

Characterization and fermentation of waste paper sludge for bioethanol production

by

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Declaration

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Abstract

The need for renewable energy sources are at an unprecedented high due to the world population and energy demand increasing drastically past the point that the remaining fossil fuels are able to supply. Biomass is a sustainable and renewable source of energy with the potential to mitigate greenhouse gas emissions and to serve as an alternative to fossil fuels when converted into biofuels, such as bioethanol or biodiesel. Paper sludge (PS) is a biomass waste stream from the paper and pulp industry that is often landfilled. By converting PS into bioethanol, landfill can be avoided and an energy stream can be produced to be used at the mill or sold.

This study investigated the conversion of PS into ethanol and how the nature of the sludge influenced a high solid loading fermentation process. Paper sludge samples from various paper and pulp mills in South Africa were collected and characterized into categories according to chemical composition and the feed utilized at each mill. Significant variation was observed in the chemical composition between mills, whereas clear correlations were observed within categories utilizing the same feed. Screening for fermentation performance also revealed substantial variation due to the differences in digestibility of the samples. Based on characterization and screening data, samples from two categories, namely corrugated recycle mills and virgin pulping mills were chosen for further investigation and optimization.

Selecting PS samples with high digestibility to ensure maximum ethanol yield and productivity is a critical requirement for process efficiency. However, the PS samples differed substantially in terms of viscosity. Virgin pulp PS, originating from a chemical pulping process, had a significantly higher water holding capacity and viscosity compared to corrugated recycle PS, originating mainly from recycling and repulping operations. These differences affected the maximum solid loading that could be achieved in fermentations, and inherently, the enzymatic hydrolysis of the material where high viscosity would limit enzyme accessibility to the fibers. Given the viscous nature of virgin pulp PS, solids loadings of between 3 to 9% (w/w) achieved the maximum PS hydrolysis to sugar, whereas for corrugated recycle PS the maximum enzymatic hydrolysis was achieved at substantially greater solids loadings of 15% (w/w) and higher.

The optimised process with corrugated recycle PS resulted in an ethanol concentration and yield of 45.5 g/L and 78.2 %, respectively, at a solid loading of 27% (w/w) and an enzyme dosage of 11 FPU/gram dry sludge. The optimised process for the virgin pulp PS required a significantly higher enzyme dosage of 20 FPU/gram dry sludge at a lower solid loading of 18% (w/w), to achieve

the optimum ethanol concentration and yield of 34.2 g/L and 66.9% (w/w), respectively. The virgin pulp PS was highly viscous at 18% (w/w) and required high agitation of 1500 rpm that, in turn, had a negative effect on enzyme activity from shear stress of the agitator. This study demonstrated that corrugated recycle PS is more suited for bioethanol production compared to virgin pulp PS, primarily due to water holding capacity, viscosity and shear stress associated with high agitation rates, which had a major influence on high solids loading fermentation processes.

Uittreksel

Die behoefte aan hernubare energiebronne het ongekende hoogtes bereik weens die aanwas in die wêreldpopulasie asook die vraag na energie wat die punt drasties oorskry het waar fossielbrandstowwe aan hierdie aanvraag kan voldoen. Biomassa is 'n volhoubare energiebron met die potensiaal om groenhuiskasvrystelling te bekamp en om as alternatief tot fossielbrandstowe te dien wanneer dit na biobrandstof omgeskakel word. Papierslyk (PS) is 'n biomassa-ryke afvalstroom van die papier en pulp industrie wat gebruiklik vir stortingsterreine bestem is. Hierdie vermorsing van potensiële energie kan verhoed word deur PS na bioetanol om te skakel. 'n Energiestroom kan dus gegenerer word wat by meule gebruik of verkoop kan word.

In hierdie studie is die omskakeling van PS na etanol ondersoek asook hoe die eienskappe van die slyk die fermentasieproses by hoë soliedemateriaalladings beïnvloed het. Papierslykmonsters is van Suid Afrikaanse papier en pulp meule ingesamel en volgens chemiese samestelling, en die aard van die voer by die meule, in kategorieë gegroepeer. Beduidende variasie in die chemiese samestelling van monsters tussen verskillende meule is waargeneem, terwyl daar duidelike korrelasies binne kategorieë was wanneer dieselfde voer vir meule gebruik was. 'n Siftingsproses op grond van fermentasiewerkverrigting het ook op aansienlike variasie gedui, weens verskille in die monsters se verteerbaarheid. Op grond van karakteriserings- en siftingsdata is monsters van twee kategorieë, nl. geriffelde hersirkulerende meule en reinpulpmeule vir verdere ondersoek en optimering gekies.

Die seleksie van PS monsters met hoë verteerbaarheid is 'n kritiese vereiste ten einde etanol opbrengs en produktiwiteit te optimeer, en die proses se effektiwiteit te maksimeer. Die PS monsters het taamlik in terme van viskositeit verskil. Reinpulp, wat vanuit die chemiese pulpproses afkomstig is, het 'n aansienlike hoër waterhouvermoë en viskositeit vergeleke met geriffelde hersirkuleerde PS, wat meestal uit hersirkulerings- en herpulpprosesse afkomstig is. Hierdie verskille het die maksimum lading van soliede materiaal moontlik in fermentasieprosesse, en wesenlik die ensiematiese hidroliese van die materiaal beïnvloed, waar hoë viskositeit toegang van ensieme tot die vesels beperk. Gegewe die viskeuse aard van die reinpulp was hidroliese van PS na suikers maksimaal by soliedemateriaalladings van tussen 3 en 9% (m/m), terwyl geriffelde hersirkuleerde PS se hidroliese na suiker maksimaal by aansienlik hoër soliedemateriaalladings van 15% (m/m) en meer was.

Die geoptimeerde proses met geriffelde hersirkuleerde PS het 'n etanol konsentrasie en opbrengs van onderskeidelik 45.5 g/L en 78.2 % by 'n soliedemateriaallading van 27% (m/m) en ensiemdosering van 11 FPE/gdm tot gevolg gehad. Daarenteen was die proses met reinpulp by 'n beduidende hoër ensiemdosering van 20 FPE/gdm en laer soliedemateriaallading van 18% (m/m) optimaal, waar 'n etanol konsentrasie en opbrengs van onderskeidelik 34.2 g/L en 66.9% (m/m) aangeteken is. Die reinpulp was by 'n lading van 18% (m/m) baie viskeus wat 'n baie hoë roersnelheid van 1500 opm vereis het. Om die beurt het die hoë roersnelheid hoë sleurkragte tot gevolg gehad wat negatiewe effekte op die stabiliteit van die ensieme gehad. In hierdie studie is gedemonstreer dat vergeleke met reinpulp, geriffelde hersirkuleerde PS meer geskik vir bioetanolproduksie is, hoofsaaklik as gevolg van waterhouvermoë, viskositeit en sleurkragte, saam met hoë roersnelhede, wat 'n groot invloed op hoësoliedlading fermentasieprosesse gehad het.

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List of abbreviations

FSC	Forest Stewardship Council
gds	gram dry substrate
GP	Gauteng
KZN	Kwa-Zulu Natal
mM	Millimolar
MP	Mpumalanga
NREL	National Renewable Energy Laboratory
PAMSA	Paper Making Association of South Africa
PS	Paper sludge
PSOM	Paper sludge organic material
SHF	Separate Hydrolysis and Fermentation
SSCF	Simultaneous Saccharification and Co-Fermentation
SSF	Simultaneous Saccharification and Fermentation
UV	Ultraviolet

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Chapter 1: Introduction

1.1. Background

Similar to most other industries, the paper and pulp industry has made considerable efforts towards sustainability and decreasing their dependence on fossil fuels. This includes having the processes and products certified by the global Forest Stewardship Council (FSC), increasing recycling, reducing waste and emission streams, reducing specific energy consumption and moving more towards renewable sources of energy (Mondi, 2013; Mpact, 2012; Sappi, 2014). Paper sludge (PS) is the term used to describe the solid waste stream emanating from the waste water treatment facility in the paper making process and mostly consists of fibers, fillers and ash (Prasetyo & Park, 2013; Nampak, 2012). This stream is an ideal feedstock for bio-ethanol production as it usually does not require harsh thermo-chemical pretreatment, as is required for the biological processing of unprocessed or virgin lignocelluloses, due to the extensive chemical and mechanical pretreatment during the papermaking process (Fan & Lynd, 2007b).

It is estimated that approximately 500 000 wet tons of PS is produced annually in South Africa by the members of the Paper Making Association of South Africa (PAMSA) (Personal communications). This organization represents 90% of all paper manufacturers in South Africa including Kimberly-Clark South Africa (Pty) Ltd, Mondi South Africa Ltd, Mpact Ltd, Nampak Tissue South Africa Ltd, and Sappi South Africa Ltd (The Paper Story, 2015a). The increasing cost and constraints associated with legal disposal of PS via landfill have created the need to reuse it in other processes (Republic of South Africa, 2013).

In 2012, Nampak land filled only 20% of their PS and supplied the rest for the manufacturing of clay bricks (Nampak, 2012). Two Mpact mills, namely Felixton and Springs recycled 77% of their PS by using it for the production of compost and concrete block making (Mpact, 2012). Kimberly-Clark set the goal to have no waste going to landfill by 2015 and re-use PS for building and insulation products, soil amendment, newsprint and corrugated packaging. However, PS still ended up contributing 90% of the manufacturing waste from Kimberly-Clark going to landfill in 2013. (Kimberly-Clark, 2013). Mondi International had a 7% reduction in total waste going to landfill in 2013 compared to 2010 but still ended up land filling 272,783 tons (Mondi, 2013).

By supplying PS as a feedstock to other processes and industries, landfill can be avoided, however a potential energy feedstock is being lost. The ideal would be if the paper and pulp industry could utilize PS for biofuel production through either biological or thermochemical processes. Thereby the industry can

reduce the amount of waste going to landfill and produce energy to be used in the mills, making them less dependent on energy from the national grid.

Previous studies clearly demonstrates the suitability of PS for successful bioethanol fermentation (Lark *et al.*, 1997; Kang *et al.*, 2010; Prasetyo *et al.*, 2010). In this study, the focus was on investigating how the nature of PS might affect industrial PS to bioethanol processes. This was done by first comparing the chemical compositions and fermentability (measured as the final ethanol concentration in fermentation broth) from all the available PS types in South Africa. From this screening, two types of PS samples from different PS categories were selected and simultaneous saccharification and fermentation (SSF) fed-batch processes, optimised for high solid loadings and low enzyme dosages, were developed for these two samples.

The fed-batch SSF processes adds to the significance of the results presented herein, as work in bioreactors with PS as feedstock are not commonly found in literature due to the high water holding capacity of PS and the accompanying viscosity issues (Dwiarti *et al.*, 2012; Kang *et al.*, 2011; Ballesteros *et al.*, 2002; Wang *et al.*, 2012). The chemical compositions and fermentability reported for PS from all the types of milling operations is valuable for further and other process development as, to our knowledge, this has not been previously reported.

1.2. Thesis layout

Chapter 1: Introduction. This chapter provides the background and context to the study. The aims and objectives for this research is given with the layout for the thesis.

Chapter 2: Literature Study. In this chapter the information relevant to this study is given. Lignocellulosic biomass and PS are discussed, while the fermentation processes, including SSF, are reviewed with the effects that key parameters, such as agitation, enzyme dosage and high solids loading have on the process with PS as feedstock as source of carbon. Information on the paper and pulp industry in South Africa is summarised and Chapter 2 concludes with the experimental approach followed in this study.

Chapter 3: Paper sludge to bioethanol: Evaluation of virgin and recycle mill sludge for low enzyme, high-solids fermentation. This research chapter contains all the experimental work in this study and is written in the format of a scientific research paper to be submitted to Bioresource Technology.

Chapter 4: Conclusions and recommendations. This final chapter provides a general summary to the study and contains the main conclusions as well as recommendations for further research.

Chapter 2: Literature review

2.1. Waste paper sludge

2.1.1. Waste paper sludge as biomass feedstock

Biomass can be described as natural material originating from living or recently living organisms. In the context of energy, it is used to describe plant or plant derived material and material from animals or vegetables (Biomass Energy Centre, 2013). Lignocellulose is the term that describes only plant or plant derived biomass. Biomass includes crops, forestry, marine products and wastes and can be divided into five general categories as can be seen in Table 2.1.

Table 2.1: Biomass categories and examples (Biomass Energy Centre, 2013).

Virgin wood	Energy crops	Agricultural residues	Food waste	Industrial waste and co-products
-Bark	-Short rotation energy crops	-Straw	-Kitchen waste	-Untreated wood
-Brash and arboricultural arisings	(<i>Eucalyptus, Poplar</i>)	-Corn stover	(<i>Peels, Shells, Husks</i>)	(<i>Construction wastes, Broken pellets</i>)
- Logs	- Grasses	- Poultry Litter	-Beverage and food industry waste	-Treated wood
- Sawdust	(Switchgrass, Reed, Rye)	- Animal manure	(<i>Spent grains, Leftover food</i>)	(<i>Furniture production wastes,</i>
- Wood pellets and briquettes	- Non-woody energy crops	-Grass silage	- Waste vegetable oils	<i>Laminated wood</i>)
- Wood chips	(<i>Hemp</i>)		- Animal fats	- Textile wastes
	- Aquatics (<i>Algae, Seaweeds, Kelp</i>)			-Sewage sludge
	- Agricultural energy crops			-Pulp and paper industry wastes
	(<i>Sugar beet, Wheat, Maize, Potatoes, Sunflower</i>)			(<i>Recycled paper, Paper sludge, Black liquor</i>)

Waste paper sludge or paper sludge (PS) is a type of lignocellulosic biomass from the pulp and paper industry that was up until recently primarily disposed of in landfills. The stream is commonly a mixture of waste streams from various processes in the mill: a primary sludge stream is collected from the primary clarifiers or settling tanks, a recycle waste stream originates from a reprocessing unit that recycles paper and a waste stream coming from the thermo- mechanical or chemical pulping plant. The stream mainly consists of degraded short fibers that is unusable in the paper making process as well as inks, glues, clay, residues and chemicals used in the recovery process (Prasetyo & Park, 2013).

2.1.2. Composition of PS

PS is classified as lignocellulosic material and it consists of mainly cellulose, hemi-cellulose and lignin. Cellulose is a glucose polymer with a mainly crystalline structure, linked by β -(1 \rightarrow 4)-glycosidic bonds. It has an average molecular weight of 100 000 and makes up 40-50% of biomass by weight. Hemicellulose is a heteropolymer composed of various monosaccharides like xylose, mannose, glucose and galactose. It represents 20-40% of biomass by weight and has an average molecular weight of less than 30 000. Lignin is an aromatic heteropolymer with a high molecular-weight and a high resistance to chemical or enzymatic degradation. It consists of p-hydroxyphenylpropanoid units and the three basic building blocks are trans p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol (Toor *et al.*, 2011; McKendry, 2002).

The composition of PS is not typical or predictable as the carbohydrate composition in paper sludge have been reported to vary from 20-70% (Fan & Lynd, 2007b). This variation in composition was reported in a study done on 15 paper sludge samples in the USA (Lynd *et al.*, 2001) and a detailed compositional analysis of the PS samples can be seen in Table 2.2. The sludge ID numbers refer to different PS samples taken from various mills and the *a*, *b*, *c* notation next to sample ID's 29, 30 and 34 refers to samples taken at different locations within the same mill. The standard deviation next to some sludge ID samples were calculated from two or more samples collected from the same mill, at the same location at one week intervals.

Table 2.2: Detailed compositional analysis of 15 paper sludge samples (Lynd *et al.*, 2001).

