Paper industry process wastewater reclamation and potential clarification from paper sludge through integrated bio-energy production

by

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ABSTRACT

The paper and pulp industry is one of the major consumers of fresh water and as such produces large quantities of contaminated process water. However, with the recent drought crisis in South Africa, there has been a growing need amongst the paper and pulp community to reduce their water footprint. One potential strategy is to reclaim water from the paper waste sludge. Paper waste sludge (PS) consists of high amounts of cellulose and ash, with about 50 to 80% moisture content. Bioprocessing methods such as fermentation and anaerobic digestion with clean water have been reported to convert paper sludge into bioenergy thereby avoiding the urge of establishing a close water loop system. Also very little information on the potential of bioprocessing technologies to recover entrapped water molecules in paper sludge have been reported. In this study, a sequential fermentation and anaerobic digestion model using process water (COD > 2000 mg/L) as make up stream was explored to ascertain the potential of water reclamation from paper sludge while simultaneously producing bioenergy.

Three paper waste sludges, i.e. virgin pulp, corrugated recycle and tissue printed recycle with their corresponding process water samples were utilized in this study. All the sludges and their process waters were obtained from the primary clarifiers of pulp mills. Fermentation and anaerobic digestion performances in terms of energy production were the same when using clean water and recycled process water in screening experiments. Paper sludge conversion to ethanol by fermentation, as performed in bioreactors, could reclaim in excess of 80% of the water present in the solids initially, but simultaneously increased the COD of the reclaimed process water from 4 780 mg/L to 86 800 mg/L. Alternatively, anaerobic digestion applied to similar paper sludge and process water samples could reclaim about 50% of water from paper sludge solids, and achieved a 20% to 40% reduction of COD in reclaimed process water.

The proposed model of sequential bioprocessing of paper waste sludge through fermentation and anaerobic digestion achieved water reclamation similar to that obtained by the fermentation process but also increased the process water COD from 4 780 mg/L to 72 500 mg/L. In addition to water reclamation, the sequential bioprocessing of paper sludge produced about 20% to 60% more bioenergy than the fermentation or anaerobic digestion could achieve by themselves. Fermentation accounted for about 50% to 80% of the bioenergy produced in the combined process; for example, fermentation of

virgin pulp paper sludge gave the highest ethanol yield of 275.4 kg ethanol/ton dry PS; which accounted for 80% of the total product energy (10 650 MJ/kg ton PS). Although corrugated recycle produced a lower ethanol yield (152.2 kg ethanol/ton dry PS) as compared to virgin pulp, the fermentation residues were better suited for anaerobic digestion, which contributed 50% of the total product energy (9 288 MJ/kg ton PS). Moreover, anaerobic digestion of fermented stillage had the added benefit of a short (5 to 10-days) biogas production period.

In conclusion, sequential biochemical processing of paper sludge as compared to individual processes was better in maximizing both bio-energy and water reclamation. Alternatively, the sequential process considerably worsened the COD of the reclaimed water. Consequently, the water reclaimed is not immediately reusable without further wastewater treatment. The sequential approach was also able to significantly reduce the amount of solid waste which also showed promising applications in the agricultural and industrial sector.

KEYWORDS

Paper sludge

Recycled process water

Fermentation

Anaerobic digestion

Sequential bioprocessing

Bioethanol

Biogas

OPSOMMING

Die papier- en pulpindustrie is een van die grootste verbruikers van vars water en produseer as sulks groot hoeveelhede gekontamineerde proseswater. Met die onlangse droogtekrisis in Suid-Afrika, is daar egter 'n groeiende behoefte in die papier- en pulpgemeenskap om hul watervoetspoor te verminder. Een potensiële strategie is om water uit die paperafvalslyk te herwin. Paperafvalslyk (PS) bestaan uit hoë hoeveelhede sellulose en as, met omtrent 50% tot 80% voginhoud. Bioprosesseringmetodes soos fermentasie en anaerobiese vertering met skoon water is berig om paperslyk in bio-energie om te kan skakel, wat daardeur die behoefte vir 'n geslote lus waterstelsel vermy. Daar is ook baie min informasie oor die potensiaal van bioprosseseringtegnologië om vasgevange watermolekules in papierslyk te herwin. In hierdie studie is 'n sekwensiële fermentasie en anaerobiese vertering model wat proseswater (COD > 2000 mg/L) as aanvullingsstroom ondersoek om die potensiaal van waterherwinning uit papierslyk vas te stel terwyl bio-energie gelyktydig vervaardig word.

Drie papierafvalslyke, i.e. nuutpulp, geriffelde herwinning en tissue-gedrukte herwinning met hul ooreenstemmende proefsteke van proseswater, is gebruik in hierdie studie. Beide die slyke en hul proseswater is verkry deur die primêre verhelderaar van pulpmeule. Fermentasie en anaerobiese vertering doeltreffendheid in terme van energie produksie was dieselfde toe skoon water en herwinde proseswater in siftingseksperimente gebruik is. Papierslykomsetting na etanol by fermentasie, soos gebruik in bioreaktors, kon aanvanklik 'n oormaat van 80% van die water teenwoordig in vastestowwe herwin, maar het gelyktydig die COD van die herwinde proseswater van 4 780 mg/L na 86 800 mg/L verhoog. Alternatiewelik het anaerobiese vertering toegepas op soortgelyke slyk en proseswaterproefsteke omtrent 50% van water uit papierslyk vastestowwe herwin, en 'n 20% tot 40% vermindering van COD in herwinde proseswater bereik.

Die voorgestelde model van sekwensiële bioprosessering van papierafvalslyk deur fermentasie en anaerobiese vertering het waterherwinning bereik soortgelyk aan dié verkry deur die fermentasieproses maar het ook die proseswater COD van 4 780 mg/L na 72 500 mg/L verhoog. Buiten waterherwinning het die sekwensiële bioprosesering van papierslyk omtrent 20% tot 60% meer bioenergie vervaardig as wat die fermentasie of anaerobiese verteerder op hul eie kon bereik. Fermentasie was verantwoordelik vir omtrent 50% tot 80% van die bio-energie vervaardig in die gekombineerde proses. Byvoorbeeld, fermentasie van nuutpulppapierslyk het die hoogste etanol

opbrengs van 275.4 kg etanol/ton droeë PS gegee, wat rekenskap gee vir 80% van die totale produkenergie (10 650 MJ/kg ton PS). Alhoewel geriffelde herwinning ’n laer etanol opbrengs gegee het (152.2 kg etanol/ton droë PS) in vergelyking met nuutpulp, was die fermentasie residu’s meer geskik vir anaerobiese vertering, wat 50% van die totale produk energie (9 288 MJ/kg ton PS) bygedra het. Buitendien, anaerobiese vertering van gefermenteerde steier het die ekstra voordeel van ’n kort (5 tot 10 dae) biogas produksie periode.

Ten slotte, sekwensiële biochemiese prosessering van papierslyk soos vergelyk met individuele prosesse, was beter om beide bio-energie en waterherwinning te maksimeer. Alternatiewelik het die sekwensiële proses die COD van die herwinde water aansienlik vererger. Gevolglik is die water wat herwin is nie onmiddellik bruikbaar sonder verdere afvalwaterbehandeling nie. Die sekwensiële benadering het ook die hoeveelheid vastestofafval beduidend verminder, wat belowende toepassings vir die landbou- en industriële sektore inhou.

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ACRONYMS & ABBREVIATIONS

Abbreviation	Description
AD	Anaerobic digesters
ANOVA	Analysis of variance
AOX	Adsorbale organic halides
BMP	Biomethane potential test
BOD	Biological oxygen demand
C/N	Carbon to nitrogen ratio
CBP	Consolidated bioprocessing
CR-PS	Corrugated recycle paper sludge
CR-PW	Corrugated recycle dirty process water
CEPPWAWU	Chemical, energy, paper, printing, wood and allied workers' union
COD	Chemical oxygen demand
CSD	Continuously stirred digester
ECF	Elemental chlorine free
HMF	5- hydroxymethylfurfural
HPLC	High Performance Liquid Chromatography
HRT	Hydraulic retention time
LAB	Lactic acid bacteria
NREL	National renewable energy laboratory
NSSC	Neutral sulfite semi chemical
OLR	Organic loading rate
PAMSA	Paper making association of south africa
PW/CW	Processed wastewater to clean water ratio
PS	Paper/primary sludge
PW	Recycled process wastewater
RCF/RPF	Recycle pulp fiber
SCFA	Short chain fatty acids
SHF	Separate (enzymatic) hydrolysis and fermentation
SS	Suspended solids
SSF	Simultaneous saccharification and fermentation

TCF	Total chlorine free
TSS	Total suspended solids
TAN	Total ammonia nitrogen
TPR-PS	Tissue printed recycle paper sludge
TPR-PW	Tissue printed recycle dirty process water
TS	Total solids
VFA	Volatile fatty acid
VP-PS	Virgin pulp paper sludge
VP-PW	Virgin pulp dirty process water
VS	Volatile solids
WHC	Water holding capacity

GLOSSARY

Acclimation. Temporary biological adjustments that happen during an organism's lifetime in response to ephemeral changes in environmental conditions

Adaptation. The development of genetic change that accumulates over a time scale of many generations in response to an organism's specific environmental niche.

Biological Oxygen Demand. The measure of the amount of oxygen used by microorganisms in the oxidation of organic matter.

Chemical Oxygen Demand. This value determines the relative oxygen requirement needed for the oxidation of all organic substances in wastewater.

Free water. Water not bounded to or trapped in fibre.

Mesophilic. Microbes growing best at temperature range within 30-40 °C.

Osmotic pressure. The applied pressure needed in a solution to prevent the inward flow of water across a semipermeable membrane of an organism.

Sequential biochemical processing. Sequential fermentation and anaerobic digestion

Thermophilic. Microbes growing best at temperature range within 50-60 °C.

Total ammonia nitrogen. The total amount of nitrogen in the forms of NH_3 and NH_{4+} in digester.

Total solids. The material residue left in a vessel after evaporation of a sample and its subsequent drying in an oven at a defined temperature.

Total suspended solids. The portion of total solids retained by a filter.

Volatile solids. The solids in a sample lost on ignition of dry solids at 550 °C.

Water reclaimed or water recovered. The amount of water recovered from bioprocessing of paper sludge. Water reclamation was based on the principle that, the treated substrate retained a lower water holding capacity compared to that of the original substrate.

THESIS OUTLINE

Chapter 1: **Introduction**. This chapter gives the background and a context to this study.

Chapter 2: **Literature review**. This chapter presents literature on paper sludge and process water production from pulp industries in South Africa and furthermore discusses bioprocessing of paper sludge. Biochemical processes such as fermentation and anaerobic digestion are reviewed relating to effects of key parameters such as enzyme dosage and solids loading.

Chapter 3: **Research methodology**. Experimental methods applied in sequential fermentation and anaerobic digestion are discussed in this chapter. Whereas analytical methods employed in this study are explained also in this chapter.

Chapter 4: **Results and discussion**. This chapter presents and discusses findings from experimental work in relation to the outlined research aims and objectives.

Chapter 5: **Conclusions and recommendations**. Conclusion based on study findings are outlined in this chapter with recommendations for future work.

CHAPTER 1: BACKGROUND

1.1 INTRODUCTION

The present project addresses the possibility of reclaiming process water from paper waste sludge through integrated bio-energy production. In South Africa, approximately 500 000 wet tons of paper sludge is generated every year by the Paper Making Association of South Africa (PAMSA) (Boshoff *et al.*, 2016). Of the 500 000 wet tons produced, the entrapped moisture content ranges from 50%-70% depending on the pulp and paper mill (Boshoff *et al.*, 2016).

Previous studies by Robus *et al.* (2016), Boshoff *et al.* (2016) and Williams (2017) have established that bio-energy technologies, such as fermentation and anaerobic digestion, can convert the carbohydrates present in paper sludge, to bioethanol or biogas, while simultaneously reducing the water holding capacity of the solids. The reduction in solids content and its holding capacity should result in the release of the entrapped water molecules in paper sludge, thus providing potential for reclamation of this water. However, these former studies utilise clean water as make-up for the bioconversion of paper sludge, which is an unattractive option that increases the amount of wastewater generated. Water is added to paper sludge to obtain a slurry suitable for fermentation and/or biogas production. The possibility of employing process water discharged from primary clarifiers as make-up water for both fermentation or biogas production and thus possibly clean-up the process water for recycling is an issue which needs to be investigated. There aren't any reported literature on the usage of recycled process water in fermentation or anaerobic digestion of biomass substrate. Hence there could be downsides to the usage of process water, as process water contains inhibitory compounds such as lignosulfonic acids, resin acids and phenolic compounds that can adversely affect microorganisms (yeast and anaerobic bacteria) in fermentation or anaerobic digestion of paper sludge. Thus, this present study seeks to investigate and optimise water reclamation through application of fermentation and anaerobic digestion of paper sludge, with recycled process water as make-up stream while simultaneously avoiding the use of freshwater. The quality of the reclaimed wastewater is a key consideration to determine the effectiveness of bio-energy processes as a water treatment strategy. Key research question relating to the gap in literature are discussed in section 2.8.1. Out of these key research questions, objectives relating to this study were formulated in section 2.8.2.

The main objective of this study is to maximise the potential to reclaim industrial waste water for re-use, the quality of the reclaimed water and the amounts of bio-energy produced. Fermentation and anaerobic digestion are used individually and sequentially to determine the potential of water reclamation from paper sludge. Another key objective is the use of recycled process water in fermentation and anaerobic digestion of paper sludge which is explored in terms of energy production and its effect on bioprocessing microorganisms.

1.2 HYPOTHESIS

1. Fermentation, anaerobic digestion or the combination of both bioprocesses would lead to water reclamation from paper sludge.
2. Sequential bioprocessing of paper sludge would produce more bioenergy than standalone fermentation or anaerobic digestion.

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

Paper sludge (PS) is a major source of landfilled waste from the pulp and paper industry which currently has no major eco-friendly solution. Considerable amounts of reclaimable water is lost in landfilling of paper sludge due to its high moisture content. Apart from landfilling of paper sludge, the industry discharges potentially reusable process water into the environment. Various South African paper sludge tested by Williams (2017) and Boshoff *et al.* (2016) showed a significant decrease in water holding capacity of the original paper sludge after fermentation and anaerobic digestion. The reduced amount of residual solids together with decrease in WHC capacity shows a potential for water reclamation from paper sludge. The recovery of entrained water in paper sludge through fermentation or anaerobic digestion produces ethanol and methane. Both methane and ethanol are valuable biofuels but there is a possibility that the aforementioned bioprocess can either worsen or improve the quality of reclaimed. Therefore, apart from water reclamation, this study would also assess the impact of both anaerobic digestion and fermentation process on the quality of water reclaimed.

2.2 THE SOUTH AFRICAN PULP AND PAPER INDUSTRY

2.2.1 Raw material for pulp production

The raw material supply for the South African pulp and paper industry is indicated **Table 2-1** below. The South African Pulp and paper Industry production totalled between 2.1 million tonnes to 2.7 million tonnes per year within 2001 to 2011 (PAMSA, 2012). Pulpwood is the primary fibre source and is supplemented with sugarcane bagasse, forest and milling residues (CEPPWAWU, 2004). Pulpwood can be either hardwood or softwood that can be employed in the manufacturing of different grades of paper. Pine is the commonly used softwood in South Africa to fulfil strength and bulk requirement in produced (paper largely newsprint, magazine and packaging grades). Eucalyptus on the other hand is the main source of hardwood fibre used in making high strength corrugated paper and board (CEPPWAWU, 2004). Recycled fibre is another important source of raw material for pulp and paper production. As a result, the South African pulp and paper industry has established mechanisms regarding its collection and recycling.

Table 2-1: Raw material Supply for the Pulp and Paper Industry (CEPPWAWU, 2004)

Fibrous Raw Material	% Supply to the Industry
Hardwood	50
Softwood	39
Recovered paper	8
Sugarcane bagasse	3

2.2.2 South African pulp and paper mill operations

The South African paper and pulp manufacturing sector has grown substantially since 1970. South Africa is now considered the 15th largest producer of pulp and ranked 24th in paper production globally (FpmSeta, 2014). In 13 years of this sector, the minimum and maximum of pulp and paper production per year totalled between 2.1 million tonnes to 2.7 million tonnes respectively as shown in **Table 2-2** below. While **Table 2-3** and

Table 2-4 give the types of pulp and paper products made by major South African pulp and paper companies.

