	R 1 689 175.87	R 8 835 285.10		R 8 835 285.10	R 56 619 373.92	R 4 968 040.76
11			-R 17 741 358.31	R 28 265 819.28	R 0.00	R 1 689 175.87	R 1 689 175.87	R 8 835 285.10		R 8 835 285.10	R 65 454 659.01	R 6 694 604.78
12			-R 17 741 358.31	R 28 265 819.28	R 0.00	R 1 689 175.87	R 1 689 175.87	R 8 835 285.10		R 8 835 285.10	R 74 289 944.11	R 8 183 022.04
13			-R 17 741 358.31	R 28 265 819.28	R 0.00	R 1 689 175.87	R 1 689 175.87	R 8 835 285.10		R 8 835 285.10	R 83 125 229.20	R 9 466 140.36
14			-R 17 741 358.31	R 28 265 819.28	R 0.00	R 1 689 175.87	R 1 689 175.87	R 8 835 285.10		R 8 835 285.10	R 91 960 514.30	R 10 572 276.85
15		-R 2 368 170.00	-R 17 741 358.31	R 28 265 819.28	R 0.00	R 1 689 175.87	R 1 689 175.87	R 8 835 285.10	R 3 007 200.00	R 9 474 315.10	R 101 434 829.39	R 11 594 811.39

Discount Rate 16%

IRR 22%

D.3 Discounted Cash Flow Sheet – 50 t d^{-1}

Year	Fixed Capital	Working Capital	Production Costs	Revenue	Depreciation	Tax (28 %)	Tax Actual	Net Income	Salvage	Cash Flow	Cum Cash Flow	NPV
0	-R 62 294 400.00	R 3 114 720.00								-R 59 179 680.00	-R 59 179 680.00	-R 59 179 680.00
1			-R 28 231 546.52	R 47 109 698.80	R 31 147 200.00	-R 5 531 455.36	R 0.00	R 18 878 152.28		R 18 878 152.28	-R 40 301 527.72	-R 42 017 723.39
2			-R 28 231 546.52	R 47 109 698.80	R 18 688 320.00	-R 2 042 968.96	R 0.00	R 18 878 152.28		R 18 878 152.28	-R 21 423 375.45	-R 26 415 944.64
3			-R 28 231 546.52	R 47 109 698.80	R 12 458 880.00	-R 298 725.76	R 0.00	R 18 878 152.28		R 18 878 152.28	-R 2 545 223.17	-R 12 232 509.43
4			-R 28 231 546.52	R 47 109 698.80	R 0.00	R 3 189 760.64	R 0.00	R 18 878 152.28		R 18 878 152.28	R 16 332 929.11	R 661 522.59
5			-R 28 231 546.52	R 47 109 698.80	R 0.00	R 3 189 760.64	R 0.00	R 18 878 152.28		R 18 878 152.28	R 35 211 081.38	R 12 383 369.88
6			-R 28 231 546.52	R 47 109 698.80	R 0.00	R 3 189 760.64	R 1 696 131.00	R 17 182 021.28		R 17 182 021.28	R 52 393 102.66	R 22 082 172.96
7			-R 28 231 546.52	R 47 109 698.80	R 0.00	R 3 189 760.64	R 3 189 760.64	R 15 688 391.64		R 15 688 391.64	R 68 081 494.30	R 30 132 798.49
8			-R 28 231 546.52	R 47 109 698.80	R 0.00	R 3 189 760.64	R 3 189 760.64	R 15 688 391.64		R 15 688 391.64	R 83 769 885.94	R 37 451 548.97
9			-R 28 231 546.52	R 47 109 698.80	R 0.00	R 3 189 760.64	R 3 189 760.64	R 15 688 391.64		R 15 688 391.64	R 99 458 277.57	R 44 104 958.50
10			-R 28 231 546.52	R 47 109 698.80	R 0.00	R 3 189 760.64	R 3 189 760.64	R 15 688 391.64		R 15 688 391.64	R 115 146 669.21	R 50 153 512.62
11			-R 28 231 546.52	R 47 109 698.80	R 0.00	R 3 189 760.64	R 3 189 760.64	R 15 688 391.64		R 15 688 391.64	R 130 835 060.85	R 55 652 198.18
12			-R 28 231 546.52	R 47 109 698.80	R 0.00	R 3 189 760.64	R 3 189 760.64	R 15 688 391.64		R 15 688 391.64	R 146 523 452.49	R 60 651 003.24
13			-R 28 231 546.52	R 47 109 698.80	R 0.00	R 3 189 760.64	R 3 189 760.64	R 15 688 391.64		R 15 688 391.64	R 162 211 844.13	R 65 195 371.47
14			-R 28 231 546.52	R 47 109 698.80	R 0.00	R 3 189 760.64	R 3 189 760.64	R 15 688 391.64		R 15 688 391.64	R 177 900 235.77	R 69 326 615.32
15		-R 3 114 720.00	-R 28 231 546.52	R 47 109 698.80	R 0.00	R 3 189 760.64	R 3 189 760.64	R 15 688 391.64	R 3 955 200.00	R 16 528 871.64	R 194 429 107.41	R 73 283 495.77

Discount Rate 10%

IRR 30%