Sludge ID #	Glucan	Xylan	Mannan	Ash	Acid soluble lignin
27	36.27 \pm 4.26	3.07 \pm 0.03	2.25 \pm 1.76	14.85 \pm 7.04	1.74 \pm 0.39
28	43.52 \pm 4.67	2.97 \pm 0.81	2.28 \pm 0.61	29.43 \pm 4.58	0.64 \pm 0.06
29a	68.6 \pm 5.86	2.43 \pm 0.08	2.65 \pm 0.20	5.56 \pm 0.06	0.87 \pm 0.01
29b	53.97	1.45	2.57	1.80	1.06
30a	47.27 \pm 0.46	3.33 \pm 0.25	2.79 \pm 0.15	19.9 \pm 0.42	1.17 \pm 0.09
30b	56.54	5.61	3.64	5.03	0.99
31	42.72 \pm 8.74	4.66 \pm 1.62	1.33 \pm 0.17	34.88 \pm 5.96	0.84 \pm 0.11
32	33.08 \pm 6.26	5.7 \pm 0.67	0.69 \pm 0.53	31.53 \pm 11.02	0.89 \pm 0.14
33	32.1 \pm 5.96	1.96 \pm 0.38	2.14 \pm 0.91	47.81 \pm 3.02	0.49 \pm 0.05
34a	50.13	4.98	3.30	13.59	0.89
34b	56.28	6.17	3.84	8.00	0.90
34c	11.66	1.29	0.80	53.98	1.57
35	23.33 \pm 14.47	1.89 \pm 1.08	1.58 \pm 0.01	57.37 \pm 29.11	0.37 \pm 0.16
36	51.44 \pm 3.71	5.09 \pm 0.54	3.53 \pm 0.46	2.35 \pm 0.36	1.65 \pm 0.02
37	27.2 \pm 2.35	3.02 \pm 0.80	3.94 \pm 1.12	22.38 \pm 1.88	1.7 \pm 0.09

It was determined that the chemical compositions are significantly different between different mills and for various locations within the same mill. The standard deviation also indicated that it changes significantly over time. This all indicates that the composition of PS wastes are not constant due to the large difference in feed utilized at different mills. The same conclusion was drawn in other studies (Scott *et al.*, 1995).

2.1.3. Processes for paper sludge utilization

The disposal of PS is viewed as a substantial problem in the pulp and paper industry. Landfill has for some time been the main way of disposing of PS but recent government regulations, environmental concerns and high tipping prices, as well as lack of tipping space, has caused the industry to evaluate other options (Prasetyo *et al.*, 2010; Nampak, 2013, Republic of South Africa, 2013). The chemical composition of paper sludges differ substantially based on the process and feed used and therefore a wide spectrum of process options needs to be available (Ochoa de Alda, 2008). Below are some processes that can utilize PS.

Agricultural applications: The ash produced from the combustion of PS can be used as a liming agent to add to the organic matter of soil. However, combustion is energy intensive due to the drying required as the moisture content of PS are generally above 65% (Table 3.1), and combustion releases pollutant gases into the atmosphere (Prasetyo & Park, 2013).

Anaerobic digestion: PS can be used as a feedstock for biogas production (Puhakka *et al.*, 1992; Rintala & Puhakka, 1994). It is proposed that co-digestion with another feedstock is used with added nutrients such as general food or fish wastes (Dalwai, 2012; Lin *et al.*, 2012a).

Biohydrogen production from anaerobic fermentation: Anaerobic fermentation of paper sludge can produce biohydrogen in quantities higher than the international reported value in 2009. (Wu & Zhou, 2012).

Incineration: The incineration of PS is commonly accepted to retain some of the energy it contains as well as reducing the volume (Ochoa de Alda, 2008). However, the high moisture content in the PS results in a low efficiency and it was found that burning of biomass causes secondary harmful organic aerosols like acids, benzene and furan derivatives (Aghamohammadi *et al.*, 2011).

Production of ethanol: PS is an attractive material for the production of ethanol through fermentation. Concentrations in excess of 40g/L have been obtained by various studies indicating that the use of PS as a feedstock for ethanol production is a viable option (Fan *et al.*, 2003; Kang *et al.*, 2011; Elliston *et al.*, 2013).

Pyrolysis: Pyrolysis is a thermochemical process that can be used for the conversion of PS into gas, bio-oil or char with various studies showing promising results (Ridout *et al.*, 2015; Mendez *et al.*, 2009). Feeding PS to pyrolysis required drying of the feedstock to less than 10% moisture, which incurs significant energy cost.

Recovery of minerals: A hyperthermal reaction can be used to recover kaoline and silica from PS. The reaction occurs under alkaline condition, is however energy intensive and was found not to be feasible for industrial scale (Hendriks & Zeeman, 2009).

2.1.4. Advantages and disadvantages of paper sludge as feedstock for bioethanol production

2.1.4.1. Advantages

Negative cost feedstock: The cost of enzymes and feedstock is known to be the main contributors to the running cost of a biomass conversion process (Kumar & Murthy, 2011; Aden & Foust, 2009). The feedstock price can be costly as it also needs to account for harvesting and transport costs. By using PS as a feedstock, the feedstock and harvesting costs can be avoided. If the processing facility is built on site, the transport cost can be eliminated as well.

No pretreatment necessary: For most biological conversion processes, the lignocellulosic biomass needs to be pretreated to make the cellulose more accessible to the enzymes. Most PS samples do not need to be pretreated because of the extensive mechanical and chemical processing done during the papermaking process (Lark *et al.*, 1997; Prasetyo *et al.*, 2011; Fan & Lynd, 2007b). PS is thus typically amenable to enzymatic hydrolysis, providing adequate hydrolysis yields for biological conversion processes, such as fermentation or anaerobic digestion. It is however important to note that the paper making process is optimised for paper production and not pretreatment of cellulose for biological conversion processes, indicating that there might be limitations imposed on the conversion process by the properties of PS emanating from the paper making process.

Potential availability of pre-existing infrastructure: The costs accompanying a waste treatment facility is one of the main factors when deciding how waste streams will be handled. Incorporating a PS treatment plant into the standing mill can significantly decrease the costs of waste handling and bio-energy production, compared to other cellulosic processing facilities (Fan *et al.*, 2003; Lin *et al.*, 2012b). An onsite PS processing facility can be linked to the mill's energy and water grid and could make significant savings on infrastructure and installation fees.

Reduction of industrial waste: Vast amounts of waste paper sludge are produced worldwide with Japan discarding 5 million tons of PS annually (Prasetyo *et al.*, 2010). The USA, UK and China accounts for respective annual amounts of 8, 2 and 12 million tons (Dwiarti *et al.*, 2012). By utilizing PS, it will decrease the amount that will end up in landfill as a result of industrial waste as well as avoiding transport/disposal costs for producers of PS. This will also in turn reduce landfill space and soil degradation, groundwater pollution and greenhouse gas emissions such as methane associated with landfill (Crespo *et al.*, 2012). The typical high moisture content of PS (>60%) also implies that significant amounts of water is lost by landfilling, which will be recuperated through PS conversion to energy.

Second generation bioethanol production: The production of bioethanol from PS is classified as second generation bioethanol production and does not result in increased food prices by competing for food supplies (Boddiger, 2007). It can make a feasible contribution to the worldwide effort to move away from fossil fuels and towards greener energy without threatening food security.

2.1.4.2. Disadvantages

Ash: Recycling mills, feeding mostly printed recycle material, is known to produce paper sludge with ash contents of more than 50% by mass due to all the fillers, inks and clay used in the printing process. Such high amounts of ash can cause irreversible binding to enzymes, resulting in a poor enzymatic hydrolysis process (Chen *et al.*, 2014, Kang *et al.*, 2010) and subsequently low ethanol concentrations and yields (Robus, 2013).

High water holding capacity: Another significant disadvantage of paper sludge is the high water holding capacity associated with paper related feedstocks (Lark *et al.*, 1997). Water holding capacity is given in g water/g substrate and is considered high when the value is more than one, thus retaining an amount of water that is more than the mass dry substrate. The problem with the high

water holding capacity is the limited free water during the process resulting in increased viscosities that in turn results in improper mixing (Fan & Lynd, 2007).

2.1.5. Effect of pulping processes on the digestibility of paper sludge

The pulping process is used to extract the cellulose fibers in the wood for use in the production of paper. The separation takes place by removing the lignin that binds the fibers together and this can be done either in chemical or mechanical pulping. In chemical pulping the fibers are separated by cooking the wood in chemical solutions at high temperatures and pressures that end up dissolving the lignin and carbohydrates and separating the cellulose fibers (Gullichsen & Fogelholm, 2000). In mechanical pulping the lignin is not dissolved but only softened and fibre separation takes place by means of grinding or refining (Sundholm, 1999).

Through dissolving the lignin and carbohydrates in chemical pulping, the fibers are more accessible to cellulase enzymes during hydrolysis, due to little to no obstruction from the lignin when compared to mechanical pulping. This will result in better digestibility for sludges from chemical pulping operations compared to mechanical pulping operations. The same conclusion was made by Lark *et al.* (1997) and Zhu *et al.* (2012).

2.2. Biological processing of biomass for ethanol production

2.2.1. Introduction

The production of bioethanol are done with the fermentation process and an overview of the most commonly used processes as found in literature can be seen in Figure 2.1 and is described below.

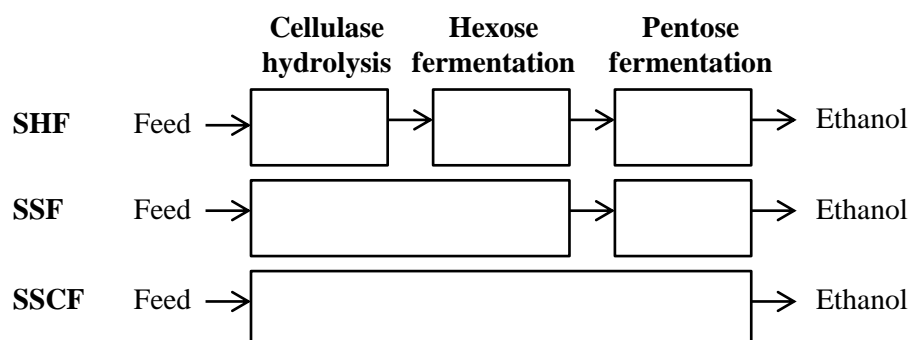


Figure 2.1: Overview of fermentation processes (Redrawn from Lynd *et al.*, 2002).

Separate hydrolysis and fermentation (SHF): SHF comprises of two separate steps. The first step entails the hydrolysis of the cellulose into monomers by the enzymes. The second entails the conversion of the sugar monomers into alcohol by the microorganisms (Dwiarti *et al.*, 2012).

Simultaneous saccharification and fermentation (SSF): SSF is a process in which the cellulose hydrolysing enzyme complex is combined with a sugar fermenting microorganism to produce ethanol in one integrated step (Lin *et al.*, 2012b). A schematic diagram of the SSF process can be seen in Figure 2.2. The first part of the process is where the cellulose is converted into glucose by the enzyme and the second part is the conversion of the glucose into ethanol by the microorganism. The conversion of cellulose into glucose occurs at the same time that glucose conversion into ethanol occurs. This results in less glucose accumulation and less inhibitory effects on cellulase and β -glucosidase (Lynd *et al.*, 2001; Philippidis *et al.*, 1993; Wang *et al.*, 2012).

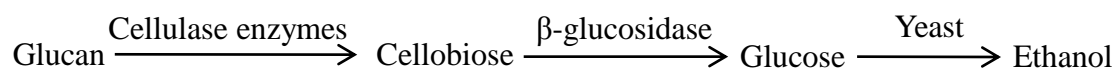


Figure 2.2: Schematic diagram of the SSF process (Redrawn from Lynd *et al.*, 2002).

Simultaneous saccharification and co-fermentation (SSCF): SSCF is essentially the same as SSF but it utilizes a microorganism that is able to convert both five- and six- carbon sugars to ethanol (Hamelinck *et al.*, 2005).

2.2.2. Influence of process parameters on ethanol concentration and yield

Operating and design factors can be manipulated and varied to increase the ethanol concentration, yield and productivity. This section describes the influence of certain factors on the production of ethanol.

Agitation: The agitation intensity was increased in a study on PS to investigate the effect it would have on the ethanol concentration and yield (Kang *et al.*, 2011). The highest agitation intensity (250 rpm) resulted in the highest concentration and yield for both de-ashed and untreated PS. This indicated, together with various other studies that efficient mixing is necessary to facilitate in mass and heat transfer, and to improve cellulose hydrolysis by cellulases (Cavaco-Paulo *et al.*, 1996; Kadic *et al.*, 2014; Palmqvist *et al.*, 2011).

Enzyme dosages: The effect of enzyme dosages (ranging from 5 to 80 FPU/g paper sludge organic material (PSOM)) on the saccharification yield was investigated and resulted in a saccharification yield that increased as the cellulase dosages increased (Prasetyo & Park, 2013). In another study the effect of increased enzyme dosages on ethanol concentration and yield was studied (Kang *et al.*, 2011). The results (Table 2.3) show that as the enzyme dosage increased the ethanol concentration and yield increased as well. Similar results were found in other studies (Prasetyo *et al.*, 2010; Prasetyo *et al.*, 2011).

Table 2.3: Results from study done by (Kang *et al.*, 2011) with 6% glucan fed.

Spezyme (FPU/g-glucan)	5	10	15
120 h SSF Ethanol yield (%)	60.5	67.1	74.5
120 h SSF Ethanol concentration (g/L)	20.5	22.8	25.3

Enzymes are, together with feedstock, the most costly running expense in a biomass conversion process (Kang *et al.*, 2011) and therefore it is not possible to increase the enzyme until the highest yield is obtained. In order to increase the yield when using low enzyme dosages, it is possible to extend the reaction time but this will in turn reduce the productivity. Minimizing the amount of enzyme used should therefore be an aim when developing a bioethanol process, but while still achieving acceptable ethanol yields, final concentrations and productivities (Aden & Foust, 2009).

High solids loading: The effect of increased PS loading from 5 to 10% (w/v) in shake flask was studied at enzyme dosages of 15 and 45 FPU/g substrate (Ballesteros *et al.*, 2002). For both the enzyme dosages, the ethanol concentration increased as the solid loading increased (Table 2.4). A fed batch feeding approach was tested at 15 FPU/g substrate where three feedings of 5%, 3% and 2% (10% w/v in total) was added to the system. The fed batch culture resulted in an ethanol concentration and yield that is higher than the 10% (w/v) batch feeding at the significantly higher enzyme dosage of 45 FPU/g substrate. When using a fed-batch feeding system, a smaller initial amount is used compared to batch systems, and the rest of the substrate is introduced into the system at times when the hydrolysis has progressed far enough in order to accept more solids. This then makes it possible to achieve higher substrate loadings than what is possible in batch culture, while avoiding unacceptably high viscosities (Kristensen, 2009).

Table 2.4: Ethanol concentration and yield for SSF runs at various substrate loadings and enzyme dosages (Ballesteros *et al.*, 2002).

Substrate loading (% w/v)	15 FPU/g substrate		45 FPU/g substrate	
	Ethanol (g/L)	Yield (%)	Ethanol (g/L)	Yield (%)
5	8.2	74.2	8.9	80.3
7.5	8.9	53.7	13.1	78.6
10	12.6	56.4	15.6	70.4
10 (Fed-batch: 5 + 3 + 2)	17.7	79.7		

In order to increase the ethanol concentration in a separate experiment, Prasetyo *et al.* (2011) increased the paper sludge organic material (PSOM) from 50 g/L to 110 g/L in increments of 30 g/L. From a surface response area given, it was clear to see that the higher PSOM loadings resulted in a higher residual sugar concentration and higher ethanol concentrations.