Table 2-2: South Africa paper and pulp production (PAMSA, 2014; PAMSA, 2012)

	Production summary, Tonnes ('000)				
Year	**Printing and Writing Papers**	**Packaging Papers**	**Tissue Paper**	**Total Paper**	**Total Pulp**
2001	863	1,245	150	2,258	2,138
2002	913	1,265	154	2,332	2,183
2003	920	1,265	152	2,337	2,317
2004	1,019	1,306	197	2,522	2,073
2005	925	1,365	193	2,483	2,193
2006	1,050	1,369	191	2,610	2,222
2007	1,132	1,400	195	2,727	2,311
2008	1,066	1,440	220	2,726	2,572
2009	922	1,097	224	2,244	2,130
2010	939	1,341	217	2,497	2,307
2011	790	1,223	219	2,233	2,321
2012	796	1419	216	2431	2277
2013	740	1356	222	2318	2016

Table 2-3: Pulp production in South Africa (PAMSA, CEPPWAWU 2004)

Company	**Mill**	**Products**	**2001 Capacity (1000ts)**
Mondi	Richards Bay	Hardwood and softwood Kraft paper	576
	Piet Retief	Hardwood and softwood NSSC pulp	60
	Flexiton	Unbleached Bagasse pulp	70
	Merebank	Thermomechanical pulp	220
		Groundwood Pulp	6
Sappi	SilvaCel	Hardwood Pulp	*
	Ngodwana	Hardwood and softwood Kraft paper	410
		Groundwood Pulp	100
	Tugela	Unbleached softwood pulp	230
		Hardwood NSSC pulp	120
	Stanger	Bleached Bagasse pulp	60
	Enstra	Bleached hardwood pulp	90
	Saicor	Dissolving pulp	500
Total			2602*

*1.9 million green metric tonnes of hardwood woodchips/annum

Table 2-4: Major paper and board mills in South Africa (PAMSA, CEPPWAWU 2004)

Company	Mill	Products	Total Capacity (1000ts)
Kimberly-Clark	Enstra	Crepe tissue	52
Mondi	Richards Bay	White top and craft liner board	260
	Felixton	Fluting medium	100
	Piet Retief	Unbleached linerboard	130
	Springs	Carton Board	125
	Merebank	News print and telephone directory Paper	230
		SC mechanical	100
		Uncoated fine paper	220
		Other grades	16
Nampak	Belville	Crepe tissue	25
	Klipriver	Crepe tissue	23
	River view	Crepe tissue	10
	Rosslyn	Fluting and testliner	50
Sappi	Ngodwana	White top and Kraft linerboard	240
		Newsprint	140
	Tugela	Kraft linerboard, fluting and other kra ft paper	390
	Cape Kraft	Testliner, fluting and ceiling board	80
	Enstra	Uncoated printing and writing paper	170
		Coated fine paper	80
		Tissue paper	30
		Uncoated industrial and packaging Paper	40
Unicell	Germiston	Testliner	80
Other	Approximately 12 other smaller mills often dealing with recycled paper		77*
Total			2648*

*Estimate

2.2.3 Water use in the industry

The pulp and paper industry is largely dependent on water in their production operations (Macdonald, 2004). All the major processes along the production line requires substantial amounts of water, between 75 to 230 m^3 of water per ton of product (Nemerow & Dasgupta, 1991). The total water consumption of some pulp and paper mills located in South Africa is indicated in **Table 2-5**. The consumption of water by this industry leads to some serious concerns about effluent discharge, and can sometimes be detrimental to the environment if not treated properly (Ali and Sreekrishnan, 2001). Lately, stricter regulations have forced many paper and pulp mills to recycle as much as process water back into the production system. This includes the recycling of white water effluents from the papermaking machine into the washing, screening and bleaching of brown pulp (Suhr *et al.* 2015). This reduces the load of water intake and also reduces the effluent discharge into the environment. Other mills also have switched to less toxic and severe pulping and bleaching techniques (Suhr *et al.* 2015). This reduces water intake and discharge mildly polluted waste water, but yet still pulp and paper industry is still considered among the sixth largest polluter of the earth's environment (Ali & Sreekrishnan, 2001).

Table 2-5: Total water consumption (SWC) for various South African mills (Macdonald, 2004)

Mill	Total water consumption in ML/d - (Mega litres per day)	
	Lower	**Upper**
Mondi Richards Bay	41.1	76.8
Mondi Merebank	11.4	44.3
Mondi Piet Retief	1.2	14.6
Mondi Felixton	2.0	6.0
Mondi Springs	2.3	5.5
Sappi Ngodwana	19.6	50.4
Sappi Enstra	10.6	27.1
Sappi Saiccor	94.5	193.5
Sappi Stanger	5.3	20.5
Sappi Cape Kraft	0.25	1.63
Sappi Tugela	9.2	45.6
Sappi Adamas	0.55	1.8
Nampak Klipriver	0.35	6.9
Nampak Rosslyn	0.06	1.14
Nampak Bellville	0.46	9.1
Nampak Riverview	0.14	2.8
Kimberly Clark Enstra	0.7	14.0

2.3 OVERVIEW OF PAPER SLUDGE AND PROCESS WASTEWATER

Paper sludge and process wastewater are some of major waste streams emanating from the pulp and paper industry (Suhr *et al.*, 2015). The pulp and papermaking process produce substantial amounts of wastewater comprising of ash, fines, short and degraded fibres (**Figure 2-1**). This effluent stream, mostly a mixture waste streams, emanate from various processes in the mill such as washing unit, bleaching unit and papermaking units (**Figure 2-1**). This effluent stream is separated into respective liquid (process water) and solid waste (paper sludge) streams by physiochemical treatments such as sedimentation and filtration clarifiers (Thompson *et al.*, 2001) (**Figure 2-1**). It is worth highlighting that the variability in composition of both process water and paper sludge are highly dependent on raw material feedstock (virgin wood or recycled paper) and production operations (chemical or mechanical pulping) employed in various pulp mills (Monte *et al.*, 2009; Martin A Hubbe *et al.*, 2016).

2.3.1 Paper Sludge Characterization

Paper sludge is the solid waste collected from primary clarifiers that is mostly disposed of in landfills. In primary clarifiers, suspended solids in effluent stream are first removed and afterwards thickened (Suhr *et al.*, 2015). The thickened stream is usually dewatered using a belt press or screw press to form to paper sludge (Mendes, Rocha and Carvalho, 2014). Mill operations can generate up to 50 kg (dry weight) of primary paper sludge per tonne of paper produced and this could vary by 20% in a newsprint mill, to 40% in a mill producing tissue paper and higher percentages of waste from recycling operations (Gottumukkala *et al.* 2016; Bajpai, 2015). **Table 2-6** show the variation in the feed, process types and amount of paper sludge emanating from different milling operations in South Africa.

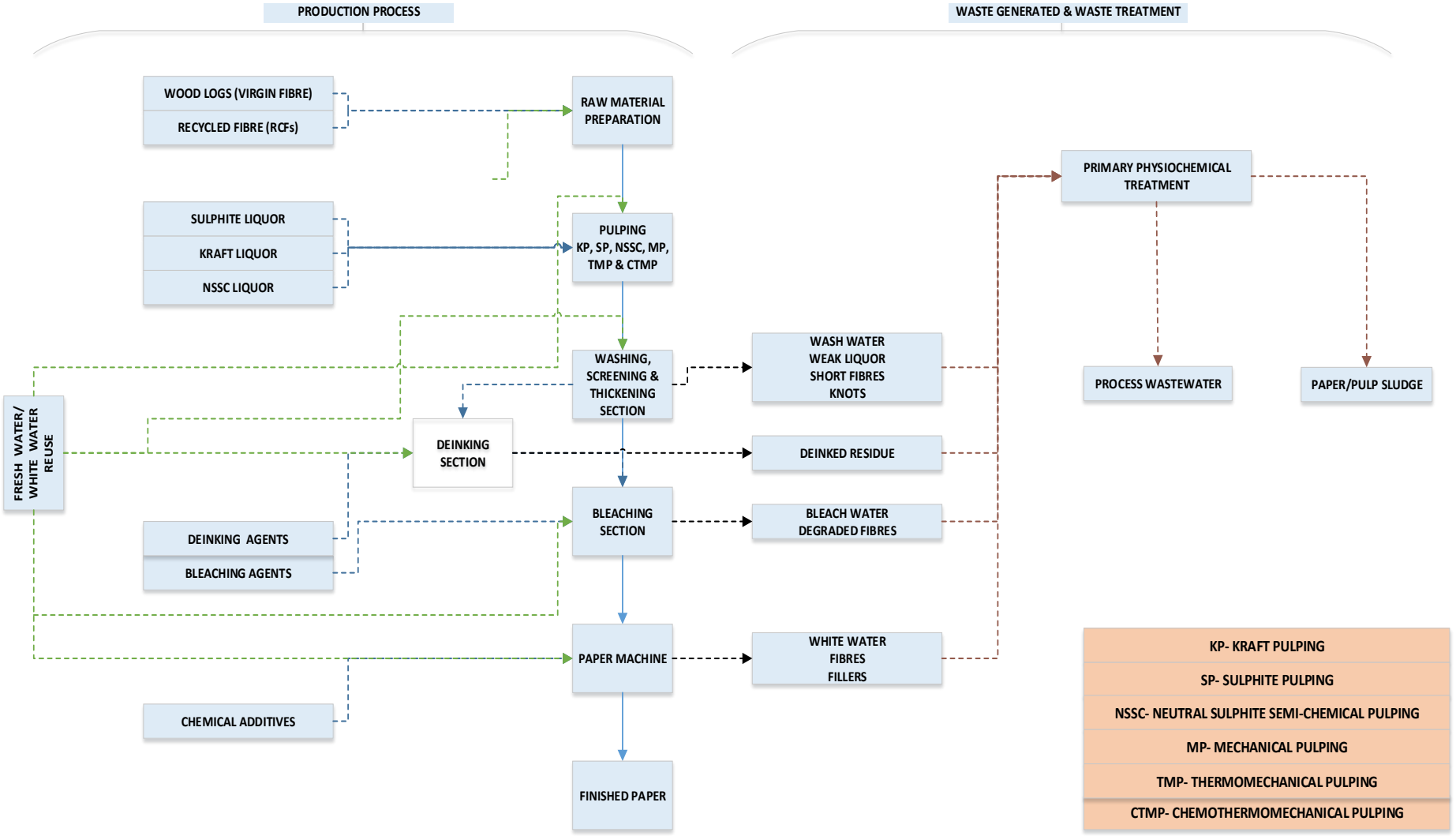


Figure 2-1: Paper and pulp making process and produced organic waste schematic representation

Table 2-6: The kind of feed, process, products and primary clarifier sludge production by 11 South African Paper and Pulp Mills (Redrawn from Boshoff *et al.* (2016))

Company: Mill	Sample number	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	6000	54
Nampak: Bellville	5, 6, 7, 8	RF, NPW, VP	RP, DI	TP	1800	54
Nampak: Kliprivier	9, 10, 11, 12	RF, NPW, VP	RP, DI	TP	1500	60
Nampak: Verulam	13, 14	RF, NPW, VP	RP, DI	TP	1500	57
Sappi: Enstra	15, 16, 17, 18	VP	RP	PO, SP, PP	7500	71
Mondi: Richardsbay	19	RF, C, VW, E	RP, K	B, KL, CB	12500	64
Mpact: Felixton	20, 21, 22, 23	BP, VW, E, P	RP	CB	4 000	43
Mpact: Springs	24, 25, 26, 27	RF, C, VP	RP, DI	WLC, LB, SCB	11000	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	7000	85
Sappi: Ngodwana	34, 35, 36, 37	VW, E, P	K, MP	NP, KL, CUP, MP, DP	15000	80

[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.

The composition of paper sludge from pulp and paper mills is difficult to determine due to several interfering factors. Generally, paper sludge is a combination of cellulose fibre (40–60% of dry solids), printing inks and mineral components (40–60% dry solids: kaolin, talc and calcium carbonate) (Bajpai, 2015). Also paper sludge mainly has carbon content around 30% dry solids and C/N ratio within 12 to 200 with low levels of fertilising elements and metal content. **Table 2-7** and **Table 2-9** below indicate the chemical, physical and compositional properties of various types of pulp and paper sludge.

Apart from the cellulosic content, paper sludge also has lower amounts of hemicellulose and lignin as indicated in **Table 2-8**. The carbohydrate content of paper sludge varies between 20 to 70% (Fan & Lynd, 2007). Cellulose is a glucose polymer with crystalline structure connected by β-(1→4)-glycosidic bonds with average molecular weight around 100,000 (McKendry, 2002). Hemicellulose on the other hand is rather a heteropolymeric polysaccharides consisting of various monosaccharides such as galactose, mannose, xylose, glucose, rhamnose, and arabinose with average molecular weight less than 30,000 (McKendry, 2002). Whiles lignin is the binding agent that fills spaces in cell walls linking cellulose and hemicellulose structures. Lignin consists of hydroxyphenylpropanoid units with three building blocks (trans p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol) (McKendry, 2002). Another class of material found in lignocellulosic biomass are extractives such as fatty acids, wax and sap.

Also, paper sludge generally has a high water holding capacity. The water holding capacity (WHC) is the amount of water that a material can saturate. The water holding capacity of paper sludge ranges between 4.8- 12.6 litres of water per gram of paper sludge (Boshoff *et al.* 2016; Williams, 2017). This is because water is connected with fibre either as trapped water or bound water (Robertson & Eastwood, 1981).

Table 2-7: Paper and pulp mill sludge (PPMS) chemical and physical properties (Primary, secondary and de-inked PPMS) (Faubert *et al.* 2016)

Parameter	Primary PPMS	De-inking PPMS	Secondary PPMS
Dry matter (%FM)	15-57	32-63	1-47
Ash content (%dry solids)	10-15	40-60	10-20
Nitrogen (%DM)	0.045-0.28	0.15-1	1.1-7.7
Phosphorous (%DM)	0.01-0.06	0.0012-0.16	0.25-2.8
Potassium (%DM)	0.02-0.09	0.0029-0.2	0.078-0.7
pH	5-11	7.2-9.2	6.0-8.5

FM- Fresh Matter; **DM-** Dry Matter

Table 2-8: Paper and pulp sludge compositional analysis (Lynd *et al.* 2001)

Compositional analysis of 15 Paper sludge samples	Glucan	Xylan	Mannan	Acid soluble lignin
	11.66 - 74.46	1.29 – 6.17	0.69 – 5.06	0.21 – 2.13

Table 2-9: Average composition of mixed Pulp and Paper Industry sludge (Gendebien. R, Ferguson. J, Brink. H, Horth. M, Davis. R, Brunet. H 2001)

ELEMENTS	Min	Max
Dry solids (%)	2	65
C/N ratio	12	200
pH	4	9
	Agricultural Value (% DS	
Organic matter	19	90
N-TK	0.4	5
N-NH_4	0	0.3
CaO	0.5	20
MgO	0.02	6
P_2O_5	0.2	8
K_2O	0.06	0.8
SO_3		1.3
	Heavy Metals (mg kg^{-1} DS)	
Cadmium – Cd	0	4
Chromium –Cr	< 1	44
Copper – Cu	2	349
Mercury – Hg	< 0.01	1.4
Nickel – Ni	< 1	32
Lead – Pb	< 1	83
Zinc – Zn	1.3	330

2.3.2 Properties of clarifier process wastewater

The quality and quantity of process wastewater from clarifiers depends on raw material and operational practices employed by various pulp mills (Pokhrel and Viraraghavan, 2004). The major contributors to process wastewater loads in mills are the pulping, washing and bleaching process with minor generation in the paper machines (Rintala and Puhakka, 1994; Ali and Sreekrishnan, 2001) (**Figure 2-1**). Depending on the mill, specific wastewater loads can vary from 5 to 180 m^3/air dry ton produced pulp or paper (Sierra-Alvarez, 1990). The properties of process wastewater are generally characterized by chemical oxygen demand (COD), biological oxygen demand (BOD) and suspended solids (SS) (Pokhrel and Viraraghavan, 2004). Process wastewater from pulp and paper mills have high strength COD (1 000 to 7 000 mg/L) and suspended solids ranging from 500 to 2 000 mg/L (De los Santos Ramos *et al.*, 2009; Eskelinen *et al.*, 2010) (**Table 2-10**). Chemical pulping produces high

strength wastewater with soluble wood material and debris. On the other hand, pulp bleaching generates the most toxic components found in process water, as it employs chemicals like chlorine dioxide and hydrogen peroxide for pulp brightening (Pokhrel & Viraraghavan, 2004).

As a result of the pulping and bleaching process, several toxic substances like lignosulfonic acids, resin acids, phenolic compounds and many other chemicals are produced in process wastewater (Pokhrel & Viraraghavan, 2004). In addition, chlorinated organic compounds are also identified in process water, if the pulp is bleached using chemical agent like chlorine dioxide (Martin A. Hubbe *et al.*, 2016). Bleach wastewater mainly comprises of degradation compounds of residual lignin in pulp after chemical pulping (Rintala & Puhakka, 1994). Furthermore, elevated levels of heavy metals have been reported in wastewater emanating from recycling pulp mills (Suhr *et al.*, 2015). The observed heavy metals content are largely in the form of stable organic complexes (Suhr *et al.*, 2015).

Table 2-10: Characteristics of process wastewater from various pulp and paper mills

	SS	BOD	COD	References
TMP mill	330–510		3343–4250	(Qu *et al.*, 2012)
TMP mill	383	2800	7210	(Pokhrel and Viraraghavan, 2004)
CTMP	350	3000	7521	(Liu *et al.*, 2011)
Bleach Kraft mill	37 - 74	128 - 184	1124 - 1738	(Pokhrel and Viraraghavan, 2004)
Bleached pulp mill	1133	1566	2572	(Ashrafi *et al.*, 2015)
Recycled paper mill		1650–2565	3380–4930	(Zwain *et al.*, 2013)
Recycled paper mill		669	4328	(Kamali *et al.*, 2016)

SS- Suspended solids; BOD- Biological oxygen demand; COD- Chemical oxygen demand; TMP- Thermochemical pulping; CTMP- Chemo-thermochemical pulping

2.4 PRODUCTION OF BIOETHANOL AND BIOGAS FROM PAPER SLUDGE

Presently, bioethanol and biogas production are two major bioenergy processes that are being explored for valorisation of paper sludge (Gottumukkala *et al.*, 2016). Ethanol production from paper sludge is a well-studied process at bench scale with limited studies at pilot scale (Gottumukkala *et al.*, 2016). Alternatively, biogas production from paper sludge have lately gathered attention due to its renewable energy capability though the research area is still in its early stages (Gottumukkala *et al.*, 2016).

2.4.1 Advantages of paper sludge as a bioenergy feedstock

Fibres in paper sludge are more accessible to enzymes and microbes during biological processes due to the chemical and mechanical pulping stages in papermaking (Boshoff *et al.*, 2016). There slight or no impediment from lignin as seen in other biomass feedstocks (Boshoff *et al.*, 2016). As a result, most paper sludge samples do not require pre-treatment technology to improve digestibility in fermentation process (Lark *et al.*, 1997; Fan and Lynd, 2007a; Prasetyo *et al.*, 2011).

Furthermore, combining the utilization of paper sludge and process water from the industry in bioethanol and biogas production into a pre-existing waste treatment infrastructure on site can significantly lessen the cost of waste handling and energy production relative to other biomass processing facilities (Fan *et al.* 2003). In addition to circumventing cost of waste handling in a pre-existing waste treatment facility, biofuel production from paper sludge can lead to significant reduction in landfill waste (Williams, 2017). Also, the high moisture content of paper sludge implies significant amounts of water can be reclaimed in addition to bioenergy production (Boshoff *et al.*, 2016).

2.4.2 Ethanol production from paper and pulp sludge

Bioethanol production from unprocessed lignocellulosic raw material involves a sequence of bioprocesses described in **Figure 2-2**. Virgin, untreated lignocellulosic biomass is pre-treated at elevated temperatures in the presence of acids, alkali or organic solvents to render the carbohydrates fractions accessible to hydrolytic enzymes (Galbe and Zacchi, 2007). But due to the extensive alkali or

acid pulping methods undertaken in the papermaking to retrieve cellulose fibres, most paper sludge samples need little or no pre-treatment (Prasetyo *et al.* 2011).

The cellulose content of pretreated lignocellulose can be converted to ethanol by using well-established bioprocessing methods such as separate (enzymatic) hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). Separate (enzymatic) Hydrolysis and Fermentation (SHF) of pretreated lignocelluloses comprises of two steps; the first step involves the enzymatic hydrolysis of cellulose into glucose at optimum temperature between 45 °C to 50 °C, while the second step entails the conversion of the resultant fermentable sugars such as glucose into ethanol also within optimum temperature of 30 ºC to 35 ºC (Vertes *et al.* 2010). Simultaneous Saccharification and Fermentation (SSF) incorporates the enzymatic hydrolysis of cellulose and the subsequent fermentation of the cellulose hydrolyzate into a single process reactor. Both the fermenting microorganism and enzymes are introduced into the reactor to convert the cellulose to ethanol. Cellulose conversion to glucose is instigated by enzymes and the resulting glucose is simultaneously also converted to ethanol. In so doing, inhibitory effects on cellulase activity by cellobiose and glucose is significantly reduced unlike in SHF (Xiao *et al.* 2004; Olofsson *et al*. 2008). The essential advantages of SSF over SHF comprise of the requirement of fewer vessels, a higher ethanol yield and less contamination (since ethanol presence reduces the risk of contamination). However, SSF has the disadvantage of operating at pH and temperature conditions that comprise between the optima for both fermentation and enzymatic hydrolysis with the temperature normally kept around 37 ºC (Lark *et al.* 1997).

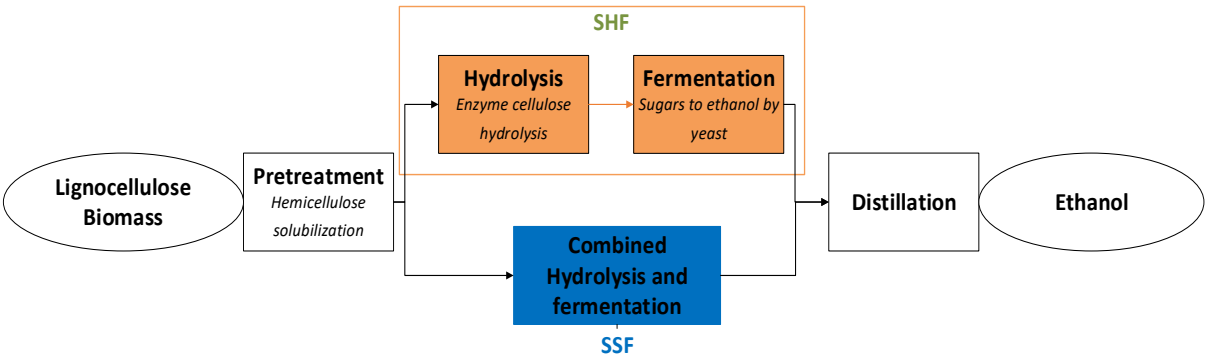


Figure 2-2: Schematic representation of ethanol production from lignocellulose biomass; SHF (Separate hydrolysis and fermentation) and SSF (Simultaneous Saccharification and Fermentation) (Vertes *et al.* 2010)

2.4.3 Process Parameters on paper sludge fermentation

Although SSF doesn't operate at optima temperature and pH for enzymatic hydrolysis. The reported ethanol concentration for SSF was almost twice as much as that of SHF under the same conditions (Prasetyo *et al.*, 2011). For SSF process of paper sludge to be economically viable, it is essential to produce ethanol concentrations more than 40 g/L, as distillation at lower concentrations would be too energy intensive, making such process not financially sensible (Kang *et al.*, 2011). Resultantly, modification of key process factors highlighted below can be helpful in reaching this goal.

2.4.3.1 Enzyme dosage

Prasetyo & Park (2013) and Kang *et al*. (2011) established that saccharification and ethanol concentration yield increased as cellulase dosage also increased. However, enzymes are major drawback with ethanol production from second generation feedstocks since enzyme cost could be as high as $ 1.47 gal^{-1} (R 3.28 l^{-1}) (Klein-marcuschamer et al. 2012). Hence for SSF to be economically feasible, it is imperative to design to compensate for low enzyme dosage while producing reasonable ethanol yields. Prolonging reaction time can help achieve high ethanol yields at low enzyme dosage but this unfortunately reduces productivity. Robus *et al.* (2016) and Boshoff *et al.* (2016) investigated the fermentability of three categories of South African pulp and paper mill sludge using Optiflow RC 2.0 enzyme from Genencor, Cedar Rapids, IA, USA. Both studies reported economic enzyme dosages ranging from 10 FPU gds^{-1} to 20 FPU gds^{1}.

2.4.3.2 Fermenting Microorganism

Various species of bacteria, filamentous fungi and yeast produce ethanol from paper and pulp sludge with the most relevant microorganisms being *Saccharomyces cerevisiae, Zymomonas mobilis* and *Pichia stipitis.* Gírio *et al.* (2010) in the **Table 2-11** pointed out the merits and demerits of the above-mentioned species with *S. cerevisiae* surpassing the other microorganisms in all relevant characteristics except for pentose sugars utilization. Robus *et al.* (2016) and Boshoff *et al.* (2016) also assessed the ethanol production of three types of strains of *S. cerevisiae* with Optiflow RC 2.0 as the enzyme cocktail and discovered there was no significant variation in ethanol production levels for MH1000, TMB3400 and D5A, although there was a noticeable lag in fermentation activity during the first 24 hours for D5A yeast strain. Another germane factor with respect to fermentative microorganism, is the inoculum

volume. Prasetyo *et al.* (2011) reported improved ethanol yield when inoculum volume was increased from 10% to 20% during paper sludge SSF with thermotolerant *S. cerevisiae* TJ14. The 10% inoculum yielded ethanol concentration of 35.7 g/L with theoretical yield of 61.8%, while the 20% inoculum produced 40.5 g/L of ethanol with theoretical ethanol yield of 66.3%.

Table 2-11: Merits and demerits of relevant fermenting micro-organisms (redrawn from (Gírio *et al*. 2010))

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

- Negative, + Positive, w Weak

* Engineered newer strains of *S. cerevisiae* that can ferment C5 sugars

2.4.3.3 Solids loading, Feeding and Agitation

High solids loading in paper sludge fermentation resultantly yields higher ethanol concentrations (Ballesteros *et al.*, 2002). However, this is hard to achieve due to the high water holding capacity of paper sludge (>60) (Boshoff *et al.*, 2016). The density of paper sludge with water rises with an increase in solid loading (Fan & Lynd, 2007), hence higher agitation speeds are required to overcome this negative effect to improve ethanol concentration and yield (Fan *et al.* 2003). A better alternative method largely used to achieve higher solids loading at moderate agitation speeds is the use of fed-batch system in paper sludge fermentation (Ballesteros *et al.*, 2002; Jørgensen, Kristensen and Felby, 2007). More free water is released as hydrolysis progresses due to biomass degradation, and as such moderate amounts of paper sludge can be fed from time to time without increasing the viscosity of the broth (Ballesteros *et al.*, 2002). **Table 2-12** below shows SSF runs for various paper sludge solid loadings and enzyme dosages by Boshoff *et al.* (2016). A fed-batch system with 3% (w/w)

intermittent feeding experimented at 11 FPU/g substrate lead to higher ethanol concentration as compared to batch culture.

Table 2-12 SSF runs at different solids loading and enzyme dosages (Boshoff *et al.*, 2016)

Substrate Loading (g/L)	5 FPU/g dry PS		15 FPU/g dry PS	
	Ethanol (g/L)	**Yield (%)**	**Ethanol (g/L)**	**Yield (%)**
20[1]	3.2	80.0	3.4	85.0
20[2]	3.1	59.6	3.5	67.3
	11 FPU/g dry PS			
Fed-batch: 30 g/L incremental	**Ethanol (g/L)**	**Yield (%)**		
270[1]	45.5	78.2		
180[2]	34.2	66.9		

[1]Corrugated recycle paper sludge
[2]Virgin pulp paper sludge

2.4.3.4 Viscosity and Water holding capacity

The water holding capacity and viscosity of the paper sludge are intrinsic characteristics that limits solids loading and hence, fermentation performance of a run (Boshoff *et al.*, 2016). Water is bound as intracellular water or by a surrounding matrix of highly hydrated extracellular polymers in paper sludge (Hagelqvist, 2013). The water holding capacity of paper sludge depends on the amount of cellulose present and the length of the cellulose fibres (Boshoff *et al.*, 2016). This consequently contributes to the high viscosity of paper sludge. Boshoff *et al.* (2016) indicated high viscosity negatively influences digestibility through physical constraints for enzyme access, thus slowing down hydrolysis and increasing the demand for enzymes.. Additionally, higher agitation rates can partly counter high viscosity levels, but leads to reduction in enzyme stability due to high shear stress of the blades on the cellulase (Fan and Lynd, 2007; Boshoff *et al.*, 2016).

2.5 BIOGAS PRODUCTION FROM PAPER SLUDGE AND FERMENTATION RESIDUE

Anaerobic digestion involves the degradation of organic materials under anaerobic conditions by microbial organisms into biogas, consisting of methane (50–75%), carbon dioxide (25–50%), hydrogen (5–10%), and nitrogen (1–2%), as well as microbial mass (Kelleher *et al.* 2002; Maghanaki *et al.* 2013). Anaerobic digestion is known to be one of the most efficient and widely used wastewater treatment technology employed in municipal waste and pulp and paper mill effluents (Parkin *et al*. 1983; Meyer & Edwards, 2014; Kamali *et al.* 2016). However, it can also be applied to solid wastes from paper and pulp processes, as discussed below. A combination of solid and liquid wastes for AD treatment will be investigated in the present project.

Several studies have established the possibility of biogas production from paper related waste, as indicated in **Table 2-13**. Williams (2017) and Dalwai (2012) studied biogas production from paper and pulp sludge generated by various South African mills employing continuous stirred digester (CSD) and bio-methane potential (BMP) assays respectively. It can be inferred from **Table 2-13** that methane yields are highly dependent on substrate composition (co-digestion), digester type and critical operating conditions such as temperature and pH. At both mesophilic (35°C) and thermophilic (55 °C) conditions, paper sludge had a bio-methane potential 2 to 3 times greater than secondary sludge, thus, making paper sludge as the more suitable for biogas production (Bayr & Rintala 2012a; Gottumukkala *et al*. 2016).

Table 2-13: Summary of anaerobic digestion of various types of pulp and paper derived substrate

Substrate type	Digester type	Temperature (°C)	HRT (days)	OLR ($kgVS/m^3d$)	Volatile Solids = (% Total Solids)	CH_4 yield (L/kg VS fed)	References
Secondary PS	280ml mini-digester[a]	30	33	-	90.6	173.60 ± 5.87	(Huiliñir *et al*. 2014)
Secondary PS	500ml -flask digester	38	76	-	70	53 ± 26	(Hagelqvist 2013)
Primary PS	5L CSTR[b]	55	23-29	1	84	200 ± 20 to 240 ± 10	(Bayr & Rintala 2012a)
Secondary PS			14-16	1.4-2.0	81.5	190 ± 20 to 220 ± 10	
Co-digestion PSPPS			25-31	1	-	150 ± 10 to 170 ± 10	
Primary PS	30L digester[c]	37	28	-	37.13	82.1 ± 11.3	(Williams 2017)
					74.07	69.9 ± 10.2	
					75.17	47.7 ± 5.5	
Primary PS	1L BMP assay	55	42	-	84	230 ± 20	(Bayr & Rintala 2012b)
Secondary PS					81.5	100 ± 10	
Primary PS	1L BMP assay	35	42	-	84	210 ± 40	(Bayr & Rintala 2012b)
Secondary PS					81.5	50 ± 0	
Primary PS	100ml BMP assay	37	60	-	67 - 97	382	(Dalwai 2012)
					31 - 40	226	

PS- Paper sludge; ***PSPPS***- Primary & Secondary pulp and paper sludge***; CSTR*** – Continuous stirred tank reactor; ***BMP***- Biochemical methane Potential

[a] Daily manual stirring; [b] 400-700 rpm magnetic stirrers; [c] 93 rpm motor driven single Rushton impeller

2.5.1 Microbial community and their metabolisms leading to biogas production

Biogas production from organic matter is driven by the metabolisms of a complex microbial community that includes bacteria, archaea and probably also fungi and protozoa (Vertes *et al.* 2010). **Figure 2-3** highlights the biomethanation process with unique functional group of microbes performing specific tasks in relation to each other. The first phase, also the rate limiting step, involves the hydrolysis of polymeric biomass by facultative anaerobic bacteria (*e.g., Clostridium, Peptococcus, Micrococcus, and Streptococcus*) into monomers and oligomers (Angenent *et al*. 2004). Monomers and oligomers resulting from the hydrolysis step are further fermented into short chain fatty acids, CO_2 and H_2 by another guild of anaerobic bacteria comprising of *Bacteroides, Clostridium, Butyribacterium, Propionibacterium, Pseudomonas*, and *Ruminococcus* (Ahring, 2003). This phase often referred to as the acidogenesis stage generally occurs rapidly and can result in short chain fatty acids (SCFA) accumulation and digester failure when feedstock fed contains large amounts of readily fermentable carbohydrates (Ahring, 2003). Fortunately, paper sludge undergoes slow hydrolysis in anaerobic digestion and SCFA accumulation will not occur in digesters. Next, acetogenesis proceeds by another special guild of anaerobes referred to as syntrophic acetogens. These anaerobes convert various types of SCFA into acetate, CO_2 and H_2 (Ahring, 2003). Lastly, methanogens, different from bacteria and belonging to the domain Archaea, produce CH_4 and CO_2 as the end-product of the biomethanation process (Vertes *et al*. 2010). Methanogens are classified as hydrogenotrophic methanogens and acetoclastic/acetotrophic methanogens depending on substrate specificity and methanogenesis pathway. Hydrogenotrophic methanogens converts methanol, formate, methylsulfides and methylamines to methane and/or also use H_2 to reduce CO_2 to methane, while acetotrophic methanogens converts acetate to methane (Demirel & Scherer, 2008).

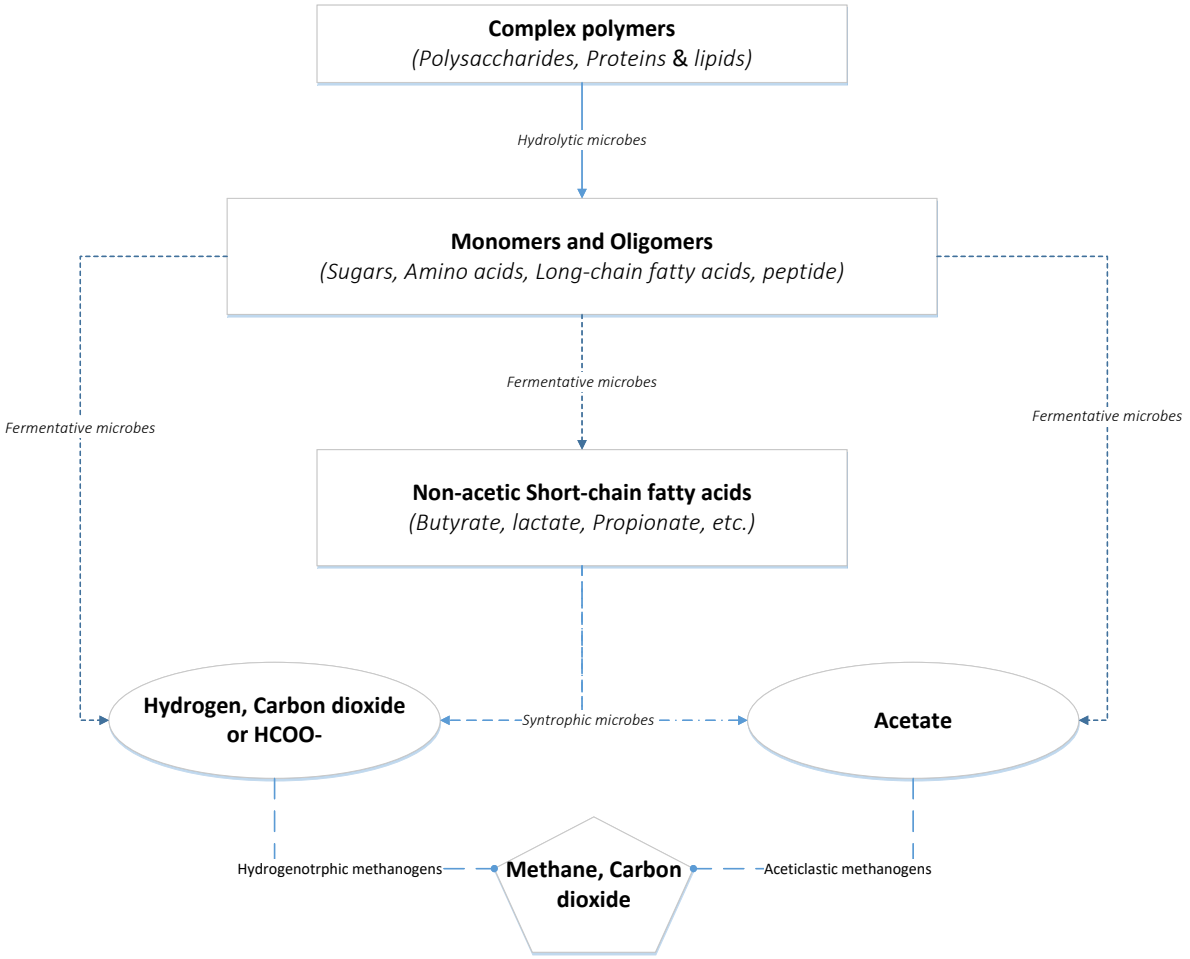


Figure 2-3: Key stages in Biomethanation process

2.5.2 Variation in operating conditions

The performance of anaerobic digesters is affected by variation in operating parameters such as pH, temperature, organic loading rate, feedstock composition, C/N ratio, hydraulic retention time (HRT) and agitation. Although various anaerobic microbes can temporarily tolerate and adapt to some extent certain changes in conditions, anaerobic digestion reactors should be designed and operated taking into consideration these important dynamics in relation to a feedstock so as to achieve optimum performance (Chen *et al.* 2008; Meyer & Edwards, 2014).