In another study, the effect of different solid loadings on the ethanol yield and concentration using normal paper sludge and de-ashed paper sludge as feedstock were studied (Kang *et al.*, 2011). With both the normal paper sludge and de-ashed paper sludge, the ethanol concentration and ethanol yield decreased as the solid loading increased (See Table 2.5) when the time was kept constant at 120 h. This can be due to ineffective mixing that in turn results in mass transfer limitations at higher solid loadings. To overcome this, the enzyme dosage can be increased, the time of the experiment can be extended or more severe agitation can be used. In the same paper by Kang *et al.* (2011), it was shown that de-ashed paper sludge resulted in higher ethanol yields and concentrations than normal paper sludge at the same glucan loading. This can be explained by the mass transfer limitations that occur due to high ash concentrations that accompanies high solid loadings.

Table 2.5: Ethanol yield and concentrations at different solid loadings (Kang *et al.*, 2011).

Glucan loading (% w/v)	Unwashed PS		Washed PS	
	3	6	3	6
120 h Ethanol yield (%)	68.6	66.1	74.3	72.8
120 h Ethanol concentration (g/L)	23.4	22.5	25.2	24.7

Inoculum volume: The inoculum volume was increased from 10% to 20% during SSF experiments on PS in an effort to improve the ethanol yield (Prasetyo *et al.*, 2011). The thermotolerant *Saccharomyces Cerevisiae* TJ14 were used as the microorganism and both the ethanol yield and concentration increased as the inoculum increased (Table 2.6). The ethanol concentration with 10% inoculum was 35.7 g/L and the theoretical ethanol yield was 61.8%. When

the inoculum increased to 20% and all other parameters held constant, the ethanol concentration was 40.5 g/L and the theoretical ethanol yield improved to 66.3%.

Table 2.6: Results from study done by Prasetyo *et al.*, (2011) with increased inoculum volume.

Inoculum volume (%)	10	20
80 h SSF Ethanol yield (%)	61.8	66.3
80 h SSF Ethanol concentration (g/L)	35.7	40.5

Nutrient medium: The effect of a nutrient solution on the ethanol concentration was studied in SSF experiments with a mixture of paper sludge waste and monosodium glutamate waste liquor (Lin *et al.*, 2012b). A nutrient solution, consisting of peeled potatoes and glucose were added to one of two identical reactor setups. The ethanol concentration in the reactor with no nutrient was less than 5 g/L whereas the ethanol concentration in the reactor with the nutrient solution resulted in more than 20 g/L.

In a different study, SSF experiments on de-ashed paper sludge wastes investigated what the effect would be if yeast extract and peptone (rich medium) is substituted with corn steep liquor (lean medium) (Kang *et al.*, 2011). The results showed that the ethanol yield with the lean medium (71.1%) was very close to the ethanol yield obtained with the rich medium (72.8%). This was in agreement with previous studies that showed that corn steep liquor is a good substitute for yeast extract and peptone (Kadam & Newman, 1997).

pH: The pH in the SSF system needs to be optimized for both the saccharification and the fermentation step. The effect of pH on ethanol concentration was studied with a mixture of paper sludge waste and glutamate waste liquor as the substrate and *Saccharomyces cerevisiae* CICC1001 as the yeast and cellulase produced from *Trichoderma viride* (Lin *et al.*, 2012b). The optimum pH for the yeast and enzyme is given as pH 6.0 – 7.0 and 4.0 – 5.5, respectively. Identical experiments were conducted at a pH of 4.5 and 6.0. The pH of 6.0 resulted in a higher reducing sugar concentration and final ethanol concentration.

Sterilization: It was found that sterilization had no significant improvement on the ethanol concentration when a mixture of paper sludge and monosodium glutamate waste were used as feed in a SSF process (Lin *et al.*, 2012b). A different conclusion was made with only paper sludge as feedstock (Kang *et al.*, 2010). The study showed how sterilization can increase the enzymatic hydrolysis of both glucan and xylan, due to it being a type of pretreatment, and resulted in higher

glucose and xylose concentrations during enzymatic hydrolysis, although ethanol yields and concentrations were not shown.

SSF, SSCF and SHF processes: The effect of various design parameters on the ethanol concentration and yield on the SSF and SSCF process using PS as feedstock was studied (Kang *et al.*, 2011). In Table 2.7 one can see that the ethanol yield and concentration is larger for the SSCF process than for the SSF process [the ethanol yield for SSCF were based on glucan and xylan and SSF were based on glucose only, (Kang *et al.*, 2011)]. This is due to SSCF processes utilizing microorganisms that are able to convert both xylose and glucose present in PS into ethanol, whereas SSF only utilizes glucose. Prasetyo *et al.* (2011) studied the difference in ethanol concentration and ethanol yield on the SSF and SHF process. The ethanol concentration for the SSF process was almost twice as much as the ethanol concentration from the SHF process executed under the same conditions.

Table 2.7: Ethanol yield and concentration for the SSF and SSCF process 3% glucan loading (Kang *et al.*, 2011).

Process	SSF	SSCF
120 h Ethanol yield (%)	68.8	72.4
120 h Ethanol concentration (g/L)	23.4	29.8

Temperature: Similar to the pH of a SSF process, the temperature needs to be in the optimum range for both the saccharification and fermentation process. To determine the optimum temperature for SSF using the thermotolerant yeast *K. marxianus*, the effect of temperature on glucose fermentation from recycled paper sludge was studied by Lark *et al.* (1997) in the range of 30-42 °C. The microorganism consumed the glucose within 8 hours for temperatures of 30 °C and 34°C and within 12 hours for temperatures of 38 °C and 42 °C. The ethanol concentration of 50 g/L was reached for all the temperatures and the ethanol productivity was the highest for 34 °C up to 8 h and decreased for temperatures 38 °C and 42 °C (Lark *et al.*, 1997). The reaction temperature was chosen as 38 °C to include good saccharification activity as well.

Other factors: Other factors that were found to significantly affect the ethanol concentration and yield were the addition of β -glucosidase (Lynd *et al.*, 2001). The maleate buffer concentration was found to affect the saccharification yield that in turn affected the ethanol yield (Prasetyo *et al.*, 2010).

2.3. Paper and Pulp industry in South Africa

In 2013 the paper and pulp industry in South Africa produced 2.31 and 2.02 million tonnes of paper and pulp, respectively. These numbers correspond to a 5% decrease from 2012 and a 30% decrease from 2008. Even though it has decreased significantly over the last seven years, it was still responsible for contributing 26.1% of the agricultural gross domestic product of South Africa and employing 187 000 people in 2013 (The Paper Story, 2014) . The decrease in the market can be partly due to the shift towards electronic media as opposed to hard copies. However, the industry is responsible for many other products that cannot be replaced by information technology, such as dissolved pulp that is used in the production of textiles and clothing, containerboard that is used for packaging and storing of products, tissue paper is a necessity for everyday living and security paper is used for the printing of currency, passports and identity documents.

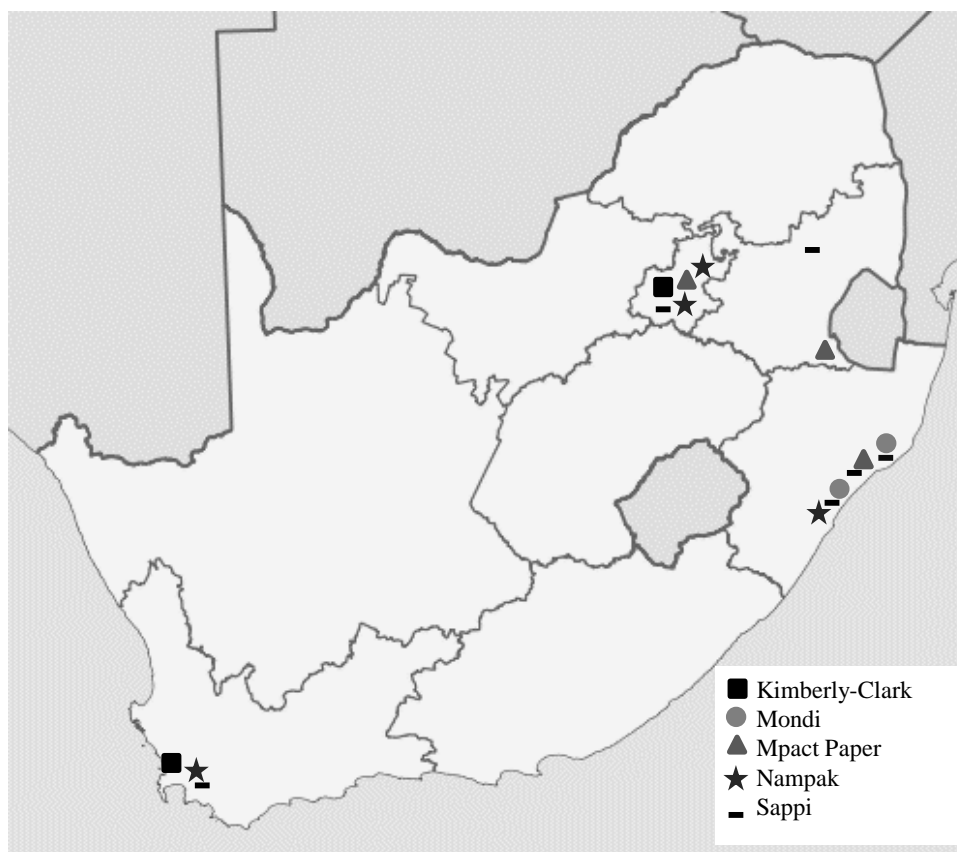


Figure 2.3: Geographical map of South Africa with the mills represented by the PAMSA organization indicated (as in 2013) (The Paper Story, 2015b).

In order for the pulp and paper industry to stay profitable it needs to constantly investigate ways to save material, chemicals and energy. Recycling is one way the industry can reduce feedstock costs, minimise the amount of waste and create jobs and income opportunities. Increased paper and fiber recycling will increase the size of waste streams as more unusable short fibers are generated (Lark *et al.*, 1997). Larger waste streams create a problem for landfill but if it is utilized for bioethanol production it can be beneficial for the industry. The location of the paper and pulp mills in South Africa that are part of the PAMSA organisation can be seen in Figure 2.3.

2.4. Gap in literature

The suitability of PS to be used as a feedstock for bioethanol production has been established (Lark *et al.*, 1997; Kang *et al.*, 2010; Prasetyo *et al.*, 2010), but the change in chemical composition and fiber properties of PS from different mills, and the associated effects on the fermentation process, have not been addressed. Most studies have been done on single PS samples with limited studies on multiple samples. There is also a lack in the processes that have been developed where the solid loading is higher than 15% due to the high water holding capacity of PS and the accompanying viscosity issues (Dwiarti *et al.*, 2012; Kang *et al.*, 2011; Ballesteros *et al.*, 2002; Wang *et al.*, 2012). In studies where high solid loadings are maintained, the enzyme dosage and fermentation time are often too high to be industrially viable (Elliston *et al.*, 2013; Zhang & Lynd, 2010). Processes need to be developed that are able to ferment PS at reasonable enzyme dosages and fermentation times, and solid loadings in excess of 15%, while still resulting in acceptable ethanol concentrations and yields. Factors affecting these processes, and how these will influence the ethanol concentrations and yields, also require further investigation.

2.5. Aims and objectives

The aim of this project is to investigate how the nature of the sludge (chemical composition, digestibility, water holding capacity, viscosity) can influence a SSF fed-batch process at high solid loadings, with the aim of minimizing enzyme dosages while still maintaining acceptable fermentation performance. To achieve this aim, the following objectives were defined:

- i) *To characterize PS samples from various paper and pulp mills in South Africa according to their chemical composition and the feedstock utilized at the mill.*
- ii) *To screen PS samples for ethanol production to determine which PS samples are more amenable to fermentation, i.e. has higher fermentability.*

- iii) *To select PS samples for optimization in a 5 L fed batch SSF process, based on chemical composition, ethanol production performance (fermentability) and suitability of using fermentation residues for subsequent energy generation through pyrolysis or biogas production.*
- iv) *To screen enzyme cocktails and different strains of Saccharomyces cerevisiae to ensure that the most suitable available strain and commercial cellulose cocktail are used to maximize the ethanol concentration and yield.*
- v) *To identify and investigate the main material properties of PS affecting the SSF fed-batch processes.*
- vi) *To develop a fed-batch SSF process for the selected PS samples by taking into consideration the maximum solid loading that is achievable while minimizing enzyme dosage and maintaining acceptable fermentation performance.*

Chapter 3: Paper sludge to bioethanol: Evaluation of virgin and recycle mill sludge for low enzyme, high-solids fermentation

Abstract

Paper sludge from paper and pulp industries consists primarily of cellulose and ash and has significant potential for ethanol production. The purpose of this study was to investigate different factors influencing a paper sludge to bioethanol process at high solid loadings. Sludges from 37 South African mills exhibited large variation in chemical composition and resulting ethanol production. Simultaneous saccharification and fermentation of paper sludge in fed-batch culture was investigated at high solid loadings and low enzyme dosages. High viscosity of sludge from virgin pulp mills restricted the solid loading to 18% (w/w) at an enzyme dosage of 20 FPU/g dry sludge, whereas an optimal solid loading of 27% (w/w) was achieved with corrugated recycle mill sludge with 11 FPU/gram dry sludge. Ethanol concentration and yield of virgin pulp and corrugated recycle sludge were 34.2 g/L at 66.9% and 45.5 g/L at 78.2%, respectively. Water holding capacity and viscosity of the sludge influenced ethanol production at elevated solid loadings where sludge from corrugated recycling operations proved to be more efficient than virgin pulp sludge.

3.1. Introduction

The potential for biofuels to contribute to energy security and environmental benefits, together with the concerns with starch-based first generation biofuel technologies have shifted the focus to biofuels produced from lignocellulose using second generation technologies. Bioethanol production from paper sludge (PS) presents a feasible contribution to sustainable clean energy generation, while also avoiding disposal of these wastes by landfill (Prasetyo & Park 2013; Jørgensen, Kristensen, *et al.*, 2007). The USA and Japan produce nearly 5 million tons of PS annually (Fan and Lynd, 2007a; Prasetyo *et al.*, 2010), China and the UK up to 12 and 2 million tons, respectively, (Dwiarti *et al.*, 2012), with PS production in South Africa a comparatively smaller amount estimated at 0.5 million tons per annum (Mill Personnel, April to August 2013).

Paper sludge is a cellulose-rich waste stream from the paper and pulp process and consists of short cellulose fiber rejects, impurities, fillers and clay removed from recycled printed paper (Kang *et al.*, 2010). It used to be primarily disposed of by landfill, but increasingly stringent environmental regulations in recent years necessitated investigation and development of new avenues for exploiting and processing of this waste stream, including brick making, agricultural applications, incineration and pyrolysis (Nampak, 2012; Republic of South Africa, 2013). A key advantage PS has over other lignocellulosic feedstocks is that the crystalline structure of cellulose has been disrupted during the paper making process (Lynd *et al.*, 2001) and is, therefore, amenable to enzymatic hydrolysis as is. Generally, harsh and energy-intensive thermo-chemical pre-treatment of lignocellulose from woody or grassy biomass is required to disrupt the crystalline structure of the cellulose polymers (Zheng *et al.*, 2009) which has been reported to account up to 30% of the total operating cost (Kang *et al.*, 2010).