2.5.2.1 C/N (Carbon to Nitrogen) ratio

Feedstock quality, characterized by C/N ratio is of prime importance for the optimal performance of AD reactors. Anaerobic microorganisms normally utilize carbon 25–30 times faster than nitrogen and the optimum C/N ratio for methane production, with no adverse effect on high-solids AD reactor, was found to be within the range of 25-30, based on largest percentage of the carbon being

readily degradable (Malik *et al*. 1987; Kayhanian & Tchobanoglous, 1992). Ammonia toxicity develops when C/N ratio is below 20, while a high C/N ratio also leads to nutrient (nitrogen) deficiency. The two-principal aqueous inorganic ammonia nitrogen responsible for toxicity is the ammonium ion (NH_4^+) and free ammonia (NH_3), with the latter suggested to be major cause for inhibition due to its free membrane permeability (de Baere *et al*. 1984). Ammonia concentrations of below 200 mg/L have been reported to be beneficial to the anaerobic process, while total ammonia nitrogen (TAN) concentrations above 1.7 g/L are inhibitory towards methanogens, leading to 50% reduction in methane production (Chen *et al*. 2008).

2.5.2.2 Temperature

Anaerobic digester temperature is of major importance in biogas production due its effect on the microbial growth rate and free ammonia concentration. Digesters can be operated at different temperature ranges*; psychrophilic (<30°C), mesophilic (30-40°C) and thermophilic (50-60°C)*. Mesophilic digestion exhibits better process stability and better richness in bacteria but produces lower methane yields and poor biodegradability as compared to thermophilic digestion (Bowen *et al*., 2014). Although temperature increases the hydrolysis rate and the methane potential, it also leads to a high free ammonia concentration (Chen *et al*. 2008; Mao *et al*. 2015). This in turn results in more easily inhibited and less stable digester at thermophilic temperatures than at mesophilic temperatures (Parkin *et al*. 1983).

2.5.2.3 pH

Similar to yeast fermentation, pH directly affects the amount and quality of biogas produced in anaerobic digestion. Several studies have reported ideal pH range for anaerobic digesters to be within 6.8-7.4 (Yadvika *et al*. 2004; Mao *et al*. 2015). Carbon dioxide and volatile fatty acids amounts produced during the anaerobic process affects the pH of the digester contents. It must be emphasized that both acidogens and methanogens have their favorable pH range of 5.5-6.5 and 6.5-8.2 respectively (Lee *et al*. 2009; Zhang *et al*. 2013).

2.5.2.4 Retention time

Hydraulic retention time (HRT) is the average duration that an input substrate spends inside an anaerobic digester before its removal. Acquiring an efficient HRT hinges on other parameters such as

substrate composition and temperature and can vary from 30–60 days for lignocellulose substrate (Yadvika *et al*. 2004; Meyer & Edwards, 2014). Shorter HRT usually potentially can lead to volatile fatty acids accumulation that can washout active bacterial population, while longer HRT demands a large digester volume and hence more capital cost (Yadvika *et al*. 2004).

2.5.2.5 Agitation

Digester agitation allows for enhanced contact between substrate and microbial community that eventually leads to temperature uniformity, efficient biogas removal from the reactor system and stratification prevention (Hoffmann *et al*. 2008; Lindmark *et al.* 2014; Tian *et al*. 2015). Earlier research studies by Stenstrom *et al.* (1983) and Karim *et al.* (2005) strongly suggested that agitation averts the formation of floating solid layers. This in turn decreases effective digester working volume. Insufficient agitation may well lead to solid layer formation, while some other research studies also indicate that high agitation intensities and period, rather have a harmful effect on digester performance, apart from intensive energy requirement (Stenstrom *et al*. 1983; Karim *et al*. 2005; Subramanian & Pagilla, 2014; Kim *et al*. 2002; Speece *et al*. 2006). Hoffmann *et al*. (2008) reported different mixing intensities (50-1500 rpm) had no influence on continuously stirred digester (CSD) performance at steady-state conditions regarding biogas production. However, severely retarded CSD performance during start-up was observed, with no considerable methane production, at agitation speeds above 500 rpm.

2.6 POSSIBLE COMPLICATIONS IN UTILIZATION OF PROCESS WATER IN FERMENTATION AND ANAEROBIC DIGESTION OF PAPER SLUDGE

Due to the combination(s) of a variety of treatment and manufacturing technologies employed in paper and pulp production, the concentrations of the major groups of compounds in process water will be mill-specific. This is of importance since some compounds as mentioned earlier will have considerable effect on process water utilization in bioethanol and biomethane production. Among the those compounds are HMF (5- hydroxymethylfurfural) and furfural, considered to be the representative inhibitors for yeast and bacterial growth. Further potential toxic class of compounds included are fatty acids, phenolic compounds (tannins), sulphur compounds, inorganics (ash) and heavy metals, that either singly or synergistically can possibly inhibit biological processes. In addition, some chemicals in used in pulping process still remains in process water and can also adversely affect biological process (**Table 2-14**). All these toxic compounds are identified alongside some other 250 chemicals in pulp mill effluents (Suntio *et al*. 1988). Additionally, concentration levels for these toxic compounds in primary clarifiers differ from mill to mill due to production practices and there no reported literature on the measured concentrations of these chemical compounds. Thus, it's difficult to determine whether process water will be inhibitory to anaerobic bacteria or yeast in anaerobic digestion or fermentation of paper sludge.

Table 2-14: Some toxic chemical components in virgin and recycled process waters

Type of Mill	Potential toxic chemical components in process water
Virgin pulp	NaS_2, Na_2SO_3, $Na_2S_2O_3$, H_2O_2, $H_2SO_4$and ClO_2
Recycled fibre	NaOH, Na_2SiO_3, Na_2CO_3 and H_2O_2

2.6.1 Potential Toxicants

Phenolic compounds and organic acids in general are more toxic to bacteria than yeast, with inorganic salts and heavy metal ions also being inhibitory towards both microorganisms (Leonard & Hajny, 1945; Mussatto & Roberto, 2004). Subsequent research studies conducted by Larsson *et al*. (1999) and Jönsson *et al*. (1998) revealed that, removal of phenolic compounds prior to fermentation with *S. cerevisiae* lead to considerable improvement of fermentability. Additional research studies by Clark & Mackie (1984), Ando *et al*. (1986) and Palmqvist *et al.* (2000) showed that low molecular mass phenolics are the most toxic to fermenting microorganisms. Heavy metals ions (copper, nickel,

chromium and iron) also present in process water can inhibit microorganism metabolic pathways (Mussatto & Roberto, 2004). Microbial activity is slightly reduced when these metal ions are presented in quantities as reported in **Table 2-15**, although heavy metal concentrations in pulp and paper mills is usually low. Long chain fatty acids show inhibitory effects on methanogenic bacteria, in particular to the acetoclastic bacteria (Lalman & Bagley, 2000; Lalman & Bagley, 2002; Ma *et al.* 2015). Additionally, resin acids and terpenes also affect bacterial activity in anaerobic digestion in concentration indicated in the **Table 2-15**.

Given that some pulp mills employ sulphate or sulphite chemical pulping, effluents contain substantial concentrations of sulphur compounds such as sulphite, sulphate, thiosulfate, sulphur dioxide, hydrogen sulphide in its dissociated form (HS-) and lignosulfonates. Sulphur dioxide especially has some level of inhibitory effect on all yeast, although the *Saccharomyces* yeast strains used in industrial alcoholic fermentation are more resistant to it, compared to the wild yeast strain (Baldwin, 1951). Sulphur compounds on the other hand are important anaerobic inhibitors (Meyer & Edwards, 2014). Sulphur reducing bacteria (SRB) compete with methane producing bacteria (MPB) for organic and inorganic substrate to reduce sulphate to sulphide (Chen *et al.* 2008). Consequent inhibition results from the sulphide production and this is toxic to various methanogenic bacteria groups (Chen *et al.* 2008). Although sulphur compounds have inhibitory effects on MPBs, SRBs have the ability to partially or completely degrade branched and long chain fatty acids, organic acids, alcohols and aromatic compounds (J.W.H. *et al.*, 1994). The latter is a desirable attribute and will be beneficial towards COD reduction from the process water clarification and reclamation perspective. Also, possible high ash content in process water and paper sludge due to repulping of recycled fibre can cause bacterial cells to dehydrate due osmotic pressure in anaerobic systems (Chen *et al*. 2008). While adequate concentrations fuel growth, excessive quantities of some light metals found in ash can individually or synergistically slow and stymie growth (Soto *et al.* 1993; Ahring *et al*. 1991).

Table 2-15: Potential process wastewater inhibitors for pulp and paper sludge biochemical processing

	Fermentation		Anaerobic digestion		References
Compound	**Critical concentration (mg/L)**				
Phenolic compounds	1000		350-3000		(Meyer & Edwards, 2014; Ando *et al*. 1986)
	(4-hydroxybenzoic acid)[a]		*Tannins*		
Fatty acids			73-1670		(Koster & Cramer 1987; Kim *et al*. 2004; Sierra-Alvarez *et al*. 1994)
Resin acids			20-600		(Field & Lettinga, 1987; McCarthy et al. 1990; Sierra-Alvarez & Lettinga, 1990)
Volatile terpenes			42-330		(Sierra-Alvarez & Lettinga, 1990)
Sulphate			500		(Meyer & Edwards, 2014)
Sulphite			50		(Meyer & Edwards 2014; Parkin *et al*. 1990)
Hydrogen peroxide			~50		(Habets & de Vegt, 1991)
Chlorinated compounds			AOX	100	(Ferguson 1994)(Patel *et al.* 1991; Blum & Speece 1991; Sierra-Alvarez & Lettinga 1991; Piringer & Bhattacharya 1999; Puyol *et al.* 2012)
			Chlorophenols	0.5-76	
Heavy metals	Copper	4	Copper	10-250	(Watson *et al.* 1984; Sanchez *et al.* 1996)
	Nickel	5-100	Nickel	200-1200	
	Chromium	100	Zinc	10-250	
	iron	150			
Inorganics (Light metals)			Aluminuim	>1000	(Cabirol *et al*. 2003)
			Calcium	2500-8000	(McCarty, 1964)
			Sodium	3500-8000	(McCarty, 1964)
			Zinc	30-150[b]	(Zheng *et al.* 2015)

[a] 4-hydroxybenzoic acid used as a model compound to study the influence of phenolic compounds on fermentation based on its abundance in hardwood hydrolysates.

[b] 30-150 mg/g-TS

2.7 GAP IN LITERATURE

2.7.1 Water reclamation from paper sludge

About 69% of paper sludge is landfilled with about 60-80% composed of water (Hagelqvist, 2013). Previous studies by Boshoff *et al.* (2016)and Williams (2017) showed significant reduction in water holding capacity of the original paper sludge after individual application of fermentation and anaerobic digestion. The decreased water holding capacity of the residual solids indicated potential for water reclamation. Thus, the determination of the amount reclaimable water from standalone and sequential fermentation and anaerobic digestion of paper sludge must be addressed.

2.7.2 Potential utilization of process wastewater in bioprocessing of paper sludge

The fermentation and anaerobic digestion of paper sludge with clean water as make up stream is well-reported in literature (Fan *et al.*, 2003; Kang *et al.*, 2011; Prasetyo *et al.*, 2011; Boshoff *et al.*, 2016; Robus *et al.*, 2016; Williams, 2017). But the possible use of clarifier process water as make up stream can be a better alternative and must therefore be explored. Processes are needed to be able to test the usability of process water in fermentation and anaerobic digestion of paper sludge and its impacts on bioprocess performance. Consequently, the quality of water reclaimed after different bioprocessing techniques needs to be determined, as this will indicated whether anaerobic digestion was a sufficient water treatment, especially for fermentation stillage in the sequential bioprocessing of paper sludge.

2.7.3 Energy yields from standalone and sequential bioprocessing of paper sludge

Recent research work by Williams (2017) on bioprocessing of paper sludge with clean water indicated fermentation as a superior bioenergy producer than anaerobic digestion. Additionally, a previous study by Vehmaanpera *et al.* (2012) showed more biofuel was extracted for a given amount of paper fibres waste in sequential bioprocessing with clean water than individual technologies.

In this study, energy yields from bioethanol and biogas will be evaluated from standalone and sequential bioprocessing of paper sludge with process water. The bioenergy yields together with the amount and quality of water reclaimed will reveal the overall best bioprocessing technique (sequential or individual fermentation and anaerobic digestion of paper sludge with process water).

2.7.4 Properties of residual solids and its potential applications

Paper sludge by its nature can be used in brick production, composting and land application (Monte *et al.*, 2009; Faubert *et al.*, 2016). Bioprocessing of paper sludge leads to the side production of residual solids. There is lack of information on the potential applications of this residual solids in the agricultural and industrial sectors. Thus, this study will evaluate the properties of the residual solids to ascertain its potential applications other industrial sectors. Finding useful applications for residual solid waste can lead to a zero waste bioprocessing technology for paper sludge.

2.8 RESEARCH QUESTIONS AND OBJECTIVES

Following comprehensive literature review, important research questions and objectives were fashioned. These are outlined below in the next sub-sections. Also, chapter 3 details an experimental design that will be used to examine research questions and objectives.

2.8.1 Primary research questions

From the gap in literature (section 2.7), four research questions were formulated;

1. **For standalone and sequential fermentation and anaerobic digestion of paper sludge, can recycled process waste water from the primary clarifier be used as is, and will it impact of bioprocess performance (*Section 2.7.2*)?**

There is lack of reported studies on the use of process waste water in fermentation and anaerobic digestion of paper sludge. Thus, it is essential to investigate the effect of process waste water on bioprocessing microorganisms with respect to ethanol and biogas production. The resulting consensus from these tests will determine whether solely utilizing process water in bioprocessing of paper sludge is achievable and if not, what co-feeding ratio of process water and clean water will permit successful fermentation and anaerobic digestion of paper sludge.

2. **How much water can potentially be reclaimed from standalone and sequential fermentation and anaerobic digestion of paper sludge with recycled process water (*Section 2.7.1*)?**

Fermentation and anaerobic digestion converts paper sludge to ethanol and biogas while simultaneously reducing the water holding capacity of the residual solids (Boshoff *et al.*, 2016; Williams, 2017). The reduction in water holding capacity should release entrapped water molecules in paper sludge, thus indicating potential for water reclamation. This study will determine the amount of water that can be reclaimed through bioprocessing of paper sludge. The amount of water reclaimed will be determined for the sequential bioprocessing and compared to individual fermentation and anaerobic digestion of paper sludge with process water.

3. **What is the water quality after standalone and sequential fermentation and anaerobic digestion of paper sludge with process water? Is anaerobic digestion a sufficient wastewater treatment? (*Section 2.7.2*)**

Biological systems such as anaerobic reactors in combination with aerobic treatment units have been used to treat wastewater in the pulp and paper industry (Rintala and Puhakka, 1994; Ali and Sreekrishnan, 2001; Meyer and Edwards, 2014; Larsson *et al.*, 2015). However, the potential to treat clarifier process water while simultaneously producing bioenergy from paper sludge is an opportunity which should be explored. Apart from the possible water reclamation and bioenergy benefits, there could be a potential treatment benefit, as COD of process water could be reduced with anaerobic digestion. Alternatively, the COD of process water is expected to significantly increase due to hydrolysis of organic content in fermentation of paper sludge (Peng and Chen, 2011; Boshoff *et al.*, 2016). Thus, this study will also ascertain whether anaerobic digestion can serve as a viable wastewater treatment for the subsequent fermented stillage produced.

4. **What are the bioenergy yields from standalone and sequential fermentation and anaerobic digestion of paper sludge with process water? (*Section 2.7.3*)**

Bioethanol and bioenergy production from fermentation of paper sludge with clean water is well-studied at bench scale with limited reports at pilot scale (Vehmaanpera *et al.*, 2012; Gottumukkala *et al.*, 2016). While research on biogas production from paper sludge is still in its earlier stages (Gottumukkala *et al.*, 2016). To further improve on this research area, this study will determine the biofuel production and bioenergy yields from standalone and sequential fermentation and anaerobic digestion of paper sludge with process water. The experiments will be conducted at both bench scale and pilot scale levels to ascertain the impacts of scaling up and the feasibility of large scale bioprocessing of paper sludge.

2.8.2 Research objectives

I. *Determine the effect of process water on fermentation and anaerobic digestion of paper sludge.*

II. *Determine the amount of reclaimable water for individual and sequential fermentation and anaerobic digestion of paper sludge in bench (5 L) and pilot scale(150 L) bioreactors.*

III. *Determine whether the quality of process water was improved after sequential and individual fermentation and anaerobic digestion of paper sludge.*

IV. *Determine the potential bioethanol and bioenergy yield from a range of paper sludge and corresponding process water in bench (5 L) and pilot scale (150 L) bioreactors.*

V. *Determine potential biogas and bioenergy yield from paper sludge and process water, and from fermentation stillage remaining after bioethanol production, using 30 L digesters.*

VI. *Characterise the final solid residues after bioprocessing of paper sludge and recommend potential industrial and/or agricultural application.*

CHAPTER 3: RESEARCH DESIGN AND METHODOLOGY

This section provides details about the experimental setup, analytical methods and characterisation procedures that will be followed during experiments.

3.1 FEEDSTOCK PREPARATION

3.1.1 Paper sludge characterization

To achieve precise conversion yields and mass balance, laboratory analytical procedures developed by the NREL was required to determine the exact composition of primary sludge samples. The NREL standards to be followed are discussed below.

3.1.1.1 Sample preparation (NREL/TP-510-42620)

Three different paper sludge samples were collected by (Williams, 2016) from three notable mills across South Africa. Virgin pulp, corrugated recycle and tissue and printing recycle paper sludges were acquired from Mondi Richards Bay, Mpact Felixton and Twincare Bellville, respectively.