In addition to savings from eliminating pre-treatment, PS often has a negative feedstock cost due to savings in transport and/or disposal fees to landfill. Infrastructure cost for biofuel production can also be mitigated by integrating PS-biofuel production with existing mill infrastructure. (Fan & Lynd, 2007a). There are, however, several disadvantages associated with PS as feedstock for ethanol production. Sludge from recycle mills often has an ash content of more than 50%, which has a negative impact on enzymatic hydrolysis, due to the irreversible binding of enzymes to ash (Chen *et al.*, 2014; Robus, 2013). The cost of enzymes is one of the largest contributions to the running cost of a lignocellulosic bioethanol plant and continued efforts are required to develop processes where this cost is minimised (Aden & Foust, 2009). The large ash fraction also adds to the bulk density of the material, leading to decreased ethanol yields and a requirement for larger

reaction vessels and higher energy input (Kang *et al.*, 2011). Furthermore, PS has a high water holding capacity (WHC), which leads to high viscosity fermentations that results in improper mixing and poor mass transfer. These are critical obstacles to overcome in order to meet the threshold value of 40 g/L for the final ethanol concentration to result in economically viable downstream processing (Fan *et al.*, 2003). These challenges can be partly addressed using fed-batch fermentation strategies where solid loadings are increased incrementally with subsequently higher product concentration compared to batch operations.

The present study illustrates the effect of PS properties (chemical composition, digestibility, viscosity and water holding capacity) from different milling operations on ethanol concentration and yield, and the adaption of process strategies to maximise bio-ethanol production. Specific emphasis was placed on minimising the enzyme dosage while maximising the solids loading to attain or exceed the 40 g/L ethanol concentration threshold, while taking into consideration the nature of the sludge samples studied. Fermentations, with selected PS samples were carried out in bench-top bioreactors using fed-batch culture where solids were incrementally fed to the culture using pulp from virgin and corrugated recycle mills, selected from a screening of 37 South African paper pulp mills, allowing a comparative performance assessment between different types of paper sludge waste.

3.2. Materials and Methods

3.2.1. Experimental approach

The experimental approach followed in this study is shown in Figure 3.1 with the shaded section indicating the significance of this study. The experimental work started with the collection of 37 samples from 11 pulp and paper mills in South Africa. Subsequently, the samples were divided into four categories according to chemical composition and the feed utilized at each mill. Fermentation screening was completed at two enzyme dosages of 5 and 15 FPU/gds. Based on experimental data, samples from two categories were selected, namely Mpact Springs (Corrugated recycle category) and Sappi Ngodwana (Virgin pulping category). Screening and selection of yeast strain and cellulase enzyme was done on the two chosen samples. From the available yeast strains in our culture collection, *Saccharomyces cerevisiae* strain MH1000 was selected as the preferred yeast strain due to strong fermentative performance and robustness in terms of ethanol tolerance, whereas Optiflow RC 2.0 was the cellulase enzyme preparation of choice based on the superior hydrolysis capacity with PS as feedstock.

The effect of solid loading on digestibility, water holding capacity and viscosity of the two chosen samples were subsequently investigated to aid in understanding the differences in the optimum fed-batch processes obtained. The large difference in viscosity indicated that a difference in agitation would be required for the two optimised processes. This in turn led to the investigation into the effect of agitation on enzymatic activity. The SSF fed-batch processes for the two selected samples were optimised using a Central Composite Design (CCD) in 5 L bench-scale bioreactors. Finally, using experimental data from fermentation runs of the two samples, the CCD statistical models were validated with final cultivations, also in 5 L bioreactors, at the optimum predicted conditions. From this data, mass balances were constructed for each of these cultivations.

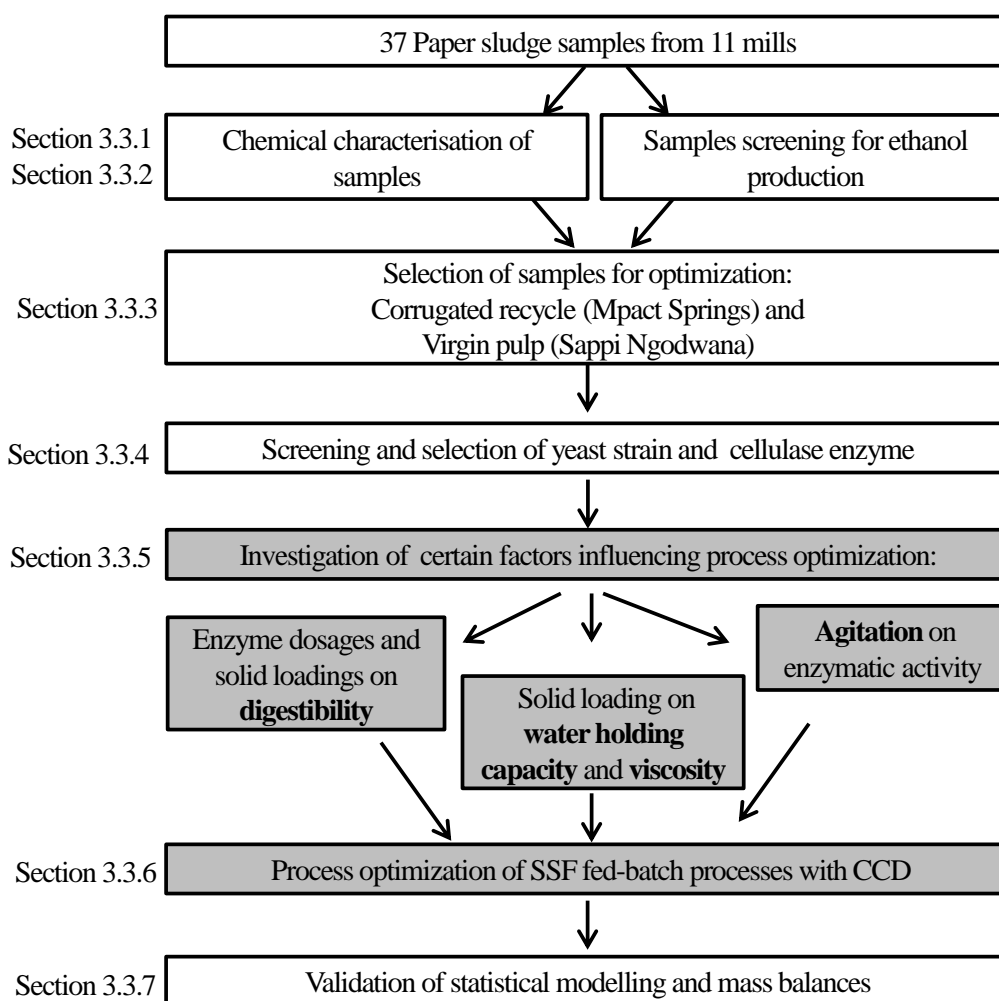


Figure 3.1: The experimental approach followed in this study. The shaded sections indicate the novelty and significance of this study.

3.2.2. Materials

3.2.2.1. Paper sludge feedstock and preparation of material

Thirty-seven PS samples were collected from 11 pulp and paper mills, representing the majority of paper and pulp companies in South Africa, namely Kimberly-Clark South Africa (Pty) Ltd, Mondi South Africa Ltd, Mpact Paper Ltd, Nampak Tissue South Africa Ltd, and Sappi South Africa Ltd. The PS samples used for hydrolysis and fermentation screening were dried at 75 °C after impurities such as plastic, pieces of paper and twigs were removed. Fed-batch SSF experiments were conducted on selected samples in 5 L bioreactors and required larger quantities of PS and were hence dried in a high tunnel (hoop greenhouse) at 40 to 45 °C. Dried samples were stored in sealed plastic bags at room temperature and chemical composition of the samples was determined according to the NREL standard procedures (Sluiter, Hames *et al.*, 2008; Sluiter *et al.* 2011; Sluiter, Ruiz *et al.*, 2008).

3.2.2.2. Yeast strain and enzyme cocktail

Saccharomyces cerevisiae MH1000 (van Zyl *et al.*, 2011), TMB3400 (Wahlbom *et al.*, 2003) and D5A ATCC-200062 (NREL-D5A) were stored at -85 °C with 30% (v/v) glycerol as cryoprotectant. Seed cultures for small and large scale fermentation were grown in medium containing (per litre): 20 g glucose, 20 g peptone and 10 g yeast extract (all Merck, South Africa) for 18 h at 37 °C in an orbital shaker at 150 rpm. Optiflow RC 2.0 (Danisco Genencor, Belgium), Spezyme CP (Danisco Genencor, Denmark) and AlternaFuel CMAX powder (Dyadic International Inc., USA) with activities of 130, 59, 37 FPU/mL, respectively, were used for SSF and enzymatic hydrolysis. All enzymes used in this study were supplemented with β -glucosidase (Novozym® 188, Novozymes, Denmark) with an activity of 929 IU/mL in a volume ratio of 10:1. β -glucosidase activity was determined by the standard filter paper assay published by IUPAC in 1984 (Ghose, 1987). Cellulase activity was determined with the microplate-based filter paper assay developed by Xiao (Xiao *et al.*, 2004) that was adapted from the standard filter paper assay published by IUPAC to use less reagents and increase throughput.

3.2.3. Methods

3.2.3.1. Batch and fed-batch fermentation

Screening of PS samples for ethanol concentration and yield, and screening of enzyme cocktails and strains, were performed in batch culture using 100 mL rubber-capped serum bottles. The medium for batch and fed-batch SSF experiments consisted of (per litre) 3 g corn steep liquor (Sigma-Aldrich, South Africa) and 0.62 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (Merck). PS at a solid loading of 20 g/L was added to media in serum bottles and autoclaved for 15 minutes at 121°C. The pH was not adjusted for fermentation and varied between pH 4-6 for all 37 samples. Filter sterilized enzymes were added to the fermentation broth after inoculating with 5 mL seed culture and were incubated at 37 °C and 150 rpm for 168 h.

Fed-batch experiments were carried out on selected samples in jacketed BIOSTAT® Bplus-5L CC twin bioreactors (Sartorius BBI Systems GmbH, Switzerland) with a final working mass of 2.5 kg and working volumes ranging from 2.5 to 2.9 L depending on the final solid loading. Reactors were fitted with a Rushton and marine-blade impeller combination for mixing and the pH was monitored with Easyferm plus K8 pH probes (Mecosa, South Africa). The pH for all the fed-batch experiments remained in the range of pH 4.8 to 5.5 and was not controlled. The initial PS solid loading upon inoculation was 3% (w/w) with further feedings of 3% (w/w) every 12 h, until the final required quantity of solids was loaded into the vessel. The bioreactors were inoculated with

125 mL (5% v/v) of *S. cerevisiae* MH1000 seed culture together with Optiflow RC 2.0 at dosages as specified in text and were allowed to continue for 168 h at 37 °C. Increases in total solid loadings resulted in decreased mixing efficiency and hence, the agitation rate was adjusted accordingly to a maximum value of 1500 rpm. The theoretical ethanol concentration and ethanol yield were calculated using Equation 3.1 and Equation 3.2, respectively.

$$\text{Theoretical ethanol concentration (g/L)} = \text{Solids fed (g/L)} * \text{Glucose fraction} * 0.511$$

(Equation 3.1)

$$\text{Ethanol yield (\%)} = \text{Experimental ethanol concentration (g/L)} / \text{Theoretical ethanol concentration (g/L)}$$

(Equation 3.2)

3.2.3.2. Enzymatic hydrolysis for digestibility of material

Solid loadings of 3, 6 and 9% (w/w) were tested for hydrolysis on selected samples in 100 mL serum bottles with the same growth medium as described in Section 3.2.3.1 and a total working mass of 100 g. Filter sterilized enzymes were added to substrate that was sterilized at 121 °C for 15 minutes. Cellulase Optiflow RC 2.0 was loaded at dosages of 5, 15 and 25 FPU/gds and the flasks were incubated at 37 °C in an orbital shaker incubator at 150 rpm for 72 h. Samples were collected at regular intervals and the glucose released is represented as a percentage of initial cellulose added.

3.2.3.3. Water Holding Capacity

The WHC of the samples were determined by using PS milled to 250-425 µm sizes and dried to constant weight at 105 °C. Dried PS samples of 3 g each were added to conical tubes containing 30 mL Reverse Osmosis (RO) water and kept at 20°C for 24 h. PS samples saturated with water were centrifuged at 4 000 rpm for 15 minutes and the excess water was decanted. The PS pellet was weighed before and after drying at 105 °C and the WHC was calculated using Equation 3.3.

$$\text{WHC (mL water/g substrate)} = [\text{Wet PS (g)} - \text{Oven dried PS (g)}] / \text{Oven dried PS (g)}$$

(Equation 3.3)

3.2.3.4. Viscosity determination at different solid loadings

The viscosities of PS slurries at different solid loadings were measured as a function of shear rate using a rheometer (Physica MCR 501, Anton Paar Southern Africa (Pty) Ltd., Midrand, Gauteng). Oven-dried PS of 250-425 µm particle size was soaked in RO water at solid loadings of 3

to 8% (w/w) for 24 h to ensure fiber saturation in water. The slurries were mixed with an impeller (ST24-2D/2V/2V-30, Anton Paar) at 25 °C in an aluminium cylindrical tube (CC27/T200, Anton Paar) at shear rates of 0 to 300 s⁻¹. Prior to viscosity measurements, the slurry was mixed at a low shear rate of 30 s⁻¹ for 10 seconds to ensure a homogenous mixture.

3.2.3.5. Enzyme deactivation at different agitation rates

The impact of agitation speed on the enzyme activity was studied in 5 L bioreactors without PS in the reactors. Seventy-five mL of Optiflow RC 2.0 enzyme was added to the reactors and the volume was adjusted to 2.5 L with RO water only. To imitate the SSF conditions, the pH was not adjusted and the temperature was maintained at 37 °C. Agitation speeds of 150, 400 and 1500 rpm were each tested for 96 h and samples were taken every 24 h for cellulase activity determination as described in Section 3.2.2.2. The change in cellulase activity is given as a percentage of the initial value at 0 h.

3.2.3.6. High performance liquid chromatography analytical methods

Ethanol, glucose and xylose concentrations were measured by a high performance liquid chromatography (HPLC) fitted with an Aminex HPx-87 column, a cation-H Micro Guard Cartridge, RI-101 detector, pump and an AS3000 AutoSampler (all Thermo Scientific Products, Bio-Rad, South Africa). The column was operated at 65 °C with 5 mM sulphuric acid as a mobile phase at the flowrate of 0.6 mL/min.

3.2.3.7. Statistical analysis

The calculating of means and standard deviations for statistical analysis were done in Microsoft Excel, version 2013. Statistica, version 10, was used to design a Central Composite Design (CCD) that used Response Surface Methodology (RSM) to predict the interaction between the two independent variables, solids loading and enzyme dosage, and the dependant variables; final ethanol concentration, ethanol yield, and ethanol productivity. Desirability surface plots were used to interpret the effect of the independent variables on the overall response desirability which is a combination of all the dependant variables. The desirability function requires each dependant variable to have a desirability value assigned to it, with 0 being very undesirable to 1 being very desirable.

3.3. Results and Discussion

3.3.1. Chemical composition of paper sludge from different milling operations

The feedstock utilised at a mill has a direct impact on the chemical composition and physical properties of the various PS samples, which impacts on the hydrolysis-fermentation process design and performance. Therefore, paper sludge samples were collected from 11 mills to include a variety of PS types and characteristics. The variation in the feed, process types and products from different milling operations can be seen in Table 3.1, together with the PS production values, as provided by the mills. Samples were categorised according to the similarity of the feed utilized at the mills (Printed recycle, Non-recycle, Corrugated recycle and Virgin pulp) and exhibited a significant correlation in each category with regards to their chemical composition (Figure 3.2).

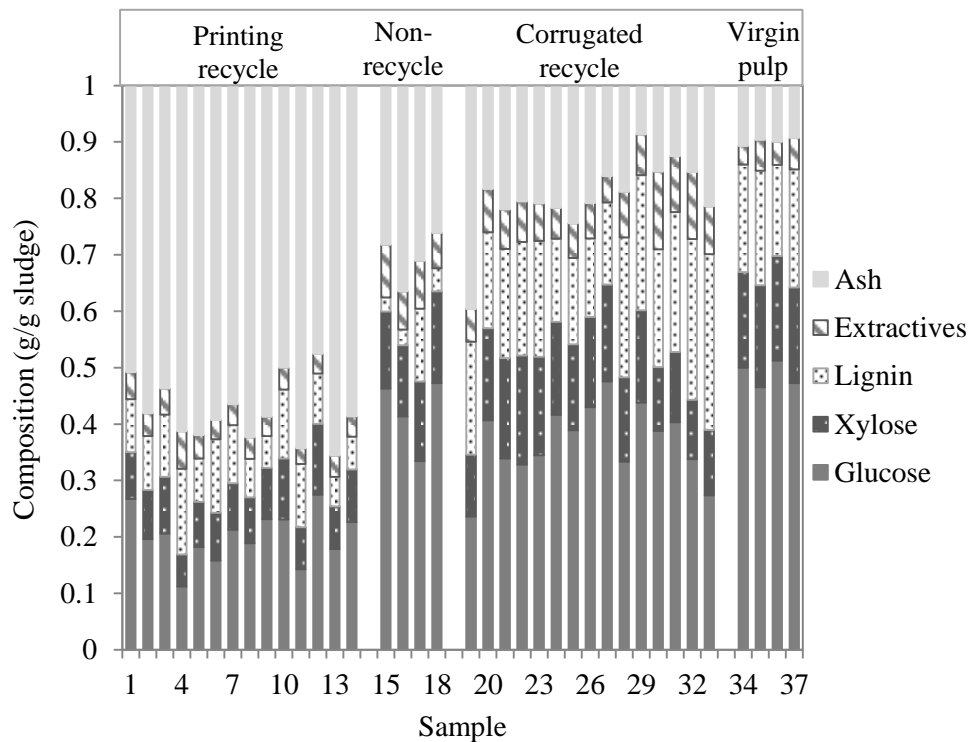


Figure 3.2: The chemical composition (g component/g sludge) of 37 PS samples collected from 11 paper and pulp mills in South Africa and categorized into four main categories with respect to the feed utilized at each mill.

Table 3.1: Paper sludge samples received from different mills and the number of samples from each. The feed, process, products, production of PS (dry ton/year) and moisture content (%) for each mill are shown¹.

Company: Mill	Sample numbers	Feed ²	Process ³	Products ⁴	Production (dry ton/year)	Moisture content (%)
Kimberly-Clark: Enstra	1, 2, 3, 4	RF, NPW, VP	RP, DI	TP	6 000	54
Nampak: Bellville	5, 6, 7, 8	RF, NPW, VP	RP, DI	TP	1 800	54
Nampak: Kliprivier	9, 10, 11, 12	RF, NPW, VP	RP, DI	TP	1 500	60
Nampak: Verulam	13, 14	RF, NPW, VP	RP, DI	TP	1 500	57
Sappi: Enstra	15, 16, 17, 18	VP	RP	OP, SP, PP	7 500	71
Mondi: Richardsbay	19	RF, C, VW, E	RP, K	B, KL, CB	12 500	64
Mpact: Felixton	20, 21, 22, 23	BP, VW, E, P	RP	CB	4 000	73
Mpact: Springs	24, 25, 26, 27	RF, C, VP	RP, DI	WLC, LB, SCB	11 000	80
Mpact: Piet Retief	28, 29	RF, C, VP, BP	RP	CB	500	70
Sappi: Tugela	30, 31, 32, 33	RF, C, VW, E, P	NSSC	CB, NSSCP, RPF	7 000	85
Sappi: Ngodwana	34, 35, 36, 37	VW, E, P	K, MP	NP, KL, CUP, MP, DP	15 000	80

¹ Data provided by individual mills.

² RF = Recycled fiber, NPW = Newsprint, Printing and Writing, VP = Virgin pulp, C = Corrugated, VW = Virgin wood, E = Eucalyptus, P = Pine, BP = Bagasse pulp.

³ RP = Re-pulping, DI = De-inking, K = Kraft, NSSC = Neutral Sulfite Semi Chemical, MP = Mechanical pulping.

⁴ TP = Tissue paper, B = Baycel pulp, KL = Kraft linerboard, CB = Containerboard, OP = Office paper, SP = Security paper, PP = Packing paper, NSSCP = Neutral Sulfite Semi Chemical pulp, RPF = Recycle pulp fiber, NP = Newsprint paper, CUP = Chemical unbleached pulp, MP = Mechanical pulp, DP = Dissolved pulp, WLB = White-lined cartonboard, LB = Laminated board, SCB = Speciality coated board.

Some significant differences were observed in the properties of PS samples within particular categories, although these differences were less pronounced than the differences between categories. Paper sludge with a high cellulose and low ash fraction is theoretically considered to be a preferred feedstock for SSF bioethanol processes, due to potential for high ethanol yields per ton dry weight (cellulose content), while avoiding the negative effect of ash content on the enzymatic hydrolysis, through irreversibly binding of enzymes (Chen *et al.*, 2014; Kang *et al.*, 2010; Kang *et al.*, 2011). Processes have been developed to remove ash from PS by washing over a screen or

series of screens prior to hydrolysis-fermentation (Kang *et al.*, 2011; Robus, 2013), although these will incur additional processing cost.

The Virgin pulp category had the highest glucose fraction of 0.46 g/g substrate (dry weight) on average, together with the lowest ash fraction of only 0.09 g/g substrate, resulting in the highest theoretical ethanol concentration, in comparison to the rest of the mills. The Printed recycle category resulted in the lowest average cellulose content, having less than half of the cellulose fraction in the virgin pulp category. The Printed recycle category also had the highest ash content of 0.55 g/g substrate on average due to the predominant utilization of newsprint, printing and writing recycle feedstock in the mills. These results were similar to a previous report on nine PS samples from printed recycling operations in South Africa, where the ash fraction was reported to range from 0.56 to 0.66 g/g substrate (Robus, 2013). In the study, the PS was de-ashed by washing over a screen, thus removing 66 to 84% of the ash, to final values as low as 10.08%. De-ashing could increase final ethanol concentrations by as much as 49 to 57%, and will likely be required for ethanol production from printed recycle PS.

3.3.2. Simultaneous Saccharification and Fermentation in batch culture

All 37 PS samples were screened in SSF batch culture for ethanol production at enzyme dosages of 5 and 15 FPU/gds. The final ethanol concentrations of all the samples showed a significant variation at a confidence level of 0.1 for both enzyme dosages. The highest ethanol concentration was obtained for the Non-recycle category at an enzyme dosage of 15 FPU/gds. The ethanol concentrations obtained over all the categories are compared to the maximum theoretical ethanol concentration in Figure 3.3 and were similar to values reported in previous studies on PS SSF (Robus, 2013; Lark *et al.*, 1997; Lynd *et al.*, 2001; Kang *et al.*, 2010). The theoretical ethanol concentration calculated with Equation 3.1 and represented by the solid line, indicated that high glucose fractions and hence high theoretical ethanol values did not necessarily imply high experimental ethanol concentrations (Figure 3.3). The difference between the experimental and theoretical ethanol concentration could be explained by the digestibility of cellulose fibers during enzymatic hydrolysis. Variations in the paper and pulp processes from which paper sludge was obtained clearly impacted on PS fiber properties, and thus the yield of fermentable sugars from enzymatic hydrolysis of these.

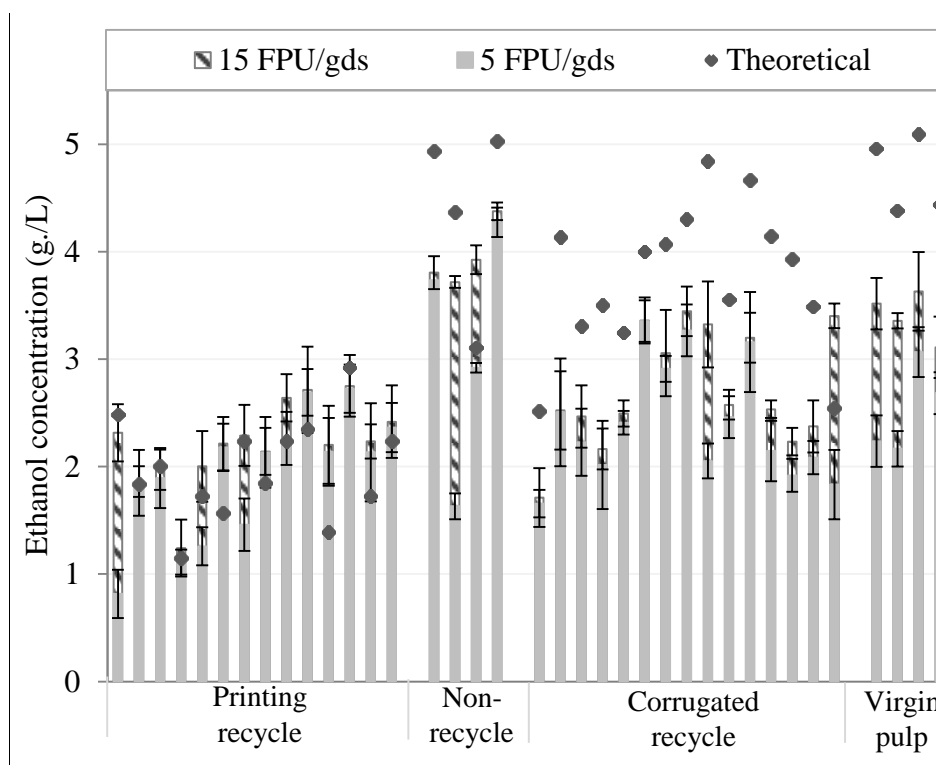


Figure 3.3: Ethanol concentrations obtained at enzyme dosages of 5 and 15 FPU/gds for all collected paper sludge samples within the four categories: Printing recycle, Non recycle, Corrugated recycle and Virgin pulp. The theoretical ethanol concentration is indicated by the round dots.

In Figure 3.3 it can be seen that with some samples the measured final ethanol concentrations were higher than the calculated theoretical maxima. This was mostly observed in the Printed recycle category where the ash fraction was very high ranging from 46 to 62 g/g substrate. It is difficult to measure ash content accurately at these high levels, which affects the accuracy of cellulose content determination.

3.3.3. Selection of paper sludge samples for optimization

Key objectives for this study were to maximise ethanol concentration and yields from PS by first using it for ethanol production via SSF at high solid loadings and low enzyme dosages and then to propose the fermentation residues as a feedstock for pyrolysis or biogas production. Hence, two mills were selected for process optimisation in 5 L fed-batch cultures based on chemical composition (Section 3.3.1), ethanol production (Section 3.3.2) and the amount of organic matter in the residue for pyrolysis or biogas production. Sappi Ngodwana from the Virgin pulp category was chosen for the low ash and high glucose content that makes it desirable as a feedstock for bioethanol production and the combined lignin and xylose fraction of 0.34 g/g substrate indicates that the fermentation residue would be suitable for pyrolysis or biogas production.

Sappi Enstra from the Non-recycle category had the second highest average glucose fraction of 0.42 g/g substrate and resulted in the highest average ethanol concentration of 3.14 and 3.96 g/L at 5 and 15 FPU/gds, respectively. However, the ash fraction was significantly higher and the combined xylose and lignin fraction (0.19 g/g substrate) in the fermentation residue might be insufficient to propose it as a feedstock for pyrolysis or biogas production. Mpact Springs from the Corrugated recycle category was therefore chosen as the second mill. The low ash and high glucose fractions make it desirable for fermentation and the lignin and xylose fraction of 0.35 g/g substrate is sufficient enough for the fermentation residue to be considered as feedstock for either biogas or pyrolysis.

3.3.4. Yeast and enzyme cocktail screening

The yeast strains and enzyme cocktail for SSF were screened for ethanol production for the selected corrugated recycle and virgin pulp PS samples. *S. cerevisiae* MH100, TMB3400 and D5A were tested with Optiflow RC 2.0. The strains showed no significant difference between the ethanol concentrations obtained (Figure 3.4) and *S. cerevisiae* MH1000 was selected for further optimization.

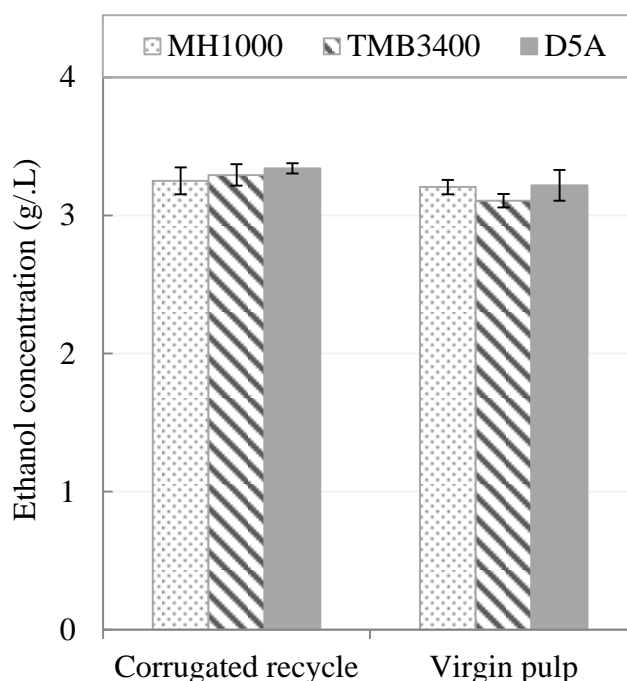


Figure 3.4: Ethanol concentration (g/L) obtained for the corrugated recycle and virgin pulp samples during screening for yeast strains to utilize during optimization. *S. cerevisiae* strains MH1000, TMB3400 and D5A were tested with Optiflow RC 2.0 as enzyme. The ethanol concentrations reported are the highest value measured after 168 h and the error bars indicate the standard deviation of triplicate runs.

The three enzyme cocktails were compared in SSF with *S. cerevisiae* MH1000 as microorganism and the highest ethanol concentration in fermentation culture was observed with Optiflow RC 2.0 and Spezyme CP, reaching values up to three times more than AlternaFuel CMAX and therefore Optiflow RC 2.0 was chosen for further optimizations (Figure 3.5). Optiflow was found to be statistically higher than Spezyme and Alternafuel CMAX at a confidence level of 0.05. The data proves that Optiflow RC 2.0 and Spezyme CP are superior in terms of hydrolysis activity for these specific substrates.

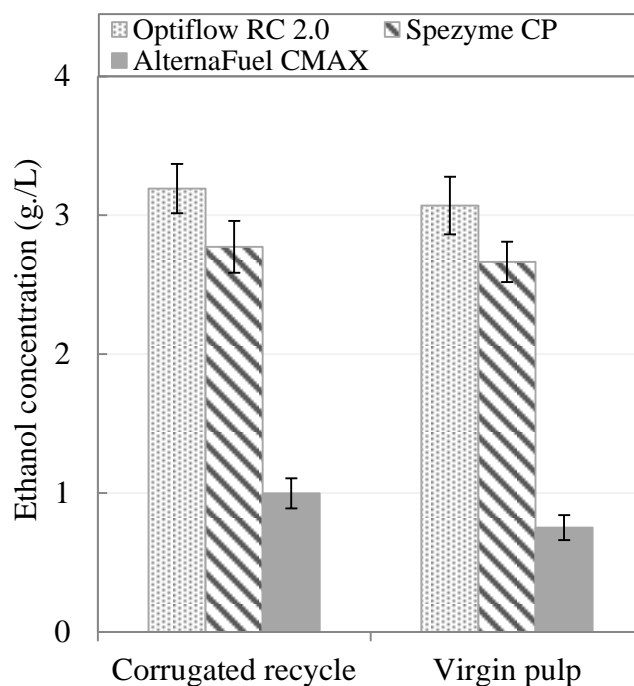


Figure 3.5: Ethanol concentration (g/L) obtained for the corrugated recycle and virgin pulp samples in cultures of *S. cerevisiae* strain MH1000 with cellulase enzymes Optiflow RC 2.0, Spezyme CP and AlternaFuel CMAX. The ethanol concentrations reported are the highest value measured after 168 h and the error bars indicate the standard deviation of triplicate runs.