Paper sludge preparation followed protocols as described by (Sluiter *et al.* 2008). The paper sludge samples were dried in a hoop greenhouse at 40-45°C after impurities such as plastic and twigs were removed. After drying, subsampling quarter-coning method was applied to make sure a homogenous mixture was attained. Next the dried paper sludge samples were milled using a hammer mill (Drotsky S1) fitted with a 2mm screen. Afterwards dried milled paper sludge samples were stored in sealed plastic bags at room temperature for later use in outlined biochemical processes.

3.1.1.2 Total solids/ moisture content (NREL/TP-510-42621)

Determination of total solids and moisture content of paper sludge also followed protocols outlined by (Sluiter *et al*. 2008). Aluminium weighed dishes were first dried in an oven at 105 ± 3 ºC for a minimum of four hours. The sample was then thoroughly mixed and 0.5-2 g of the sample carefully added to the pre-dried aluminium dish and weighed again. The aluminium dish with weighed sample was placed into a convection oven at 105 ± 3 °C for a minimum of four hours. The dish was allowed to cool down in a desiccator, and weighed again after drying in convection oven. Repeating of heating, cooling, desiccating, and weighing

procedure was done until the constant weight achieved. The constant weight was defined as ± 0.1% change in the weight percent solids upon one hour of re-heating the sample. Paper sludge samples used in total solids and moisture content analysis would not be subjected to further analysis due to the possible occurrence of thermal degradation of samples that has been exposed to elevated temperatures. The total solids and moisture content was then evaluated using equations 3.1 and 3.2 below.

$$\%\boldsymbol{total\ solids} = \frac{\boldsymbol{weight_{dry\ pan\ plus\ dry\ sample} - weight_{dry\ pan}}}{\boldsymbol{weight_{sample\ as\ received}}} \times \mathbf{100} \quad \text{.....3-1}$$

$$\%\boldsymbol{Moisture} = \mathbf{100} - \left(\frac{\boldsymbol{weight_{dry\ pan\ plus\ dry\ sample} - weight_{dry\ pan}}}{\boldsymbol{weight_{sample\ as\ received}}} \times \mathbf{100}\right) \text{.....3-2}$$

3.1.1.3 Ash content (NREL/TP-510-42622)

Ash determination of paper sludge followed protocols described by (Sluiter *et al.* 2005). Weighed crucible dishes were first placed in a muffle furnace at 575 ± 25 ºC for a minimum of four hours, afterwards placed in a desiccator for an hour and weighed again. This was repeated until constant weight is attained. Oven dried sample was then thoroughly mixed and 0.5-2 g of the sample carefully added to the pre-ignited crucible and weighed. The crucible with sample was placed into a muffle furnace at 575 ± 25 °C for 24 ± 6 hours. The crucible with sample was allowed to cool down in a desiccator for a minimum of four hours after ashing, and weighed until constant weight was achieved. The ash content was evaluated using equations 3.3 and 3.4 below.

$$\boldsymbol{Oven\ dried\ weight\ (ODW)} = \frac{\boldsymbol{weight_{air\ dried\ sample}} \times \%\boldsymbol{Total\ solids}}{\mathbf{100}} \quad \text{.....3-3}$$

$$\%\boldsymbol{Ash} = \frac{\boldsymbol{weight_{crucible\ plus\ ash} - weight_{crucible}}}{\boldsymbol{ODW_{sample}}} \times \mathbf{100} \quad \text{.....3-4}$$

3.1.1.4 Volatile and fixed solids (EPA Method 1684-821/R-01-015)

Volatile solids was determined by following protocols outlined by (Telliard, 2001). Ignited weighed clean watch glasses or crucibles at 550°C for an hour in a muffle furnace. Evaporating dishes were cooled and stored in a desiccator. Each dish was weighed and stored prior to use. Oven dried sample was then thoroughly mixed and 0.5-2 g of the sample carefully added to the pre-ignited crucible and weighed. Mass of duplicate aliquots did not differ by 10%. The evaporating dishes containing the dried residues were placed in a muffle furnace and the furnace heated to 550°C and ignited it for 2 hours. The crucible with sample was allowed to

cool down in a desiccator and weighed. Repeated igniting was done for 30 minutes, cooling, desiccating, and weighing steps followed until constant weight was achieved. The volatile and fixed solids was calculated from Equations 3.5 and 3.6 below.

$$\%\boldsymbol{volatile\ solids} = \frac{\boldsymbol{weight_{oven\ dried\ sample\ and\ dish} - weight_{residue\ and\ dish\ after\ ignition}}}{\boldsymbol{weight_{oven\ dried\ sample\ and\ dish} - weight_{dish}}} \times \mathbf{100} \quad \textbf{.....3-5}$$

$$\%\boldsymbol{fixed\ solids} = \frac{\boldsymbol{weight_{residue\ and\ dish\ after\ ignition} - weight_{dish}}}{\boldsymbol{weight_{oven\ dried\ sample\ and\ dish} - weight_{dish}}} \times \mathbf{100} \quad \textbf{.....3-6}$$

3.1.1.5 Water holding capacity

Water holding capacity of sample was determined following modified protocols outlined by Boshoff *et al.* (2016). Solid material was dried at 105°C in an oven for 24 hours. 1 g of oven dried sample was carefully weighed and added to a previously weighed 15 ml conical tube. 10 ml of water was added to the conical tube with paper sludge and weighed. The mixture was vortexed to allow proper mixing and allowed to saturate for 24 hours at 20°C. Afterwards, the conical tube with mixture was centrifuged at 2500 relative centrifugal force and supernatant decanted. The water holding capacity was determined from Equation below.

$$\boldsymbol{Water\ holding\ capcaity\ (ODW)} = \frac{\boldsymbol{wet\ sample\ (kg) - dry\ sample\ (kg)}}{\boldsymbol{dry\ sample\ (kg)}} \quad \textbf{.....3-7}$$

3.1.1.6 Structural carbohydrates and lignin (NREL/TP-510-42618)

The determination of structural carbohydrates and lignin followed protocols outlined by (Sluiter *et al.* 2012). This method involved acid hydrolysis of biomass sample followed by analysis of acid soluble and insoluble material. For the duration of the hydrolysis step, polymeric carbohydrates were hydrolysed into monomers that were soluble in the hydrolysis liquid and could be detected by HPLC. Also, lignin fractionated into acid soluble and insoluble components. The proportion of acid insoluble residue (AIR), acid insoluble lignin (AIL), acid soluble lignin (ASL) and lignin were determined using the equations 3.8 – 3.12 below.

$$\%\boldsymbol{AIR} = \frac{\boldsymbol{weight_{crucible\ and\ residue} - weight_{crucible}}}{\boldsymbol{ODW_{sample}}} \times \mathbf{100} \quad \textbf{.....3-8}$$

$$\%\boldsymbol{AIL} = \left(\frac{\boldsymbol{weight_{crucible\ and\ residue} - weight_{crucible}}}{\boldsymbol{ODW_{sample}}} - \frac{\boldsymbol{weight_{crucible\ and\ ash} - weight_{crucible}}}{\boldsymbol{ODW_{sample}}}\right) \times \mathbf{100} \quad \textbf{.....3-9}$$

$$\%\boldsymbol{ASL} = \frac{\boldsymbol{UV_{absorbance} \times volume_{hydrolysis\ liquor} \times dilution}}{\boldsymbol{absorptivity}(\epsilon) \times \boldsymbol{ODW_{sample}} \times \boldsymbol{pathlength}} \times \mathbf{100} \quad \text{.....3-10}$$

$$\boldsymbol{dilution} = \frac{\boldsymbol{volume_{sample} + volume_{diluting\ solvent}}}{\boldsymbol{volume_{sample}}} \quad \text{.....3-11}$$

$$\%\boldsymbol{lignin} = \left((\%\boldsymbol{AIL} + \%\boldsymbol{ASL}) \times \frac{\mathbf{100} - \%\boldsymbol{extractives}}{\mathbf{100}}\right) \quad \text{.....3-12}$$

3.1.1.7 Extractives (NREL/TP-510-42619)

The determination of extractives in biomass sample was outlined by (Sluiter et al. 2008). This method comprised of two-step extraction process to take out water soluble and ethanol soluble material. Ethanol extraction was necessary to eliminate interfering waxy components that precipitated during the filtration of the acid hydrolysate in further analyses. All glassware were dried prior to use. Biomass sample weighing between 2 - 10 g was added to a tared extraction thimble, and inserted into the soxhlet tube. Water extractives were analysed using water in the tared receiving flasks, with reflux of 6-24 hours. The ethanol extractives were analysed by placing water in the ethanol receiving flask, with reflux taking place for 16-24 hours. The extracted solids were placed on filter paper in a Buchner funnel. The percentage extractives were calculated using Equation 3.13 below.

$$\%\boldsymbol{extractives} = \frac{\boldsymbol{weight_{flask\ and\ extractives} - weight_{flask}}}{\boldsymbol{ODW_{sample}}} \times \mathbf{100} \quad \text{.....3-13}$$

3.1.2 Ultimate analysis

Ultimate analysis were performed to determine the elemental composition of the paper sludge. The analysis was conducted with Vario EL Cube Elemental Analyser, based on ASTM D4239 and ASTM D5373 standard methods. Dried paper sludge samples were combusted in. a column filled and enriched with Tungsten Trioxide (WO_3) and oxygen at a temperature of 1050 °C. The combustion produced CO_2, H_2O, NOX, SO_2 and SO_3 from which the amounts of different elements were determined.

3.1.3 Calorific value

The calorific values of the paper sludge and solid residues after biochemical processing were determined using an Eco Cal2K bomb calorimeter. The calorimeter works by loading 0.2 g of the sample into the crucible followed by the crucible being placed inside a cylinder and afterwards the cylinder was pressurised with oxygen within the pressure range of 1500 to 2500 kPa.

3.1.4 Water quality analysis for process water (liquid sample)

Water quality analysis was central to this research study, since the constituents of process wastewater from pulp and paper mills was dependent on several factors as established in the literature review.

3.1.4.1 Process wastewater storage

The corresponding process water were also obtained from the same paper and pulp mills with the only exception being that of tissue printed recycle process wastewater that was acquired from Kimberly-Clark Enstra mill. Both Twincare Bellville and Kimberly-Clark Enstra mill utilize similar feedstock and milling processes and thus produce comparable effluent streams. After collection from the indicated mills, the different process wastewater samples were stored in a cold storage room at -8°C in order to thwart the development of any microbial activity.

3.1.4.2 pH

pH of process water was measured by Crison pH meter GLP 21 purchased from Lasec, South Africa. The pH meter was calibrated with standard buffer solutions, pH of 4, 7 and 9 prior to use. After calibration, the pH electrode was dipped into a sample volume and the pH value recorded.

3.1.4.3 Chemical Oxygen Demand (COD)

Chemical oxygen demand (COD) is defined as the amount of oxygen required for the oxidation of all organic substances in water. Due to its special properties, the dichromate ion is the specified oxidant in COD determination. The Spectroquant Prove 300 (Merck, Darmstadt, Germany) equipment with Chromosulfuric acid oxidation method was used to determine the COD of process water in mg/L.

3.1.4.4 Light and Heavy metals

Light and heavy metal analysis was conducted for various metals expected in process water and paper sludge. Inductive coupled plasma mass spectrometry (ICP-MS) was utilized to determine the amount of various metals, some listed in **Table 3-1**. ICP-MS comprises of a flowing stream of argon gas ionized by an applied radio frequency field typically oscillating at 27.1 MHz. An aerosol of the sample is generated a pneumatic nebulizer and spray chamber and carried into the plasma chamber by an injector. Ionization of high percentage of atoms at 6000 to 8000 K produces ionic emission spectra and are converted into analyte concentration using ICP-MS calibration standards.

Table 3-1. Heavy metal elements concentration range

Element	Estimated Concentration Range
Magnesium (ppm)	(- 100) mg/L
Aluminium (ppb)	(- 100) µg/L
Boron (ppb)	(- 700) µg/L
Chromium (ppb)	(5 - 700) µg/L
Cadmium (ppb)	(3 – 1500) µg/L
Cobalt (ppb)	(- 50) µg/L
Copper (ppb)	(50 - 1500) µg/L
Mercury (ppb)	(<1) µg/L
Lead (ppb)	(10 - 500) µg/L
Manganese (ppb)	(- 7100) µg/L
Nickel (ppb)	(10 - 900) µg/L
Zinc (ppb)	(50µg/L – 15 ppm)

3.1.4.5 Total Suspended solids (APHA Method 2540 D)

Solids analysis in process water is important in the control of biological wastewater treatment. A well-mixed sample volume that would yield between 2.5 to 200 mg dried residues was chosen and filtered through a weighed glass-fiber filter. The residues retained on the filter was dried to constant weight at 103 to 105°C. The increase in filter weight indicated the total suspended solids and was calculated from the equation below.

$$\boldsymbol{mg\ total\ suspended\ solids/L} = \frac{weight_{filter+dried\ residues,mg} - weight_{filter,mg}}{sample\ volume,mL} \times 1000$$

3.2 PRODUCT STREAM ANALYSIS

3.2.1 Fermented and digested paper sludge solid residues

All analysis described in section 3.1.1 was also performed on fermented and digested paper sludge residue. This would indicate the amount of water reclaimed and help with mass balance for water and solid over the entire proposed research outline.

3.2.2 Water analysis after sequential fermentation and anaerobic digestion

All water quality analysis recommended in section 3.1.4 was performed for separated liquid obtained after sequential fermentation and anaerobic digestion of paper sludge with process water. Supernatant liquid would be obtained after centrifugation at 6000 rpm. This would indicate whether, wastewater treatment is being achieved on reclaimed water after sequential fermentation and anaerobic digestion.

3.2.3 HPLC analysis for ethanol and sugars produced from fermentation and volatile fatty acids (VFAs) production during anaerobic digestion of fermented stillage

VFAs, sugar and ethanol concentrations was measured by 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 ethanol yield and percentage theoretical yield of ethanol was evaluated as indicated in **Table 3-2** below.

Table 3-2. Ethanol yield and % theoretical yield determination

Ethanol/Sugar Concentration (g/L)	Ethanol yield (g ethanol/g glucose consumed)[1]	% Theoretical yield	Ethanol productivity (g/L hr)[2]
Determined from fermentation broth with HPLC	$\frac{Ethanol\ concentration\ (\frac{g}{L})}{Total\ glucose\ consumed\ (\frac{g}{L})}$	$\frac{Ethanol\ yield}{0.511}$	$\frac{Ethanol\ concentration}{time}$

[1]Determined from straight line section (at least 3 data points) of ethanol concentration profile

[2]Time when ethanol production levelled off

3.2.4 Biogas measurement and analysis

Important biogas and methane yield parameters are summarized in **Table 3-3**. Biogas from bio-methane potential (BMP) bottles were measured every 24 hours using 50 ml syringes. The amount of displaced biogas was recorded daily and the required biogas production evaluated as indicated in table (**Table 3-3**). The biogas composition was determined every 5 days using compact GC 4.0 Gas Chromatography (GC)

equipment. From the biogas production and GC compositional analysis, the cumulative bio-methane production was calculated as indicated in **Table 3-3**. Also, the elemental composition of paper sludge could be used to determine the theoretical methane potential (BMP_{ThAtC}) of paper sludge (Raposo *et al.*, 2011). This in turn would be used to determine the biodegradability (BD_{CH4}) of the various paper sludge.

Regarding 30 L digesters, the biogas production rate was determined from the Data Acquisition System of the digester set-up. Likewise, biogas was collected in tedlar bags from 30 L digesters every 5 days and analysed using compact GC 4.0 Gas Chromatography (GC) equipment for the different gases and their respective fractions.

Table 3-3. Biogas and bio-methane determination

	Evaluation formulae
Cumulative biogas produced	$\sum_{Day\ 0}^{Day\ N} (biogas\ produced\ per\ day(mL))$
Cumulative biogas production rate	$\frac{Cumulative\ biogas\ produced\ (m^3)}{Total\ solids\ fed\ (kg)}$ & $\frac{Cumulative\ biogas\ produced\ (m^3)}{volatile\ solids\ fed\ (kg)}$
Methane percentage	*Determined from Biogas GC analyser*
Cumulative methane produced	$\sum_{Day\ 0}^{Day\ N} (\%methane \times biogas\ produced\ per\ day(mL))$
Cumulative Methane production rate	$\frac{Cumulative\ methane\ produced\ (m^3)}{Total\ solids\ fed\ (kg)}$ & $\frac{Cumulative\ methane\ produced\ (m^3)}{volatile\ solids\ fed\ (kg)}$
	$\frac{Cumulative\ methane\ produced\ (L)}{COD\ removed\ (kg)}$
Theoretical methane potential (BMP_{ThAtC})	$\left(\frac{\left(\left(\frac{a}{2}\right)+\left(\frac{b}{8}\right)-\left(\frac{c}{4}\right)\right)*22400}{(12a+b+16c)}\right)\frac{m^3}{kg\ volatile\ solids\ fed}$
Biodegradability ($\%BD_{CH4}$)	$\frac{Cumulative\ methane\ yield}{Theoritical\ methane\ protential}*100$

3.3 EXPERIMENTAL APPROACH

This research study followed the experimental approach as indicated in **Figure 3-1**. The experimental work began with the collection of three paper sludge samples from three major mill operators across South Africa. This collection task was done previously by (Williams, 2017) from our research group. The corresponding process water samples were also collected recently from the same mills except for one type of process wastewater obtained from another different mill.

At the onset, the effect of process water on yeast, enzyme and bacteria performance was investigated. This is designated by the yellow shaded section in **Figure 3-1**. The screening stage, of fundamental importance to this research study was undertaken to observe whether solely employing process water in biochemical processes was applicable and if not, what co-feeding ratio of process water and fresh water would be suitable for both fermentation and anaerobic digestion.

Yeast adaptation screening were performed using five distinct processed water to clean water ratios (0, 25, 50, 75,100% process water) with glucose as carbon source. A yeast inoculum dosage of 5% (v/v) was used. Based on the results gathered from yeast adaptation screening, batch PS SSF experimental runs with four different enzyme dosages (5, 10, 15 and 20 FPU/gds) was conducted in shake flask.