3.3.5. Effect of sludge properties on ethanol production

Digestibility (Section 3.3.5.1), water holding capacity (Section 3.3.5.2) and viscosity (Section 3.3.5.3) are sludge properties that severely influence ethanol production with PS as feed. The effect of these properties on hydrolysis-fermentation with virgin pulp and corrugated recycle PS were investigated in the following sections.

3.3.5.1. Digestibility of paper sludge

The digestibility of the corrugated recycle and virgin pulp samples were studied (Figure 3.6) with enzymatic hydrolysis runs in serum bottles at enzyme dosages of 5, 15 and 25 FPU/gds and the

maximum possible solid loadings in batch culture of 3, 6 and 9% (w/w). The virgin pulp PS resulted in significantly greater glucose yields than that of the corrugated recycle PS with solid loadings of 3 to 9% (w/w) (Figure 3.6) and could be related to the severity of the pulping processes from which the fibers originated.

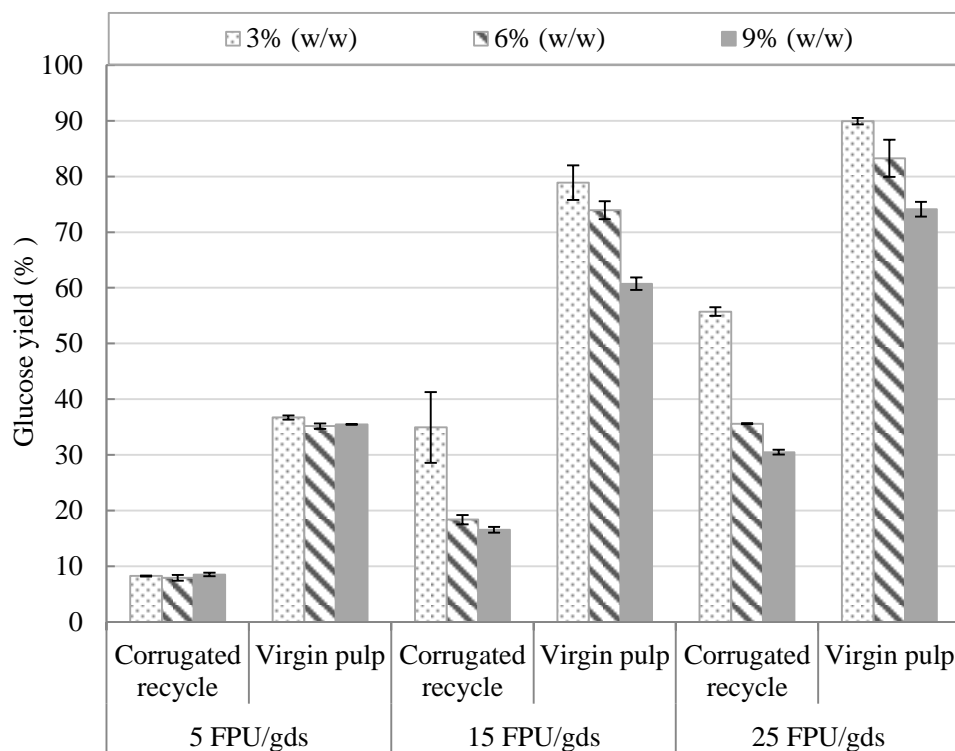


Figure 3.6: Glucose yield (%) at solid loadings of 3, 6 and 9% (w/w) and enzyme dosages of 5, 15 and 25 FPU/gds for corrugated recycle and virgin pulp PS. Glucose yield was measured at 72 h and the error bars indicate the standard deviation of triplicate runs.

The virgin pulp PS originates from a chemical pulping operation where virgin wood is chemically modified in the Kraft process to remove the lignin and separate the cellulose fibers. Corrugated recycle PS originates from the recycling and re-pulping of mainly corrugated material that consists of fibers originally separated by mechanical pulping. The fibers in the two sludges originate from two different pulping operations that employ two different methods to free the fibers from the lignin. In the chemical pulping process the lignin in the wood is dissolved, in a highly alkaline process, whereas in the mechanical process the lignin is not dissolved but only softened enough for the fibers to be separated by mechanical grinding or refining (Sundholm, 1999, Gullichsen & Fogelholm, 2000).

The fibers in the corrugated recycled PS is not as accessible to enzymes as the fibers in the virgin pulp PS, since a substantial amount of the lignin and hemicellulose still remain in the corrugated recycle PS (Gullichsen & Fogelholm, 2000). The remaining lignin and hemicellulose obstruct the working of the enzymes and thus result in poor digestibility and lower glucose yields when compared to the virgin pulp PS. The lower hydrolysis yields, due to poor digestibility, result in less available sugar for ethanol production. The pulping process is thus very important due to the direct impact it has on the amount of glucose and subsequent amount of ethanol that can be produced.

A decrease in glucose yield was observed with an increase in solid loading at all enzyme dosages although these differences were marginal with the enzyme dosages of 5 FPU/gds (Figure 3.6). It is expected that the glucose yield would continue to decrease at solid loadings higher than what was tested in this section and what would be required to reach the ethanol concentration threshold of 40 g/L. By feeding in a fed-batch manner, this problem can be partly addressed. The decrease in yield with increase in solid loading was previously reported for a range of feedstocks including olive tree biomass, pretreated corn stover, soft wood and wheat straw (Cara *et al.*, 2007; Jørgensen, Vibe-Pedersen, *et al.*, 2007; Kristensen *et al.*, 2009; Hodge *et al.*, 2008) and have been attributed to ineffective enzyme adsorption to the substrate (Kristensen *et al.*, 2009), improper mixing leading to poor mass and heat transfer (Palmqvist *et al.*, 2011) and enzyme inhibition due to the accumulation of glucose in the fermentation broth (Hodge *et al.*, 2008).

3.3.5.2. Water holding capacity and viscosity of paper sludge

The highly viscous slurries at increased solid loadings have various implications on a SSF process including ineffective mass and heat transfer and ineffective hydrolysis. The high viscosity also creates mixing difficulties at the high solid loadings required to achieve ethanol concentrations of 40 g/L. The WHC of corrugated recycle and virgin pulp PS was determined as 6.62 and 8.61 g water/g PS and was reduced to 2.55 and 4.54 g water/g PS, respectively, after fermentation.

A difference in fiber length could aid in explaining why WHC values for corrugated recycle operations were smaller than virgin pulping operations. Chemical pulping processes produce pulp with higher fiber lengths when compared to mechanical pulping processes, when utilizing the same wood (Pulp and paper resources and information, 2015). This can be explained by the grinding and refining that occurs in mechanical pulping. The fibers are damaged and shortened during mechanical pulping in the grinding and refining stages in which fibrillation takes place. Internal and external fibrillation is necessary for the fibers to be delaminated and the secondary wall to be

fibrillated (Sundholm, 1999). The longer fibers from virgin pulping operations will retain more water compared to the fibers from the corrugated recycling operations. This again points to the large impact pulping processes have on the fermentation process and the amount of ethanol that can be produced.

Fermentation significantly decreased the WHC resulting in moisture recovery of up to 80 and 150 L per ton of PS for virgin pulp and corrugated recycle PS, respectively. This water would otherwise have been disposed of through landfilling but is rather to be recycled back into the papermaking process. A decrease in the WHC of PS was also reported in a study where hydrolysis of PS with cellulase dosage of 5 FPU/gds resulted in a 65% decrease in the WHC of PS after 72 h (Lark *et al.*, 1997).

The intrinsic viscosity of the two sludges were tested at 25 °C and can be seen in Figure 3.7. The viscosity of corrugated recycle PS was tested at solid loading of 3-8% (w/w) (Figure 3.7A), while due to the highly viscous nature of the virgin pulp PS, viscosity measurements were only possible at solid loadings of 3-6% (w/w) (Figure 3.7B). The curves in Figure 3.7 indicate a pseudoplastic or sheer thinning fluid as PS had been found to be of a sheer thinning nature irrespective of substrate loading or cellulose conversion (Zhang *et al.*, 2009).

The viscosity curves for both sludges increased with an increase in substrate loading, while the virgin pulp PS (Figure 3.7B) resulted in viscosities that were between four and twenty times more than the corrugated recycle PS, at solid loadings of 3 and 6% (w/w), respectively. This indicates that viscosity is likely to restrict the solid loading possible with virgin pulp PS in a bioprocess, to a larger extent than with corrugated recycle PS. These results concurred with a previous report on the viscosity of PS where it was also found to increase as the solid loading increased (Fan & Lynd, 2007b).

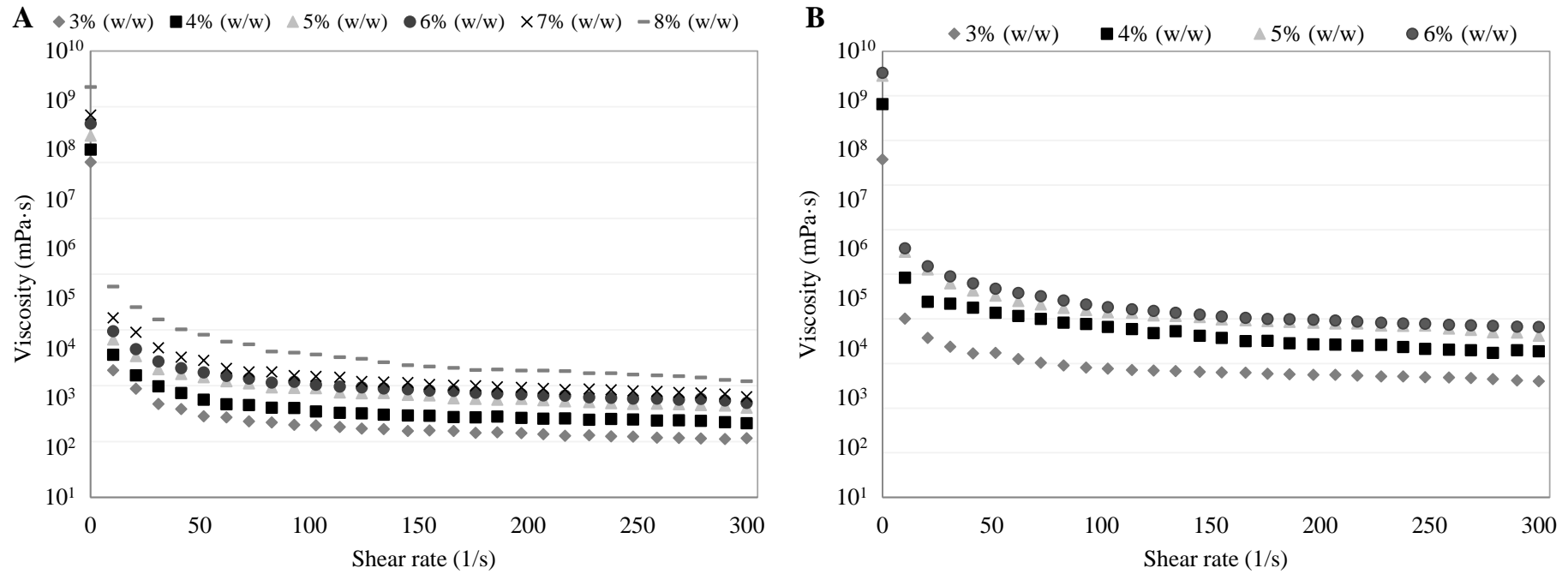


Figure 3.7: Viscosity values as a function of shear rate for corrugated recycle PS (A) at solid loadings of 3-8% (w/w) and virgin pulp PS (B) at solid loadings of 3-6% (w/w).

3.3.5.3. Effect of agitation rates on enzymatic activity

Agitation is used in fermentation to assist in mass and heat transfer and can be adjusted or increased to overcome viscosity at high solid loadings. However, the shear force at high stirring rates may impact cellulase activity and result in a decrease in enzyme activity due to high shear rates (Kaya *et al.*, 1994; Kadic *et al.*, 2014; Brethauer *et al.*, 2011). In order to investigate this, the change in activity of Optiflow RC 2.0 at increasing agitation rates was studied. The decrease in relative activity of Optiflow RC 2.0 for agitation rates of 150, 400 and 1500 rpm are given in Figure 3.8

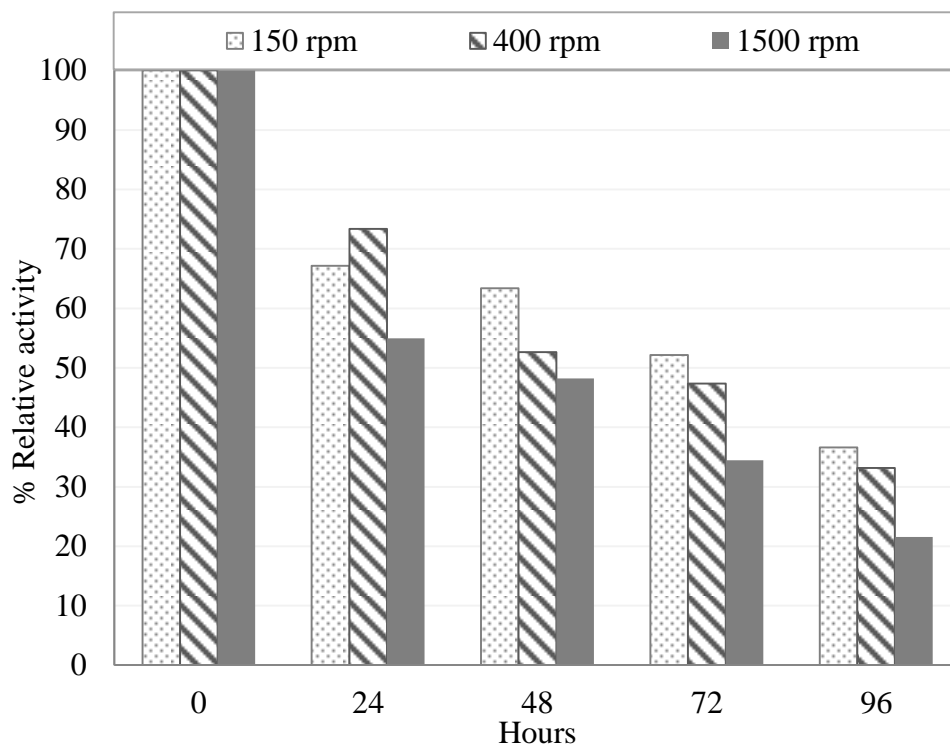


Figure 3.8: The relative activity of Optiflow RC 2.0 at agitation rates of 150, 400 and 1500 rpm.

The agitation rate and relative enzyme activity can be seen to be inversely proportional throughout the experiment with the exception of the first 24 h, where 400 rpm resulted in the highest relative activity. The highest agitation of 1500 rpm resulted in an average activity that was 15% less than the value at 150 rpm. Cellulase activity was also found to decrease over time even at the lowest agitation rate of 150 rpm indicating that enzymes become progressively inactive over time. These results indicated that higher agitation denatured the enzymes due to the larger shear force that was expressed on the enzymes and further suggested that high-viscosity PS is difficult to ferment, not only due to poor mass transfer, but also due to decreased enzyme activity when agitation is increased to overcome viscosity at high solids loading. Ganesh *et al.* similarly reported that the rate of enzyme deactivation was higher with increased agitation speed (Ganesh *et al.*, 2000).