To test the process for high solids loading and scale-up issues, fed-batch SSF runs were performed in 5 L and 150 L bioreactors based on results obtained from yeast and enzyme screening processes. In the same manner, anaerobic digestion of paper sludge with process water as make stream was also tested in 30 L digesters and was compared to the results obtained in serum bottles. Stillage obtained after fermentation in 150 L fermenter was fed into 30 L anaerobic digesters for further water reclamation and biofuel production. The blue shaded regions in **Figure 3-1** indicates the scale up stage.

Concurrent treatment of process water and water reclamation via biochemical processes is of prime importance to this study. Hence, water quality analysis indicated in **Section 3.1.4** (brown shaded region in **Figure 3-1)** was performed before and after both fermentation and anaerobic digestion to ascertain water reclamation and water quality improvements were being achieved.

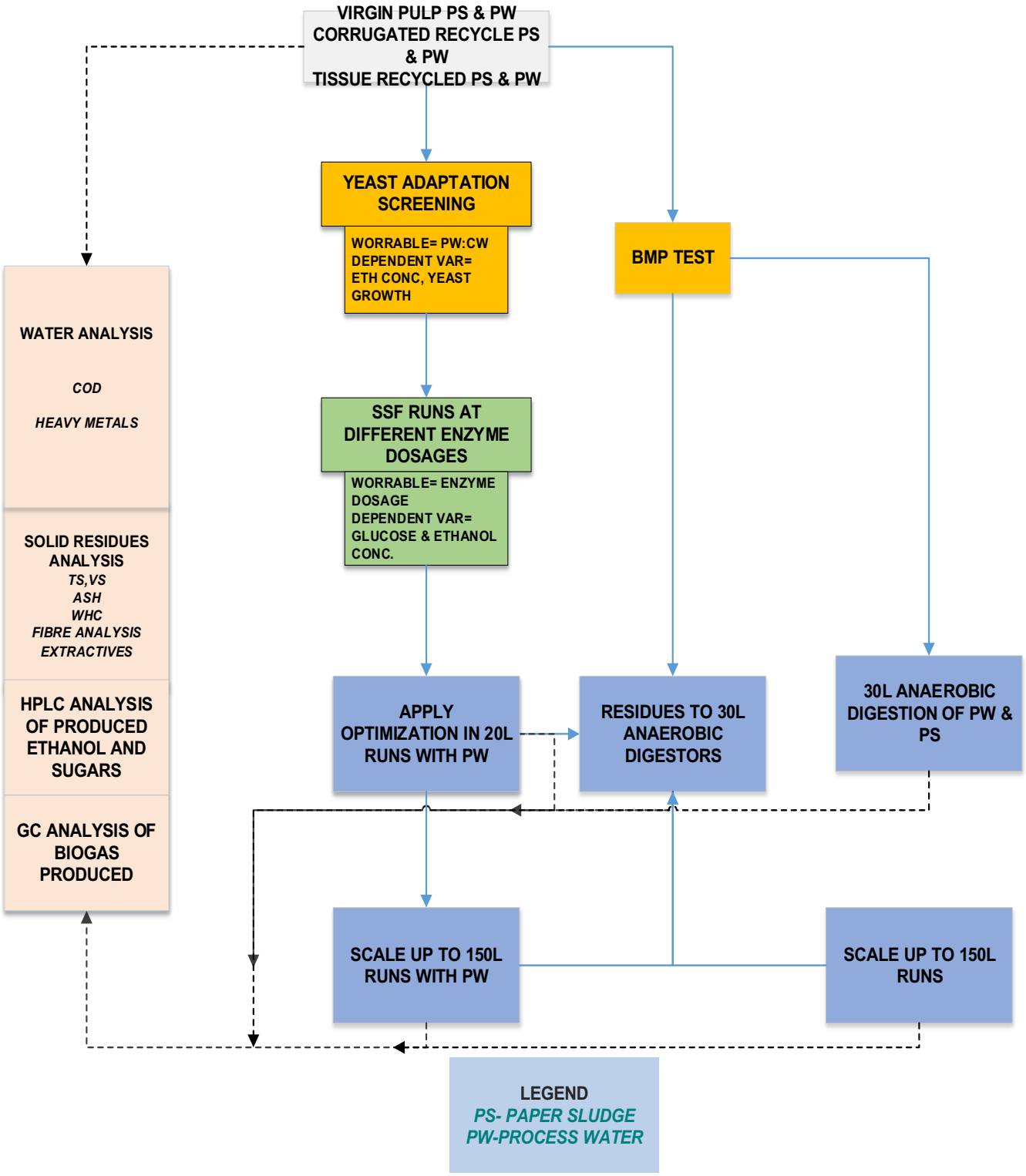


Figure 3-1: Experimental approach to study

3.3.1 Process water yeast adaptation screening

Yeast adaptation screening in process water samples for ethanol concentration was performed in batch cultures using 250 ml cotton plugged Erlenmeyer shake flasks. Batch media comprised of five distinct PW/CW (0, 25, 50, 75,100% process water) for each type of process water. Each batch fermentation media also included 3 g/L corn steep liquor (Sigma-Aldrich, South Africa), 0.62 g/L magnesium sulphate heptahydrate (Merck, South Africa) as nutrient source. Moreover, a carbon source of glucose (Merck, SA) at loading of 50 g/L was further added to the media with a final working volume of 100 ml and autoclaved for 15 minutes at 121°C. Yeast inoculum was grown in media containing 10 g/L yeast extract, 20 g/L, peptone and 20 g/L glucose for 18 hours at 37°C and 150 rpm in an orbital shaker. Finally, the prepared media was inoculated with 5 mL *S. cerevisiae* MH1000 seed culture and incubated at 37°C and 150 rpm for 144 h in an orbital shaker. Sampling was done at regular intervals to determine the yeast growth and experimental ethanol concentration.

3.3.2 Process water SSF at different enzyme dosages with paper sludge

Enzymatic screening was carried out dependent on the process water performance at section 3.3.1. Paper sludge solids loading of 6% (w/w) was investigated for the three kinds of process water at four different enzyme dosages (5, 10, 15 and 20 FPU/gds). Each media consisted of the best performed PW/CW co-feeding ratio from the yeast adaptation screening with the same nutrient source as described in section 3.3.1 and subsequently autoclaved at 121°C for 15 minutes. Viscamyl Flow enzyme was introduced at a dosage of 5, 10, 15 and 20 FPU/gds. Afterwards, each media was inoculated with 5 mL *S. cerevisiae* MH1000 seed culture and incubated at 37°C and 150 rpm for 144 h in an orbital shaker. Sampling was done at regular intervals to determine ethanol and sugar concentration.

3.3.3 Process water batch and fed-batch SSF at different reactor levels at optimum conditions

Fed-batch SSF runs were carried out in 150 L and 5 L bioreactors to determine the effect of high solid loading and scaling up the process (**Figure 3-2**). The fermentation media consisted of same nutrient source as described in screening processes. Optimum conditions (solids loading, process water and clean water co-feeding ratio) were based on screening processes and previous studies reported by Boshoff *et al.* (2016) and Robus *et al.* (2016). Similarly, samples were taken every 12 hours and prepared for HPLC analysis to determine ethanol and sugar concentration.

Figure 3-2: 150L fermenter (*left*) and 5L bioreactor (*right*)

3.3.4 Bio-methane potential (BMP) tests for process water and paper sludge

Bio-methane potential test is used to determine the maximum methane potential of a substrate (**Figure 3-3**). The set of BMP tests conducted for this research study followed the protocol defined by (Angelidaki *et al,* 2009). The PW/CW ratios employed in the BMP tests were identical to section 3.3.1. Total solids loading of 6% was investigated for each corresponding paper sludge and process water. An inoculum concentration of 6.25% (v/v) was used for 6% solids loading. This corresponded to 10% (w/w) of the total solids in the serum bottles. A pH within 6.8-7.4 is essential for anaerobic digestion. Therefore, pH adjustment was done for virgin pulp BMP set, since its corresponding process water had an initial pH of 2. The other set of BMPs for the other two substrates and their corresponding process water did not require any pH adjustment, since their initial pH was already perfect for anaerobic digestion. The serum bottles were then plugged with thick butyl rubber stoppers and sealed with aluminium crimps (**Figure 3-3**). Finally, the sealed serum bottles were flushed with nitrogen for 5 minutes to purge oxygen from the serum bottles using two needles pricked into the butyl stopper, one connected to a nitrogen gas pipeline and other serving as a gas outlet (**Figure 3-3**). The BMP tests was conducted was at mesophilic temperature range, i.e. 37°C. The temperature of the BMP tests was maintained by using an oven incubator with an incubation period of 30-45 days.

Figure 3-3: Biomethane potential test (schematic diagram obtained from Angelidaki *et al,* 2009)

3.3.5 Batch anaerobic digestion of raw paper sludge and fermented residue in 30 L digesters

The laboratory scale-up of batch anaerobic digestion were conducted in eight custom built 30 L continuous stirred digesters (CSD) manufactured by Thermodynamics and Fluid Design (TFD) Ltd, South Africa. The digesters are an upgraded version of the similar equipment utilized by (Williams, 2017) from our research group (**Figure 3-4**). The CSD was made of rectangular shaped jacketed stainless steel vessel with working volume of 21 L. Placed on top of each digester lid was an improved motor connected to a shaft fitted with single impeller at the bottom of the digester that now has functionality of rotational speed control, a detachable feeding funnel, temperature probe, level indicator and two gas outlet valves with one connected to the gas flow manometer system (**Figure 3-4**). Jacketed vessels of the digester had water circulating in the jacket for temperature control. Liquid sampling and drain valves for the digester were located underneath the vessel, with a jacket drain valve also located underneath the vessel. Data from sensors for temperature control and gas flow manometer system was read and logged by the “Data Acquisition System” connected to the eight digesters.

Figure 3-4: 30 L anaerobic digesters

3.3.5.1 Parameters and Conditions

The total solids loading for the scale up in the CSDs differed from the BMP tests, raw tissue printed recycle paper sludge and corrugated recycle paper sludge was 10% (w/v) while virgin pulp paper sludge was 6% (w/v). Also, fermentation and anaerobic digestion of fermented stillage ran in parallel, hence there was no pressing, drying and preparation of fermented residue. Instead, after evaporation of ethanol, fermented stillage was transferred carefully into digesters to start the digestion process. Evaporation was conducted at the end of the fermentation at 70°C to get rid of ethanol with 5-10% water loss expected when compared to industrial distillation. The exact conditions as in the BMP tests were also applied to CSD digesters with intermittent agitation for 30 days.

3.4 MASS BALANCE FOR SEQUENTIAL FERMENTATION AND ANAEROBIC DIGESTION OF PROCESS WATER AND PAPER SLUDGE

Table 3-4. Mass balance for proposed study

Mass Balance for Fermentation
$$Mass\ In = Mass\ Out$$ $$Solids\ Fed\ + Water\ Fed = Fermented\ Residues + Water\ Out$$ $$Wet\ Paper\ sludge + Process\ water\ Fed = Fermented\ Residues + Process\ water\ Fed + Released\ Water_1 + Ethanol\ produced + Soluble\ Sugars$$
Where $$Wet\ Paper\ sludge = Dry\ Soilds + Entrapped\ Water$$ $$Dry\ Solids = Volatile\ Solids + Ash$$ $$Volatile\ Solids = Glucan + Xylan + Lignin + Extractives$$ $$Fermented\ Residues = Residual\ Glucan + Residual\ Xylan + Lignin + Extractives + Ash$$ $$Soluble\ Sugars = Residual\ Unconverted\ glucose\ and\ xylose$$ $$Released\ Water_1 = \%Entrapped\ water\ released\ after\ fermentation$$
Mass Balance for Anaerobic digestion
$$Mass\ In = Mass\ Out$$ $$Fermented\ Residues + Released\ Water_1 + Soluble\ Sugars = Digested\ Residues + Released\ Water_1 + Relaesed\ Water_2 + Biogas\ produced$$
Where $$Digested\ Residues = Residual\ Volatile\ Solids_{after\ digestion\ of\ Fermented\ Residues} + Ash$$ $$Released\ Water_2 = \%Entrapped\ water\ released\ after\ anaerobic\ digestion$$
Total Solids Balance
$$Dry\ Solids\ Fed = Digested\ Residues + Ethanol\ produced + Biogas\ produced$$
Total Water Balance
$$Water\ Fed = Water\ Out$$

$$Entrapped\ water\ in\ Paper\ sludge\ Fed + Process\ water\ Fed = Process\ water + Reclaimed\ Water + Residual\ Entrapped\ Water\ in\ Paper\ Sludge$$

Where

$$\boldsymbol{Reclaimed\ Water} = Released\ Water_1 + Released\ Water_2$$

$$\boldsymbol{Potential\ Recovarable\ Water}_{\boldsymbol{depending\ on\ Separation\ process\ applied}} = Process\ Water\ Fed + Reclaimed\ Water$$

CHAPTER 4: RESULTS AND DISCUSSION

4.1 CHARACTERIZATION OF PROCESSED WASTEWATER AND PAPER SLUDGE

4.1.1 Characterization of paper sludge

4.1.1.1 Compositional analysis of paper sludge.

Compositional analysis was performed to determine the carbohydrate, lignin and ash content of paper sludge derived from three different sources (**Table 4-1**). Virgin pulp PS (VP-PS) had the highest glucan fraction of 58.2% (w/w), a value at least 21% (w/w) higher that the glucan content of corrugated recycle paper sludge (CR-PS) and tissue printed recycle (TPR-PS). The superior glucan content of VP-PS is related to pulping technology used, this is aimed at producing high quality paper by removing lignin thereby enriching cellulose in the process (Sixta, 2008). Alternatively, CR-PS and TPR-PS produce low quality paper derived through a mechanical pulping process which do not remove lignin. TPR-PS consist predominantly of ash (i.e. 62.9%) whereas the ash content of VP-PS and CR-PS is only a quarter of its weight. The high ash content of TPR-PS could be attributed to ink and filler that accumulates when waste paper (i.e. newsprint and printing paper) is recycled (Boshoff *et al.*, 2016; Robus *et al.*, 2016).

Table 4-1: Chemical composition of the types of paper sludge

Paper sludge	Glucan (% w/w)	Xylan (% w/w)	Lignin (% w/w)	Extractives (% w/w)	Ash (% w/w)
VP-PS	58.2 ± 0.4	12.2 ± 0.1	4.1 ± 0.1	5.4 ± 0.1	20.8 ± 0.1
CR-PS	37.5 ± 0.4	13.1 ± 1.1	13.1 ± 0.1	10.4 ± 0.1	25.9 ± 0.3
TPR-PS	20.8 ± 0.1	4.9 ± 0.2	6.4 ± 0.1	5.1 ± 0.1	62.9 ± 0.4

4.1.1.2 Elemental analysis of paper sludge

Elemental analysis was conducted to determine the carbon, nitrogen, hydrogen and oxygen content of paper sludge (**Table 4-2**). The carbon and nitrogen content of a feedstock plays an important role in anaerobic digestion. Ideally the carbon to nitrogen (C/N) ratio of a substrate should vary between 20 to 30 (Malik *et al*. 1987; Kayhanian & Tchobanoglous, 1992). TPR-PS is the only substrate that fell within that range (**Table 4-2**). The other two substrates (i.e. VP-PS and CR-PS) displayed much higher C/N ratios (i.e. C/N > 57) which may negatively affect the overall biogas production (section 2.5.2.1).

Table 4-2: Elemental analysis of paper sludge

Paper sludge	Carbon (% w/w)	Hydrogen (% w/w)	Oxygen (% w/w)[1]	Nitrogen (% w/w)	C:N ratio
VP-PS	38.0 ± 0.6	5.6 ± 0.1	55.9 ± 0.8	0.5 ± 0.0	76:1
CR-PS	34.4 ± 0.0	5.0 ± 0.1	60.0 ± 0.2	0.6 ± 0.0	57:1
TPR-PS	23.5 ± 0.6	2.0 ± 0.0	73.7 ± 0.6	0.8 ± 0.0	29:1

[1]Oxygen % (w/w) determined from difference (100 – C – H – N)

4.1.1.3 Water holding capacity (WHC) of paper sludge

The water holding capacity refers to the amount of water a substrate can retain. The water holding capacities of VP-PS, CR-PS and TPR-PS were 8.0, 6.7 and 3.8 kg water/kg PS respectively. The high WHC of VP-PS and CR-PS could be a direct consequence of the morphology of fibres in paper sludge (Boshoff *et al.*, 2016). The higher the WHC, the greater the moisture content of paper sludge emanating from of various mills (Boshoff *et al.*, 2016). Consequently, the greater the amount of water that could be reclaimed instead of ending up in landfills.

4.1.2 Constituents of process water

The constituents of process water (PW) are displayed in **Table 4-3**. COD was chosen as the main parameter for identifying the quality of wastewater. VP-PW had the lowest COD concentration of 1 194 mg/L followed by TPR-PW and finally CR-PW at 4 775 mg/L. There is no direct evidence to support the wide variation in COD but could be due to the starting material or the pulping process or a combination of both factors. The process waters also contained heavy and light metal elements. These, however, were well below inhibitory concentrations discussed in section 2.6.1.

Table 4-3: Characteristic summary of recycled process water

	Virgin pulp PW	Corrugated recycle PW	Tissue printed recycle PW
COD (mg/l)	1194	4775	2618
pH	2.3	7.2	7.4
TSS (mg/l)	NA	343	NA
Boron (µg/l)	162	3810	3986
Vanadium (µg/l)	11	0.8	3.4
Chromium (µg/l)	1098	3.6	29
Cobalt (µg/l)	1.8	0.2	0.9
Nickel (µg/l)	27	1.8	4.7
Copper (µg/l)	43	1.0	4.5
Arsenic (µg/l)	3.1	1.7	2.7
Selenium (µg/l)	2.7	0.4	0.6
Strontium (µg/l)	964	815	297
Molybdenum (µg/l)	2.9	0.5	5.2
Cadmium (µg/l)	1.8	0.0	<0,02
Antimony (µg/l)	0.6	0.6	1.8
Barium (µg/l)	91	221	105
Mercury (µg/l)	<0.2	0.2	<0,2
Lead (µg/l)	17	0.2	0.2
Uranium (µg/l)			
Zinc (mg/l)	0.2	<0.2	<0.2
Aluminium (mg/l)	1.8	0.8	<0.2
Manganese (mg/l)	3.9	0.7	<0.2
Iron (mg/l)	5.3	1.6	<0.2
Calcium (mg/l)	280	476	188
Potassium (mg/l)	87	15	11
Magnesium (mg/l)	31	24	17
Sodium (mg/l)	1421	258	300
Phosphorous (mg/l)	8	<1	<1
Silicon (mg/l)	11	6.1	4.7

TSS- Total suspended solids; NA- Not Applicable

4.2 EFFECT OF PROCESS WATER ON YEAST, ENZYME AND ANAEROBIC BACTERIA

The potential effects of process water (PW) on yeast, enzyme and anaerobic digestion of paper sludge (PS) was investigated by conducting a series of fermentative screening and BMP tests. Bioconversion processes with recycled process water were compared to the same processes using clean water.

4.2.1 Effect of process water on *S. cerevisiae* MH100 yeast strain (Fermentation in batch culture)

Biomass and ethanol yield were investigated at different ratios of recycled process water (PW) to clean water (CW). Batch fermentations were conducted at different PW/CW co-feeding ratios ranging between 0 to 100% over 6 days' incubation period. Glucose was added as the sole carbon source at a concentration of 50 g/L. Biomass yield was measured at the end of fermentation while ethanol concentration was measured twice daily.

4.2.1.1 Effect of process water on yeast growth

Specific growth rate was not affected by process water. Similar growth rates were calculated for yeast growing in clean as well as process waster (**Table 5-1, Appendix**). Similarly, process water had no effect on the duration of the lag phase. Alternatively, measured biomass concentration in clean water was 20% to 35% higher than that of process water at any concentration (p-value (0.001) < 0.05) (**Figure 4-1**). This suggested that process water had some form of adverse effect on yeast although growth rates were unaffected. Process water is known to contain all kinds of toxic compounds and could have affected the final biomass concentration. This however didn't seem to affect ethanol production. This suggested the biological activity of yeast was increased in the presence of process water and that led to similar ethanol production in clean water. This observation was consistent with reported studies by Vertes *et al.* (2010), Zaldivar *et al* (2000) and Palmqvist and Hahn-Hägerdal (2000) who suggested successful ethanol production could achieved amid a decrease in biomass concentration.

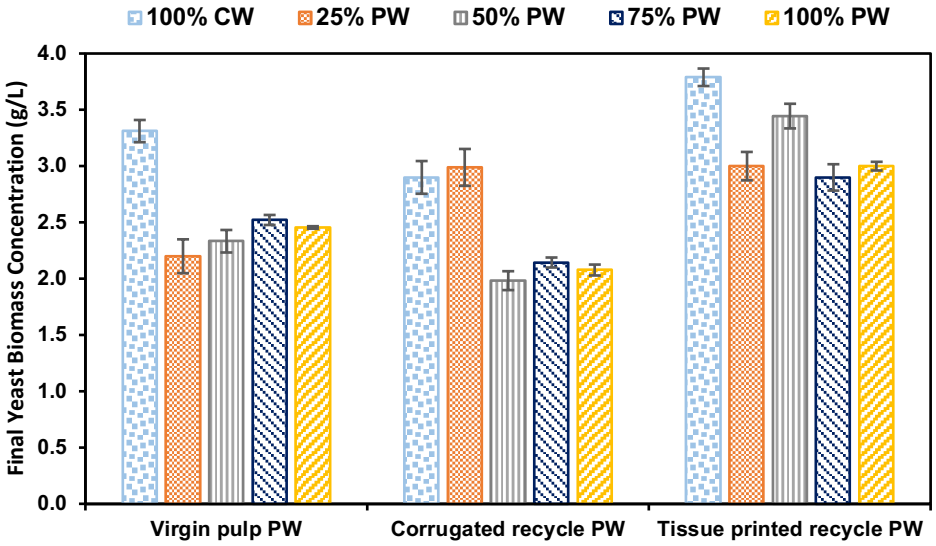


Figure 4-1: Final yeast biomass yield at different co-feeding of process water (PW) and clean water (CW) ratios after fermentation

4.2.1.2 Effect of process water on ethanol production

Process water had no effect on ethanol production. An ANOVA analysis conducted on the ethanol concentrations showed no statistical significance (*p*-value (0.114) > 0.05). Both clean and process water produced final ethanol concentrations ranging from 20 to 23 g/L. The produced ethanol concentration were relatively closer to the theoretical ethanol yield of 25 g/L (**Figure 4-2**). Although synergistic repression led to reduced yeast biomass concentration, successful production of ethanol was be attained. These observations were consistent with previous literature studies on various engineered *S. cerevisiae* strains that established successful ethanol production could be attained albeit a reduction in the biomass yield due minimum concentrations of inhibitory compounds (Vertes *et al.*, 2010).

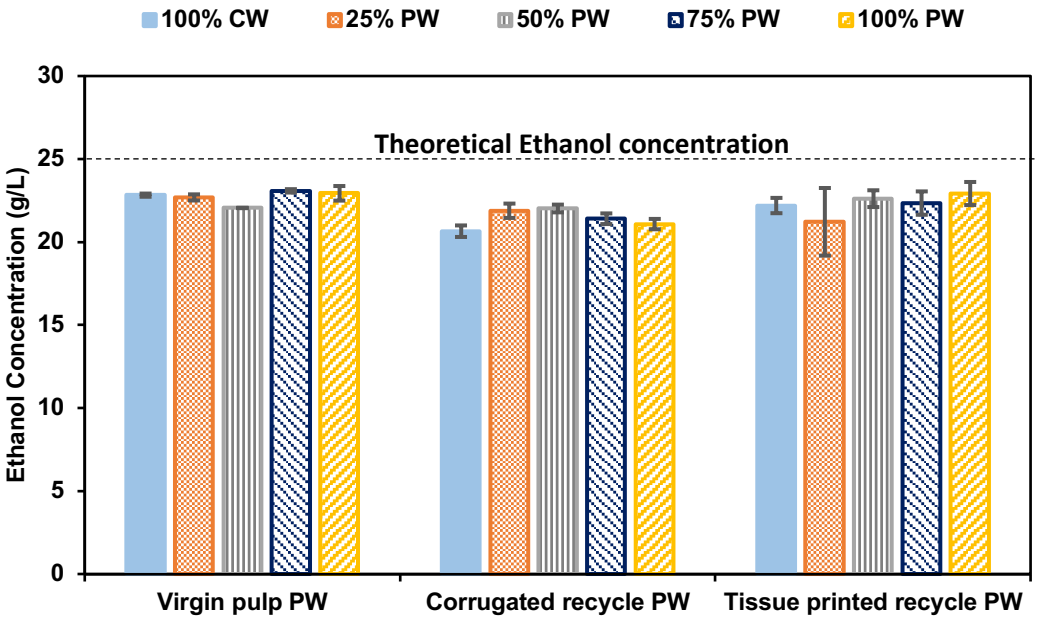


Figure 4-2: Ethanol production at different co-feeding ratios of process wastewater and clean water

4.2.2 Effect of process water on ethanol production from paper sludge

Batch fermentations were carried out with process water and paper sludge at a 6% (w/w) solids loading. An enzyme dosage ranging between 5 to 20 FPU/gds were administered. A control fermentation with clean water was conducted at 15 FPU/gds. Ethanol concentration was measured twice daily over a 6-day incubation period.

Process water had no effect on ethanol production from paper sludge, as compared to the control experiment with clean water (**Figure 4-3**). A linear correlation was also observed between ethanol production and enzyme dosage. An increase in enzyme dosage resulted in a higher ethanol concentration (**Figure 4-3**). This was similar to reported studies by Boshoff *et al.* (2016) in fermentation of paper sludge at different enzymes dosages of 5 and 15 FPU/gds. In addition Kang *et al.* (2011) observed a similar correlation of higher enzyme dosages leading to greater ethanol concentration in fermentation of paper sludge at different enzyme dosages of 5, 10, and 15 FPU/g-glucan. Furthermore, VP-PS generally produced higher ethanol concentrations as compared to CR-PS and TPR-PS. This could be due to the superior glucan content of VP-PS (**Table 4-1**).

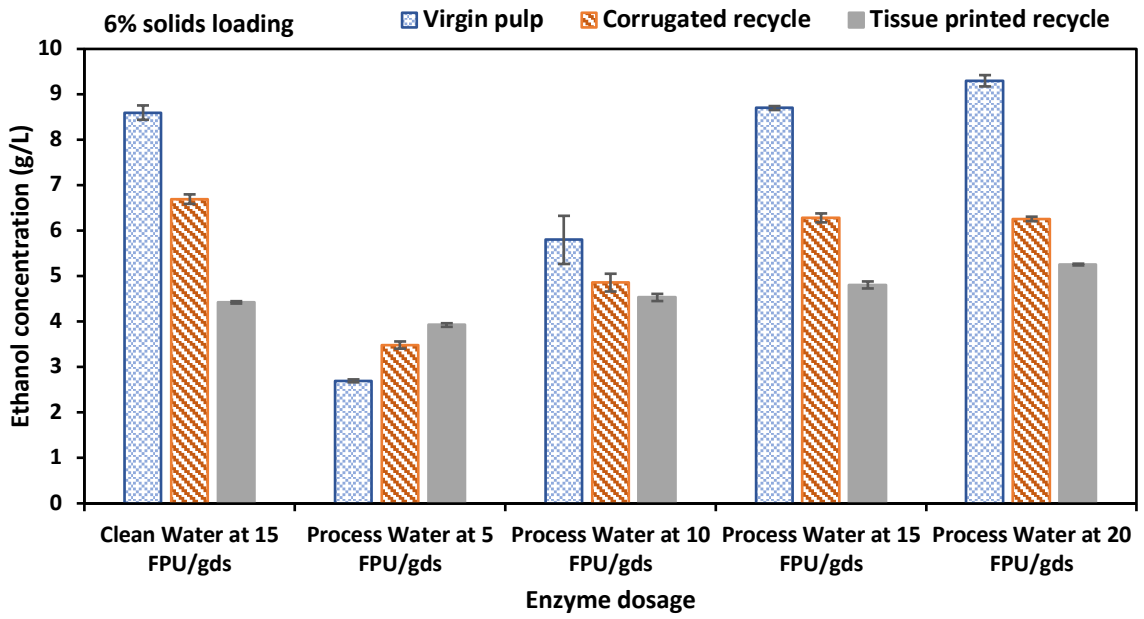


Figure 4-3: Ethanol yield at different cellulase dosages for fermentation of paper sludge (PS) with process water (PW) as make-up stream

4.2.3 Effect of process water on biogas production (Biomethane potential Screening)

Biogas and methane production were investigated as part of a biomethane potential (BMP) test. The tests were conducted at different PW/CW co-feeding ratios ranging between 0 to 100% with paper sludge being kept constant at a solid loading of 6% (w/w). The cumulative biogas and methane production for each were determined over a 30 to 45-day digestion period.

4.2.3.1 Biogas and methane production from paper sludge with different process water concentrations

Process water affected biogas and methane production, compared to clean water digestion, for CR-PS and VP-PS. Except for TPR PS, an ANOVA analysis conducted on the cumulative biogas production on basis of total solids fed showed statistical significance (*p*-value (0.018) < 0.05). Biogas and methane yields in VP-PW tests were at least 37% higher than clean water digestion of VP-PS (**Figure 4-4)**. Virgin pulp process water (VP-PW) had an auspicious effect on the digestion process and could be noticed in enhanced anaerobic biodegradability (BD_{CH4}) of VP-PS (**Table 5-2, Appendix**). This could be attributed to the low COD concentration of VP-PW (**Table 4-3**). Sierra-Alvarez and Lettinga (1991) reported that virgin pulp (Kraft) wastewater with COD concentration lower than 3 000 mg/L were largely not harmful to methanogenic activity and biogas production. Additionally, as compared to clean water, VP-PW have beneficial concentrations of macronutrients (light metals such as Na, K, Mg, Ca and Al) that are essential for microbial growth and better digestion performance (Chen, Cheng and Creamer, 2008). For example, calcium and potassium concentration

of VP-PW (**Table 4-3**) were within the optimum stimulatory range (about 200 mg/L for Ca and less than 400 mg/L for K) reported to enhance the performance of mesophilic digestion of a substrate (Chen, Cheng and Creamer, 2008).

Conversely, biogas and methane yields with CR-PW were about 10 to 23% lower than clean water digestion of CR-PS (**Figure 4-5**). The negative effect noticed in CR BMP assays could be attributed to the toxicity of inhibitory compounds found in CR-PW wastewater (McCarthy, Kennedy and Droste, 1990). Similar observations were reported by McCarthy, Kennedy and Droste (1990) while checking for the toxicity of inhibitory compounds found in chemithermochemical pulp wastewater on anaerobic bacteria. McCarthy, Kennedy and Droste (1990) and Sierra-Alvarez and Lettinga (1990) suggested the synergistic effect of lignin derivatives and resin acids (largely responsible for the dark brown color observed CR-PW) can partially inhibit methanogenic activity. Sierra-Alvarez & Lettinga (1991) also indicated that while some lignin compounds were nontoxic, others especially low molecular weight lignin model compounds can cause up to 50% inhibition of microbial activity at concentrations ranging from 3320 to 5950 mg COD/L. CR-PW had a COD content of 4 775 mg COD/L and disintegration of macromolecular lignin by pulping processes could likely leads to the generation of low molecular weight lignin derived compounds (Sixta, 2008). Furthermore, tissue printed recycle process water (TPR-PW) had no effect on biofuel production as biogas and methane yields ranged between 190 to 205 L/kg TS_{fed} and 95 to 108 L CH_4/kg TS_{fed} (**Figure 4-6**). This could be attributed to lower COD concentration and harmless metal concentration of TPR-PW (Chen, Cheng and Creamer, 2008).

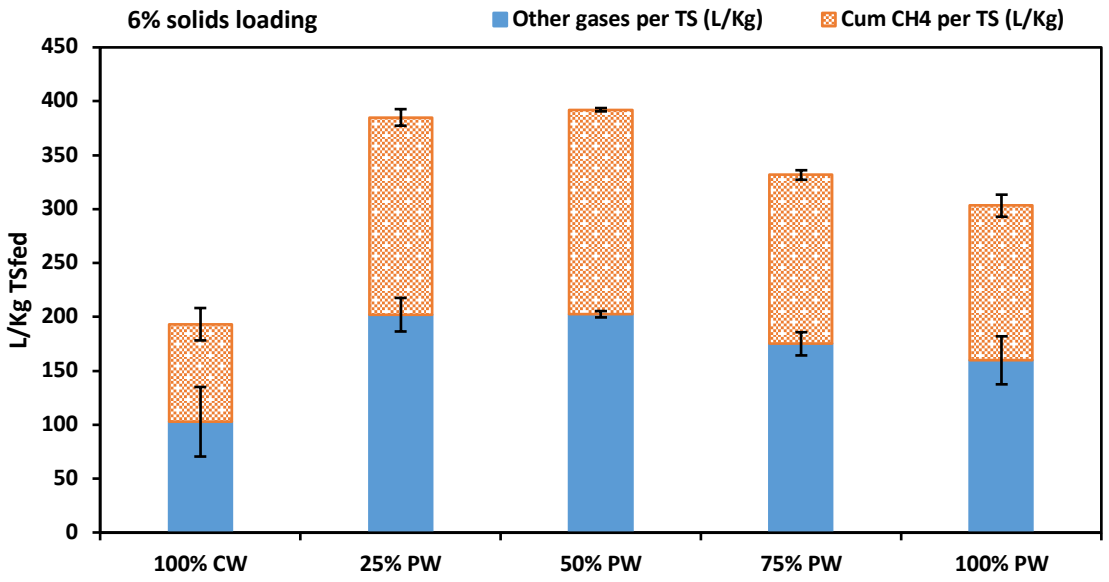


Figure 4-4:Cumulative biogas (CH_4 + other gases) and biomethane production for virgin pulp PS (VP-PS) at different co-feeding ratios of virgin pulp process water (PW) and clean water (CW)

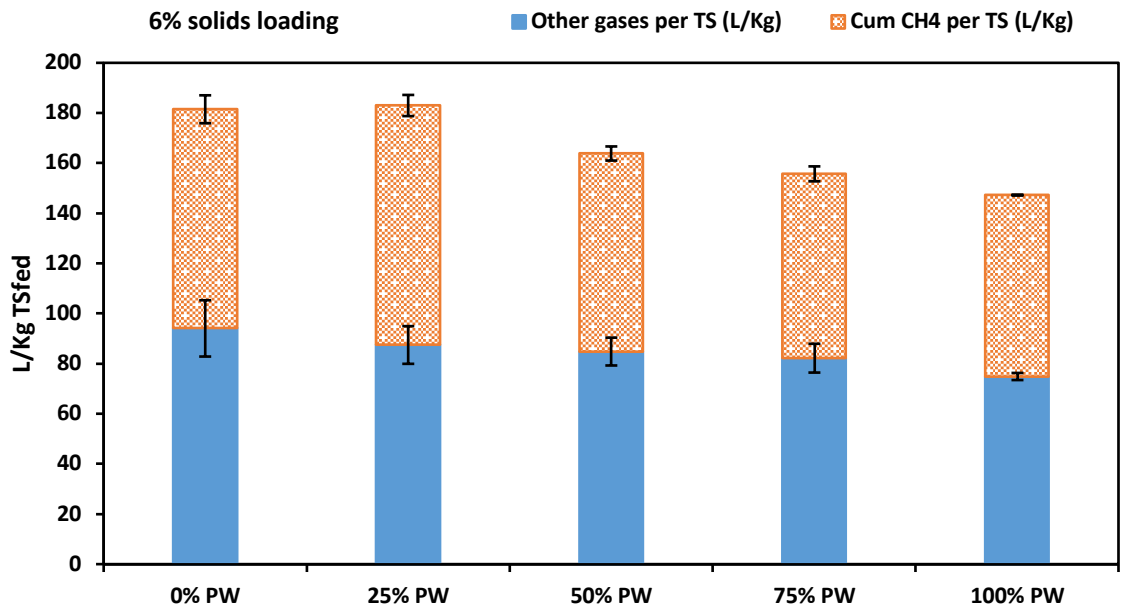


Figure 4-5: Cumulative biogas (CH_4 + other gases) and biomethane production for corrugated recycle PS (CR-PS) at different co-feeding ratios of corrugated recycle process water (PW) and clean water (CW)