3.3.6. Optimization with Central Composite Design: minimising enzyme dosage and maximizing solid loading

The preferred solids loading and enzyme dosage for each of the selected PS samples were determined by Response Surface Methodology (RSM) using Central Composite Design (CCD) as a tool, aiming to maximise solids loading and minimize enzyme dosage, as a means to achieve the desired final ethanol concentration in excess of 40 g/L. The properties of the two types of sludge influenced the process behaviour and optima. The CCD boundaries were determined by investigating the maximum solid loadings possible for each PS sample, based on the physical constraints due to increased viscosity with increased solid loadings. The corrugated recycle PS achieved a maximum solid loading of 33% (w/w) at a low enzyme dosage of 5 FPU/gds, while a maximum solids loading of 20% (w/w) was achieved with the virgin pulp PS at a significantly higher enzyme dosage of 25 FPU/gds. The high viscosity of the virgin pulp PS restricted the solid loadings above 20% (w/w) where additional feedings was no longer possible.

The response surface plots for fed-batch SSF with corrugated recycle PS as a feedstock are shown in Figure 3.9 with models for ethanol concentration (A), ethanol yield (B) and ethanol productivity (C), as well as the desirability plot (D). The models fit the data with R^2 values of 0.946, 0.857 and 0.829 for the concentration, yield and productivity, respectively, which indicated a relatively low degree of unexplained error and high suitability of the models to describe the data. The ethanol concentration model for the corrugated recycle PS (Figure 3.9A) indicated that a simultaneous increase in solid loading (% w/w) and enzyme dosage (FPU/gds) resulted in linear increases in ethanol concentration. However, a threshold enzyme dosage was apparent at 11 FPU/gds at the maximum possible solid loading of 33% (w/w) where the highest ethanol concentration of 53 g/L was obtained.

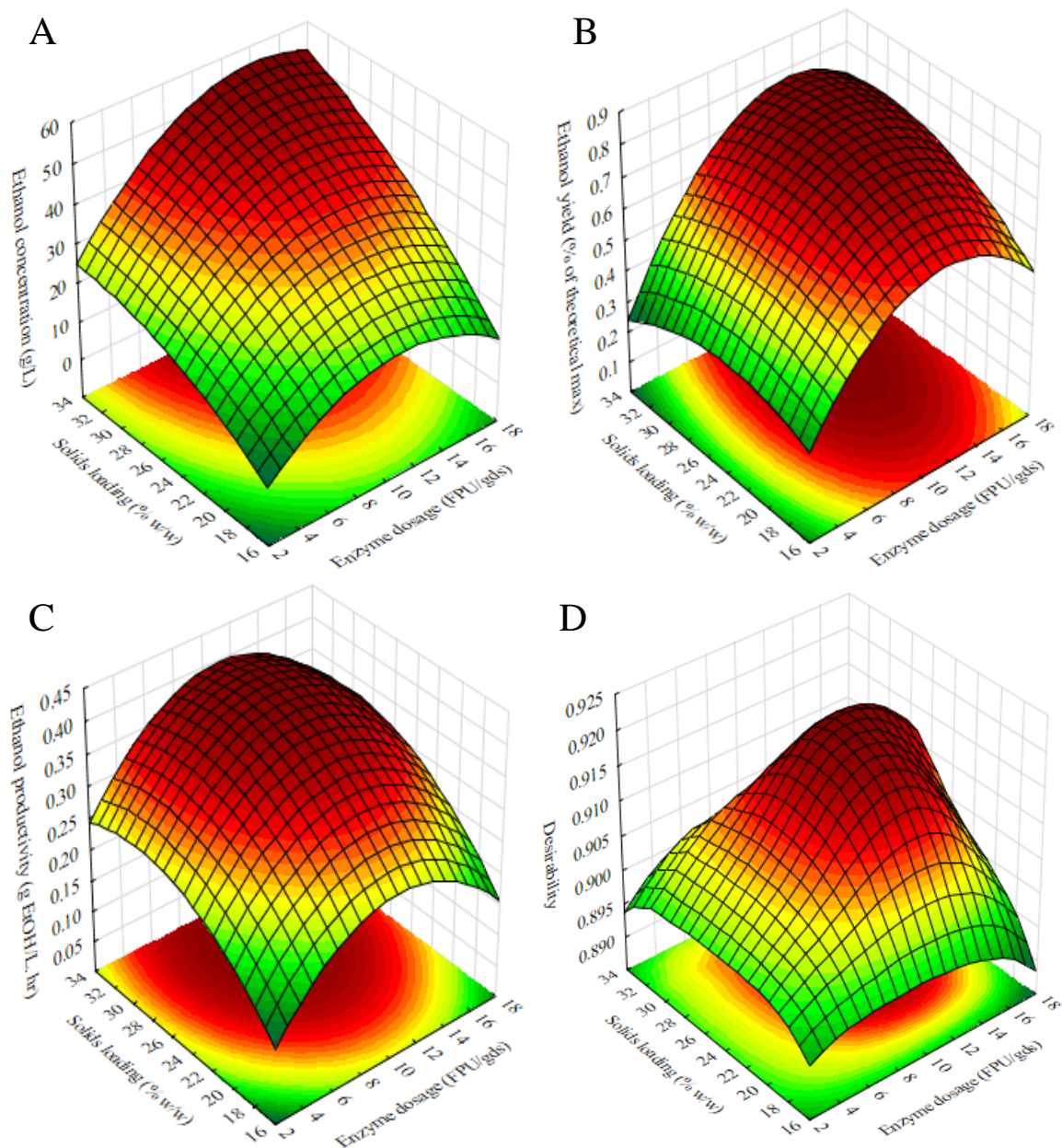


Figure 3.9: Surface plots predicting the final ethanol concentration in g/L (A), the ethanol yield as a percentage of the theoretical maximum (B), ethanol productivity in $\text{g.L}^{-1}.\text{hr}^{-1}$ (C) and desirability (D) of corrugated recycle PS with solid loading (% w/w) and enzyme dosage (FPU/gds) as the two independent variables.

The ethanol yield model (Figure 3.9B) indicated that an enzyme dosage of 11 FPU/gds was sufficient for substrate hydrolysis across the whole range of solid loadings tested, up to 34% (w/w), as evident from the ethanol yields that remained above 70% of the theoretical maximum and reached a maximum of 80% at 13 FPU/gds and 28% (w/w). This indicates that by feeding PS incrementally into the system, much higher yields were obtainable at much higher solid loadings than what was found in Section 3.3.5.1. Inefficient mixing could be the cause for the ethanol yield

levelling off at solid loadings greater than 28% (w/w), as well as irreversible binding of enzyme to the substrate (Jørgensen, Kristensen, *et al.*, 2007). The great fit between the model and the data is indicated by the high R^2 value of 0.857 whereas the validity of the yield model is indicated by the quadratic model p-value of 0.020471, which is noticeably smaller than 0.05. This coherently indicates the accuracy of this statistical model, however an incorrect assumption was made in the ethanol yield RSM at enzyme dosages higher than 14 FPU/gds. The design software showed the ethanol yield to decrease, where it actually levelled off when tested (Section 3.3.7).

The surface plot depicting ethanol productivity (Figure 3.9C) corresponded to that of the yield model at the optimum conditions where the maximum productivity was $0.404 \text{ g.L}^{-1}.\text{hr}^{-1}$. The desirability model (Figure 3.9D) combined all the dependant variables, ethanol concentration, yield and productivity models and resulted in the optimum enzyme dosage and solid loading of 11 FPU/gds and 27% (w/w), respectively. In this optimum region the models predicted the ethanol yield, final ethanol concentration and productivity to be 80.0%, 46.1 g/L and $0.41 \text{ g.L}^{-1}.\text{hr}^{-1}$, respectively.

High ethanol production at high solids loading and low enzyme dosage proved that corrugated recycle PS is a good potential substrate for bioethanol production via SSF fed-batch culture. In another study on shredded paper, a very high ethanol concentration of 91 g/L was achieved at very high solid loadings of 65% (w/v) at enzyme dosages as low as 3.7 FPU/gds in a process developed in 10 L bioreactors (Elliston *et al.*, 2013). However, the theoretical yield was 54% with an experimental time of 408 h resulting in a productivity of 0.22 g/L.hr . The low yield and extended reaction time are the drawbacks of this process to be industrially feasible. In another study an ethanol concentration of 53 g/L was obtained at a solid loading and enzyme dosage of 25% (w/w) and 10 FPU/gds, respectively (Robus, 2013). However, the PS feedstock was subjected to a washing step prior to fermentation that decreased the ash content with more than 45% (w/w) and increased the glucose concentration with more than 30% (w/w).

The response surface plots for the second chosen sludge, virgin pulp PS can be seen in Figure 3.10 and they are noticeably different from the corrugated recycle surface plots. Models were developed for ethanol concentration (A), ethanol yield (B) and productivity (C). The concentration and productivity models obtained R^2 values of 0.923 and 0.948, respectively, and the yield obtained a low R^2 value of 0.675, indicating a poor fit between the data and the yield model. In the ethanol concentration surface plot, the solid loading can be seen to affect the ethanol concentration more

severely than the enzyme dosage (Figure 3.10A). This indicates that the high viscosity of the virgin pulp PS obstructs the enzyme substrate interaction regardless of the enzyme dosage.

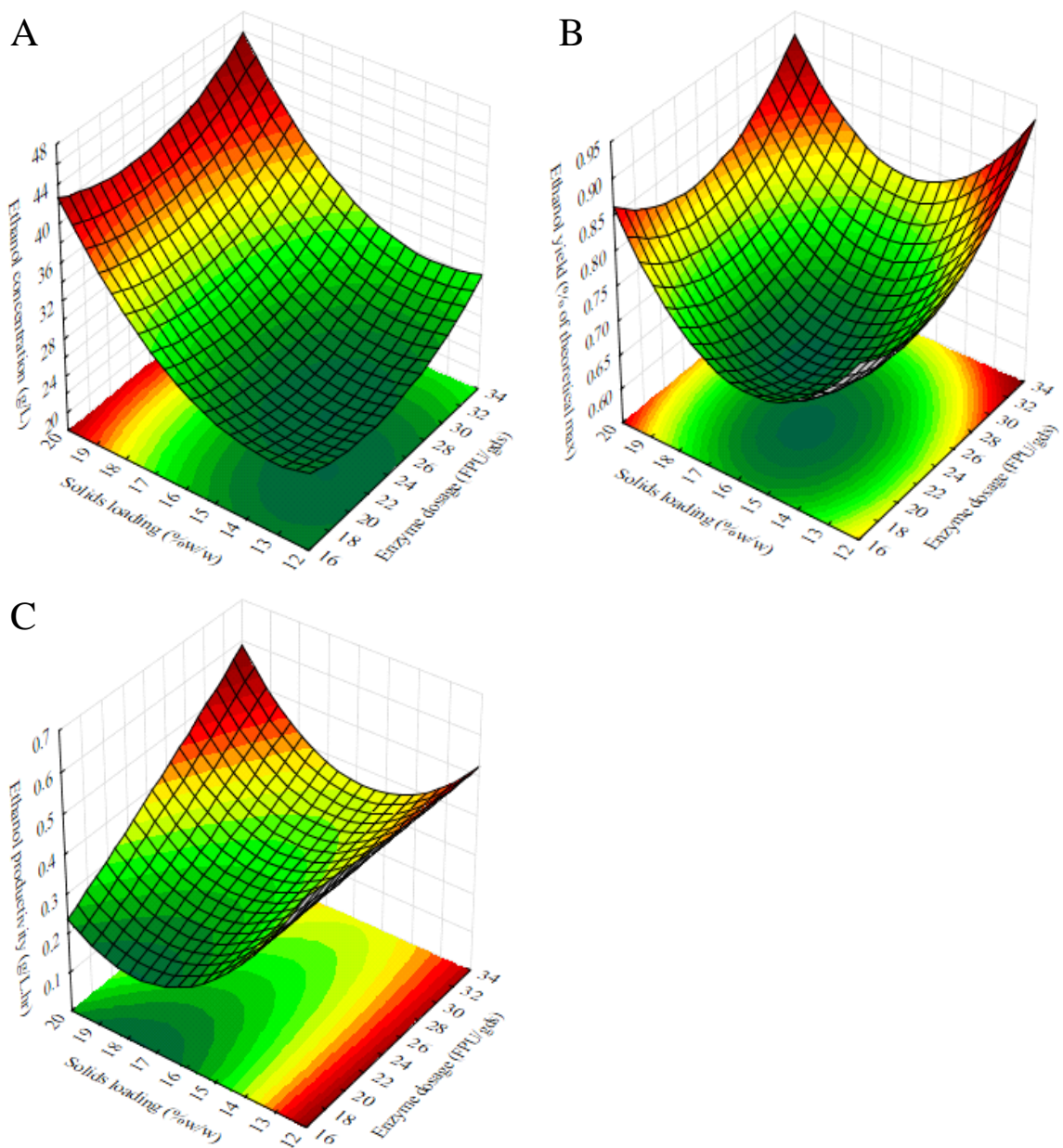


Figure 3.10: Surface plots for virgin pulp PS predicting the ethanol yield as a percentage of the theoretical maximum (A), the final ethanol concentration in g/L (B) and ethanol productivity in $\text{g.L}^{-1}.\text{hr}^{-1}$ (C) with solid loading (% w/w) and enzyme dosage (FPU/gds) as the two independent variables.

The ethanol yield surface plot (Figure 3.10B) showed an inverse parabolic shape indicating that the ethanol yield could not be optimised within this solid loading and enzyme dosage range. Reasons for this can be that the high viscosity levels encountered at the solid loadings tested, resulted in highly ineffective mixing and subsequently poor digestibility across all solid loadings

and enzyme dosages. However, the productivity surface plot (Figure 3.10C) showed to be significantly affected by both solid loading and enzyme dosage but resulted in a surface plot that did not aid in selecting the optimum conditions.

No response desirability profiling was used with virgin pulp PS due to the inaccurate fit of the ethanol yield model. The optimum values proposed by the ethanol concentration and productivity models are situated outside the borders of the CCD which indicates that these values were extrapolated and not based on actual experimental values as values higher than 19% (w/w) is not possible due to high viscosity levels. Therefore, in order to find the optimum range for a fed-batch SSF process with virgin pulp PS as feed, the surface plots were studied individually taking into consideration that 19% (w/w) solid loading is the highest value possible and the little effect the increased enzyme dosages had on the ethanol concentration (Figure 3.10A). The solid loading of 18% (w/w) and enzyme dosage of 20 FPU/gds was therefore chosen and the ethanol concentration and productivity models predicted values of 32.41 g/L and 0.22 g.L⁻¹.hr⁻¹.

The inherent digestibility of the virgin pulping PS was found to be higher than the corrugated recycle PS in Section 3.3.5.1 due to the difference in pulping processes, but due to the viscosity problems and accompanying mass transfer limitations, this was not realised in the bioreactors and resulted in noticeably smaller ethanol concentration and productivity than what was obtained with the corrugated recycle PS. The viscosity of the virgin pulp PS significantly limited the fermentation process by restricted effective mixing, even at the largest agitation rate of 1500 rpm that was used. This agitation rate resulted in the best possible mixing in this system and for this type of PS, although not completely effective, and decreased the activity of the enzymes due to the shear force of the blades.

3.3.7. Validation of statistical models and mass balances of optimised processes

Validation runs using optimum conditions predicted by the models differed by less than 2% from the experimental value for both the ethanol yield and concentration and 9% for the productivity whereas all the values for the virgin pulp PS, including the ethanol yield model that resulted in a poor fit, differed by not more than 6% (Table 3.2). The optimum conditions for corrugated recycle PS resulted in the same final ethanol concentration of 5.7% (v/v) that was found by Kang *et al.* (Kang *et al.*, 2010), but with 10% higher ethanol yields. Similar processes performance in bioreactors for conversion of paper-related feedstocks into ethanol has not been reported.

Table 3.2: Mass balance for the CCD model validation runs for the corrugated recycle and virgin pulp PS at an enzyme dosage of 11 and 20 FPU/gds and solid loading of 27 and 18% (w/w), respectively.

Experimental values	Units	Corrugated recycle	Virgin pulp
Enzyme dosage	FPU/gds	11	20
Mass dry PS fed	g/L	270	180
Percentage dry PS fed	% (w/w)	27	18
Glucose fraction ¹	%	42.24	55.71
Xylose fraction ¹	%	14.16	16.80
Total glucose fed ²	g/L	114.0	100.3
Glucose in residue ³	g/L	24.4	28.9
Soluble residual glucose ⁴	g/L	0	2.4
Total glucose consumed ⁵	g/L	89.6	69.0
Conversion of total cellulose ⁶	%	78.6	68.9
Total xylose fed ²	g/L	38.2	30.2
Xylose in residue ³	g/L	12.0	13.5
Soluble residual xylose ⁴	g/L	23.2	15.3
Total xylose lost ⁷	g/L	3.0	1.4
Percentage of initial xylose lost ⁸	%	8.0	4.8
Ethanol concentration ⁴	g/L	45.5	34.2
Theoretical ethanol yield ⁹	%	78.2	66.9
Productivity ¹⁰	g.L ⁻¹ .hr ⁻¹	0.448	0.230
Ethanol yield ¹¹	g ethanol/g glucose consumed	0.508	0.495
Overall ethanol yield ¹²	kg Ethanol/ton dry PS	168.6	190.0
Predicted values ¹³			
Total ethanol produced	g/L	46.05	32.4
Total ethanol yield	%	80.0	69.8
Productivity	g.L ⁻¹ .hr ⁻¹	0.408	0.222

¹ Fractions as given in Figure 3.2.

² [Fraction * Mass dry PS fed].

³ Determined from fermentation residue.

⁴ Determined from fermentation broth with HPLC.

⁵ [Total glucose fed – Glucose in residue – Soluble residual glucose].

⁶ [Total glucose consumed/Total glucose fed].

⁷ [Total xylose fed – Xylose in residue – Soluble residual xylose].

⁸ [Total xylose lost/Total xylose fed].

⁹ [Ethanol concentration/ (Total glucose fed * 0.51)].

¹⁰ Determined from the ethanol profile where the ethanol production levelled off.

¹¹ [Ethanol concentration/Total glucose consumed].

¹² [1000 kg dry PS * Glucose fraction * Conversion of total cellulose * Ethanol yield].

¹³ Determined from the CCD models.

Based on mass balances of the model validation runs (Table 3.2), the corrugated recycle PS resulted in a 33% higher final ethanol concentration, 11.3% higher yield and 54% higher productivity than the virgin pulp PS. Furthermore, the maximum concentration and yield with corrugated recycle PS was attained at 1.5 times higher solids loading and 1.8 times lower enzyme dosage of 11 FPU/gds. These results collectively suggest that a process with corrugated recycle PS provides a higher process performance for fed-batch SSF bioethanol production than virgin pulp PS and that the nature of the PS feedstock will directly affect the process performance due to the digestibility, viscosity and WHC of the sludge.

The overall ethanol yield for the virgin pulp PS (190.0 kg ethanol/ton dry PS) was higher than the corrugated recycle PS (168.6 kg ethanol/ton dry PS) due to the larger fraction of cellulose in the former. Although *S. cerevisiae* MH1000 does not possess xylose utilizing capabilities, the fermentation of xylose to ethanol could increase the overall ethanol yield from 168.6 and 190.0 kg to 185.12 and 202.35 kg ethanol/ton dry PS for corrugated recycle and virgin pulp PS, respectively, assuming an ethanol yield on xylose of 80% of the theoretical maximum. The mass balance showed a xylose loss of 8.0% and 4.8% for corrugated recycle and virgin pulp PS, respectively, which could be attributed to an analytical inaccuracy caused due to the very low concentration values or utilization in other reactions.

The processes were optimized in different solids loading and enzyme dosage boundaries due to the distinct difference in the properties of the two PS samples, but to investigate how the sludges would compare under similar conditions, corrugated recycle PS was subjected to the optimum conditions for the virgin pulp PS (Figure 3.11). Performance was the same for both the sludges in the first 24 h, but later virgin pulp PS exhibited slightly higher ethanol concentrations and levelled off after 96 h, whereas ethanol concentration for corrugated recycle constantly increased until 168 h without levelling off (Figure 3.11A). Yield decreased for both samples after 24 h and could possibly be due to large fed-batch feedings too frequently, although it did not affect the corrugated recycle PS final yield that was able to reach the maximum before 168 h (Figure 3.11B) indicating that the ethanol yield RSM model had incorrectly assumed the drop in ethanol yields at enzyme dosages higher than 12 FPU/gds. The ethanol concentration and yield from the virgin pulp PS levelled off right after the last feeding indicating that the viscosity was too high to result in further hydrolysis.

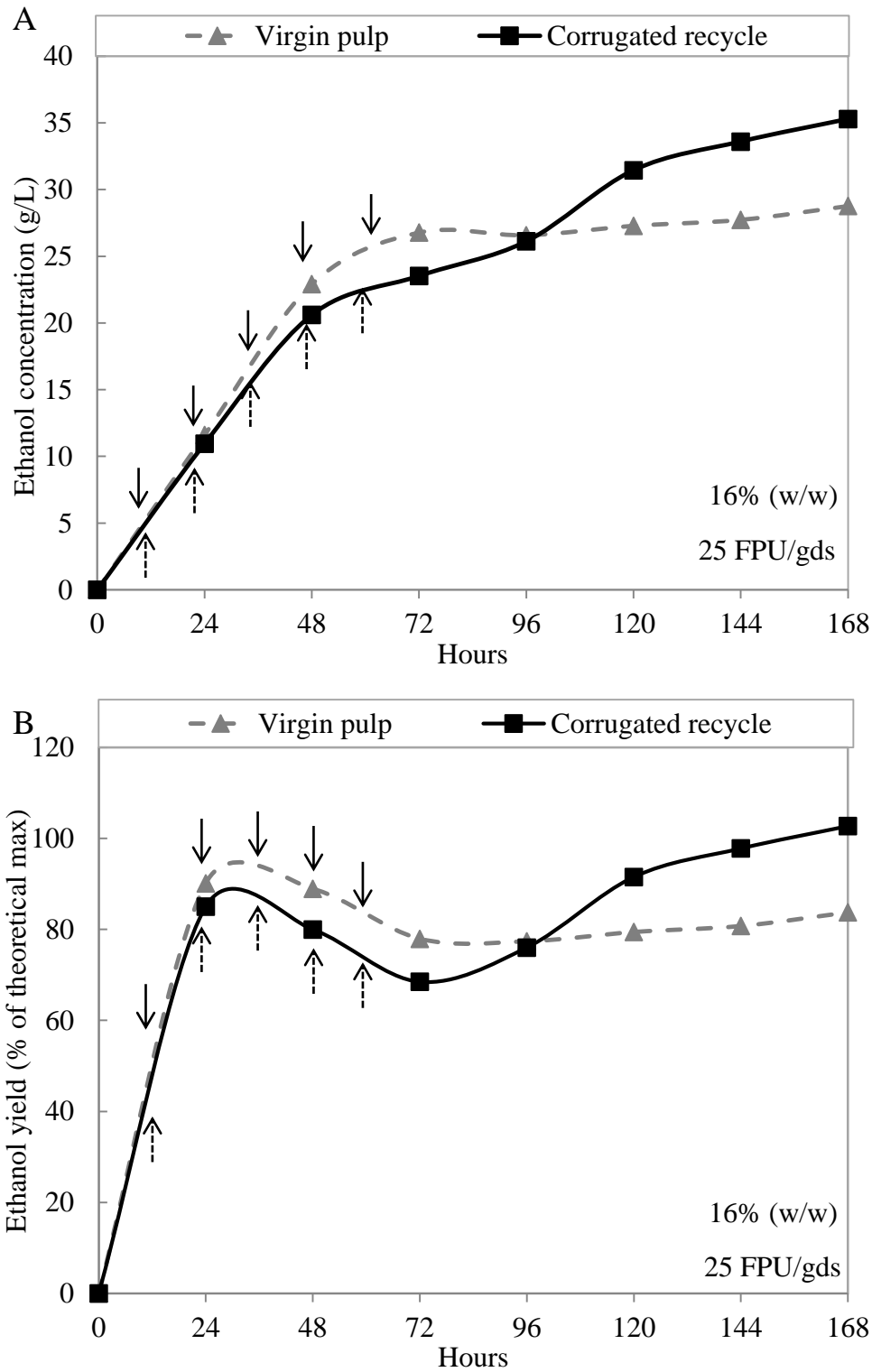


Figure 3.11: Comparative runs done with corrugated recycle (squares) and virgin pulp PS (triangles) at an enzyme dosage of 25 FPU/gds and a solid loading of 16% (w/w). The ethanol concentration can be seen in A and the ethanol yield can be seen in B. Feedings were done in 3% intervals with the last feeding of only 1% and are indicated with arrows.

Chapter 4: General conclusions and recommendations

The main aim of this study was to investigate how the nature of various types of paper sludges can influence the process performance of a paper sludge to bioethanol process and to establish how these properties can be exploited to aid in accurately designing an industrial process. This was done by collecting waste paper sludge samples from various mills in South Africa, determining the chemical composition and screening for ethanol production. From the chemical composition and screening data two samples were chosen for optimization and the processes were studied to determine how they varied due to the nature of the sludges.

4.1. Conclusions

The conclusions from this study are given below with reference to the Aims and objectives given in Section 2.5.

i) Characterization of PS samples

The composition of PS is not typical or constant as it varies greatly between mills, with the large difference in chemical composition adding to the challenge of working with PS as substrate. The chemical composition of 37 PS samples were found to vary significantly for all samples and components. The glucose fraction varied from 11.24 to 49.86 g/g substrate whereas the ash fraction varied even more from 8.54 to 61.97 g/g substrate. The samples were characterized into 4 categories namely Printed recycle tissue paper mills, No recycle paper mills, Corrugated recycle paper and pulp mills and Virgin pulp and paper mills according to the feed used at each mill. The difference in the chemical composition was found to be less significant within the categories and least significant within samples for a particular mill.

ii) Screening for ethanol production

High theoretical ethanol concentrations did not necessarily result in high experimental ethanol concentrations due to the difference in digestibility of the samples at the low solid loadings used for screening. The PS samples were screened at enzymes dosages of 5 and 15 FPU/gds and obtained significantly different ethanol concentrations. Ethanol concentrations at 15 FPU/gds were significantly higher than the value obtained at 5 FPU/gds or not significantly different at all.

iii) *Selection of PS samples for optimization*

Through the characterization and screening of a variety of samples, it was possible to select sludges that showed great potential for bioethanol production, evident from above average ethanol concentrations. Sludges from two mills in different categories were chosen for optimization. Sappi Ngodwana was chosen from the Virgin pulp and paper mill category and Mpact Springs was chosen from the Corrugated recycle paper and pulp mill category as the second mill. Both samples obtained average ethanol concentrations that were above the average for all the mills and had a sufficient organic fraction in the fermentation residue to be proposed as a feedstock for pyrolysis or biogas production.

iv) *Screening and selection of optimum enzyme cocktail and microorganisms*

It is important to select the correct enzyme cocktail and yeast strain for bioethanol production to be maximized. From the available microorganism and enzymes, *S. cerevisiae* MH1000 and Optiflow RC 2.0, respectively, resulted in the highest ethanol concentration for the selected PS samples and were used during optimization.

v) *Factors influencing SSF fed-batch processes*

Digestibility, water holding capacity, viscosity and shear stress associated with high agitation rates were identified as major factors that influenced the fermentation of paper sludge to ethanol at high solid loadings. Whereas a high degree of digestibility is crucial for attaining desired concentration, yield and productivity, the water holding capacity and viscosity of the sludge, which are inherent characteristics of the material, limits the solids loading and hence, production performance per run. High viscosity also influences digestibility through physical constraints for enzyme access. Furthermore, high viscosity requires high agitation rates, which appear to have negatively affected enzyme activity.

The high agitation rates used with virgin pulp PS to overcome high viscosity levels reduced the enzyme stability, due to high shear stress of the blades on the cellulase. The digestibility of virgin pulp PS was found to be superior to corrugated recycle PS at solid loadings between 3-9% (w/w) but corrugated recycle PS was found to be superior at solid loadings of 16 (w/w) and higher. The water holding capacity and viscosity of the virgin pulp PS was significantly higher than corrugated recycle PS due to the fibers in virgin pulp PS originating from chemical pulping compared to fibers in corrugated recycle PS originating from mechanical or repulping operations pulping. SSF decreased the water holding capacity of both sludges significantly indicating that more water could be recycled back into the papermaking process.

vi) *Fed-batch SSF process development*

The significant difference in the two optimum processes indicate how severely the nature of the sludge can influence a SSF bioethanol process. The optimised process for the virgin pulp PS resulted in an ethanol concentration and yield of 34.2 g/L and 66.2%, respectively, at a solid loading of 18% (w/w) and an enzyme dosage of 20 FPU/gds with a productivity of 0.23 g.L⁻¹.hr⁻¹. The optimised process for the corrugated recycle PS was at a significantly higher solid loading and lower enzyme dosage of 27% (w/w) and 11 FPU/gds, respectively. An ethanol concentration and yield of 45.5 g/L and 78.2% was obtained with a productivity of 0.448 g.L⁻¹.hr⁻¹. Validation runs indicated that the models developed with CCD as a tool predicted values that differed no more than 9 and 6% with the experimental value for corrugated recycle and virgin pulp PS, respectively, indicating the models to be accurate for process development.

Summary

Corrugated recycle PS is more suitable for bioethanol production than virgin pulp PS. Corrugated recycle PS achieved higher solid loadings at lower enzyme dosages and resulted in higher ethanol concentration, yield and productivity. This is due to corrugated recycle mills feeding mainly recycled material, resulting in PS that has been pulped multiple times. This resulted in lower water holding capacity and viscosity and higher digestibility at solid loadings that is viable for industrial application.

4.2. Recommendations

Production of cellulase from PS

The production of cellulase from PS could significantly decrease bioethanol production costs as enzymes are known to contribute greatly to the total operating expenses. A previous study reported that cellulase can be produced from PS by *Acremonium cellulolyticus* C-1 and used for SSF ethanol production (Prasetyo and Park, 2013). Ethanol production was estimated at 51.1 kg from 1000 kg PS using the cellulase produced with PS as carbon source and compared to a commercial *Acremonium* cellulase, the reduced sugar concentration was not significantly different.

Biorefinery approach

In order to fully utilize PS as a feedstock, an integrated process system or biorefinery must be considered (Ohara, 2003). Integrating a bioethanol process with either a pyrolysis or a biogas production process using the fermentation residue as feed could offer promising results and could add further value. (Kemppainen *et al.*, 2012; Dalwai, 2012).

Optimize feeding size

Fed-batch feedings of 3% (w/w) was used as a starting point to study a generic PS to ethanol process via SSF. But some of the results indicated that overfeeding might have occurred with a 3% (w/w) addition every 12 h. Smaller fed-batch feedings added more often might improve mixing efficiency, hydrolysis yields and total ethanol produced. It is noted that a feeding regime closest to a continuous system would be best.

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