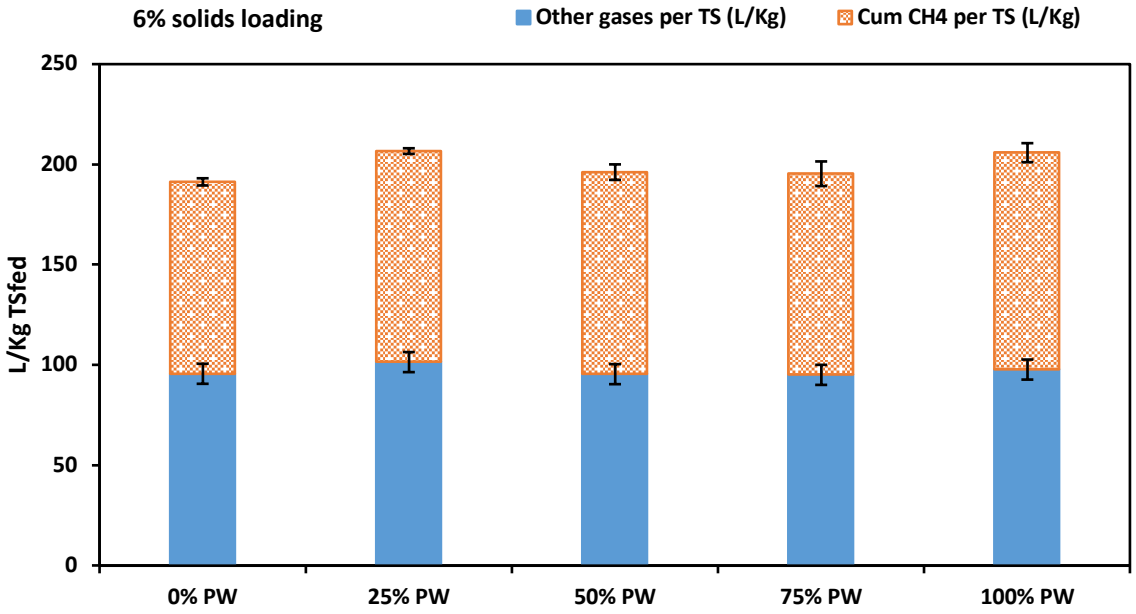


Figure 4-6: Cumulative biogas (CH_4 + other gases) and biomethane production for tissue printed recycle PS (TPR-PS) at different co-feeding ratios of tissue printed recycle process water (PW) and clean water (CW)

4.3 STANDALONE AND SEQUENTIAL FERMENTATION AND ANAEROBIC DIGESTION OF PAPER SLUDGE

This section compares the standalone fermentation and anaerobic digestion (AD) processes to a sequential fermentation-AD process, to determine impact on energy yield, water reclamation and water quality. The goal is to determine, which process gives the best technical benefits, and whether both fermentation and AD contribute significantly. The criteria for comparison of standalone fermentation and anaerobic digestion process to combined process are stated below:

1. Biofuel production. Paper sludge contain readily available carbon, through enzymatic and microbial activity can be converted to ethanol and methane.
2. Water reclamation. Raw paper sludge retains large amounts of water and this is discarded during landfilling. The water retaining capacity of paper sludge reduces with treatment. Thus, reducing the amount of discarded water.
3. Water quality. Some microbial activity reduces the chemical oxygen demand (COD) of water. The quality of the water can therefore be improved before it is reused.
4. Water reusing. Usage of process water instead of municipal water for bioprocessing.

Fermentation and anaerobic digestion, with paper sludge as the starting substrate, were covered in sections 4.3.1 and 4.3.2. The combined process was detailed in section 4.3.3, where it was compared to the individual technologies.

4.3.1 Fermentation of paper sludge in 5 L and 150 L bioreactors

Fed-batch simultaneous saccharification and fermentation (SSF) experiments were conducted in 5 L and 150 L bioreactors. Operating conditions such as solids loading, enzyme dosage and feeding approach were based on optimization studies conducted by Boshoff *et al.* (2016) and Robus *et al.* (2016) from our research group. Boshoff *et al.* (2016) recommended an optimum solids loading of 18 and 27% (w/w) for fermentation of virgin pulp PS and corrugated recycle PS respectively with a feeding approach of 3% (w/w). In addition, enzyme dosages of 20 and 11 FPU/gds were recommended for virgin pulp PS and corrugated recycle PS respectively (Boshoff *et al.*, 2016). Robus *et al.* (2016) based on optimization studies on fermentation of tissue printed recycle PS recommended a solids loading of 33% (w/w) with cellulase dosage of 15 FPU/gds. The notable difference is the use of process water instead of clean water, as the former had no effect on fermentation (sections 4.2.1 and 4.2.2). Samples were taken twice daily to determine the ethanol and sugar concentration over a 7-day period.

4.3.1.1 Ethanol production from paper sludge with process water in 5 L bioreactors

An ANOVA analysis conducted on the produced ethanol concentration from the three paper sludge fermentations showed statistical significance (*p*-value (0.028) < 0.05). Virgin pulp PS (VP-PS) produced the highest ethanol concentration of 49.6 ± 0.8 g/L as compared to the other paper sludges **(Figure 4-7)**. At the final feeding of 60 hours, the ethanol concentration was 38.7 g/L ± 3.2 for VP-PS **(Figure 4-7)**. Following the last feeding point for VP-PS, ethanol concentration increased by 28% to a stationary ethanol concentration of about 50 g/L with a productivity of 0.459 g/(L.hr) **(Figure 4-7)**. Tissue printed recycle PS (TPR-PS) yielded the second best ethanol concentration of 44.6 ± 0.1 g/L with a productivity of 0.362 g/(L.hr) (**Figure 4-9**). After the last feeding was added at 120 hours, ethanol concentration stabilized for about 24 and then finally increased to about 49 g/L at the end of fermentation for TPR-PS (**Figure 4-9**). Furthermore, corrugated recycle PS (CR-PS) yielded a peak ethanol concentration of 42.9 ± 1.8 g/L after suffering from lactic acid contamination (**Figure 4-8**).

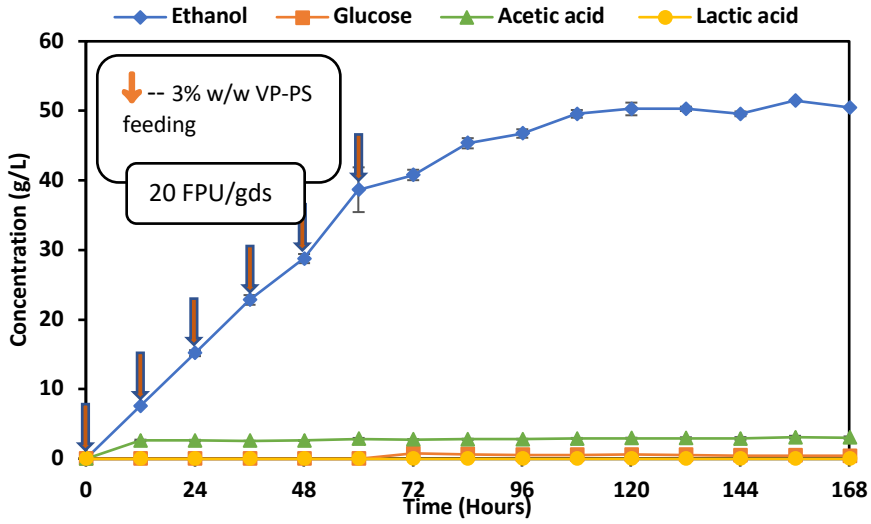


Figure 4-7: Ethanol concentration profile for 5 L fermentation of virgin pulp PS with PW; arrows ↓ represents feeding points

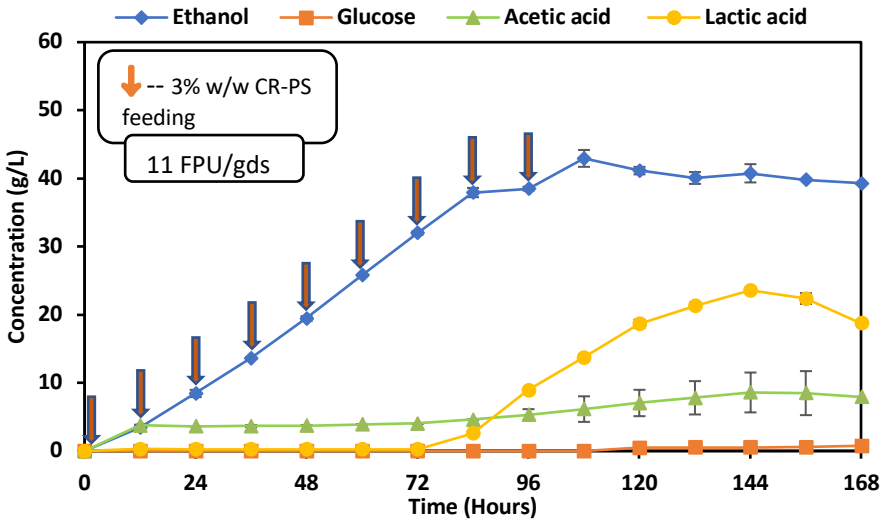


Figure 4-8: Ethanol concentration profile for 5 L fermentation of corrugated recycle PS with PW; arrows ↓ represents feeding points

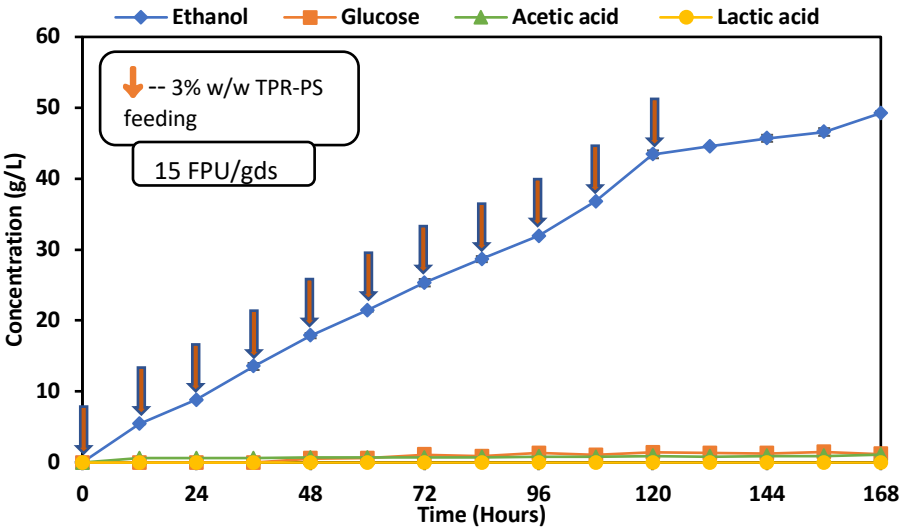


Figure 4-9: Ethanol concentration profile for 5 L fermentation of tissue printed recycle PS with PW; arrows ↓ represents feeding points

A peculiar observation noticed was the increase in lactic acid concentration to a high of 23.5 g/L after 72 hours of fermentation of CR-PS (**Figure 4-8**). Such high concentration of lactic acid indicated the fermentation broth became contaminated after 72 hours of fermentation. Although SSF was not thoroughly conducted in aseptic conditions due fed-batch mechanism employed, the lack of lactic acid production in SSF of VP-PS and TPR-PS suggested contamination in CR-PS fermentation stemmed from lactic acid bacteria (LAB) already present in CR-PS. Even with frequent sterilization of CR-PS in autoclavable bags, this phenomena was consistently observed and fermentation could not go beyond 4 days without lactic acid production. The ineffectiveness of sterilization could be attributed to contaminated CR-PS originating from the mill as a result of its production and handling of paper sludge. Therefore getting rid of the background bacterial load (LAB) already present in CR-PS before fermentation became problematic. Reported study by Jordan and Cogan (1999) established, it is possible, given enough time, that survived but injured lactic acid bacteria cells after sterilization could sufficiently recover and produce lactic acid.

The production of lactic and acetic acid were due to diversion of produced glucose for bacteria growth (Narendranath *et al.*, 1997). Lactic and acetic acid concentration above 9 g/L and 4 g/L respectively appears to synergistically inhibited performance of *S. Cerevisiae* MH1000 yeast strain resulting in the plateauing of ethanol production (**Figure 4-8**). This was similar to observations made by Dreyer (2013), as ethanol production stagnated as a result of acetic acid concentration above 3.5 g/L inhibiting *S. Cerevisiae* MH1000. In addition, Ngang *et al.* (1990) and Beckner *et al.* (2011) established that about 10 g/L of lactic acid in combination with acetic acid could lead to inhibition of *S. Cerevisiae* yeast and decrease in the rate of ethanol production. The LAB contamination had a significant impact on ethanol production as a result of inhibition of *S. Cerevisiae* yeast and certainly would affect subsequent anaerobic digestion of stillage (discussed in section 4.3.3). Provided the fermentation of CR-PS was stopped at 84 hours, an ethanol concentration of 37.9 ± 0.7 g/L at this point was only 8% lower than the peak ethanol concentration of 42.9 ± 1.8 g/L at 108 hours (**Figure 4-8**). At 84 hours of fermentation, the lactic acid concentration was 2.6 g/L, this was just 6% of ethanol production as compared to 32% of ethanol production at 108 hours. Subsequent calculation of yield and productivity for CR-PS were therefore determined using an ethanol concentration of 37.9 ± 0.7 g/L.

The chemical composition of PS significantly affects fermentation yield markers such as ethanol concentration (Boshoff *et al.*, 2016). VP-PS has the highest glucan content (58.2% w/w), almost double that

of the other substrates (**Table 4-1**). This favoured ethanol production and thus VP-PS gave the highest ethanol yield, productivity and cellulose conversion (**Table 4-4**). Although, CR-PS had the second highest glucan fraction (37.5% w/w), it produced the least ethanol yield of 126.5 kg ethanol/ton dry PS (**Table 4-4**). This could be attributed to the substantial amount of lignin and hemicellulose still present in CR-PS (**Table 4-1**). The residual lignin and hemicellulose in CR-PS impede enzymatic hydrolysis of cellulose resulting in poor digestibility and less available glucose for ethanol production (Boshoff *et al.*, 2016). This resulted in less cellulose conversion (73.8%) and more glucan (11.8%) still remaining in fermented residual solids of CR-PS (**Table 4-5**). TPR-PS produced the second best ethanol concentration with a cellulose conversion 8% higher than that of CR-PS. This could be attributed to the low lignin and hemicellulose content of TPR-PS (**Table 4-1**), this leads to easily accessible cellulose fibres for enhanced enzymatic hydrolysis (Boshoff *et al.*, 2016, Bester, 2018). Although TPR-PS had the highest solids loading of 33% (w/w), the low water holding capacity (3.8 kg water/kg PS) as compared to the other paper sludges improved hydrolysis due to availability of more free water in the fermentation broth and also prevented high viscosity that leads to mixing difficulties (Boshoff *et al.*, 2016).

Another contributing factor to fermentation performance is the cellulase dosage for each paper sludge (optimized dosages recommended by Boshoff *et al.* (2016) and Robus *et al.* (2016)). Apart from the favourable compositional properties of VP-PS and TPR-PS (**Table 4-1**), the higher enzyme dosages of 20 and 15 FPU/gds also contributed to the better fermentation performance as compared to CR-PS (**Table 4-4**). Paper sludge fermentation studies by Prasetyo & Park (2013) and Kang *et al*. (2011) indicated that higher ethanol concentrations were achieved with increasing enzyme dosages.

Table 4-4: Mass balance for SSF of PS with PW in 5L Fermenters

Operating Conditions	Units	Virgin pulp	Corrugated recycle	Tissue printed recycle
Enzyme dosage	FPU/gds	20	11	15
	FPU/g-glucan	38.4	29.3	72.2
Mass of dry PS fed	g	450	675	825
	g/L	180	270	330
Percentage dry PS fed	w/w	18%	27%	33%
Glucose fraction	w/w	58.2%	37.5%	20.8%
Xylose fraction	w/w	12.2%	13.1%	4.9%
Total glucose fed	g	261.9	253.1	171.6
Glucose in residue	g	32.1	64.3	24.7
Soluble residual glucose	g	1.4	1.9	3.4
Total glucose consumed	g	228.4	186.9	143.5
Conversion of total cellulose	w/w	87.2%	73.8%	83.6%
Total xylose fed	g	54.9	88.1	46.3
Xylose in residue	g	6.1	15.2	0.0
Soluble residual xylose	g	34.7	20.4	43.8
Lactic acid	g	0.0	6.5[2] (46.9)[3]	0.0
Acetic acid	g	7.2	11.5[2] (19.7)[3]	2.0
Glycerol	g	12.5	7.2[2] (2.75)[3]	11.3
Ethanol concentration	g/L	49.6	37.9[2] (39.3)[3]	44.6
Theoretical ethanol yield/ Y_{Et}	w/w	92.6%	73.3%	< 100%[1]
Productivity	g/(L.hr)	0.459	0.451	0.362
Ethanol yield	g ethanol/g glucose consumed	0.489	0.452	-
Ethanol yield	g ethanol/g glucose fed	0.426	0.337	-
Overall ethanol yield	kg ethanol/tonne dry PS	275.4	126.5	135.1

[1]High ash fraction in TPR-PS caused underestimation of glucan content by NREL method. This resulted in Y_{Et} being greater than 100% (Boshoff *et al.*, 2016)

[2]At 84 hours of fermentation

[3]At the end of fermentation

Table 4-5: Chemical composition of dried fermented residues from 5 L bioreactors

Fermented solids	Glucan (% w/w)	Xylan (% w/w)	Lignin (% w/w)	Extractives (% w/w)	Ash (% w/w)
VP-PS	7.8 ± 0.2	2.2 ± 0.0	23.0 ± 2.0	23.7 ± 1.0	43.4 ± 0.4
CR-PS	11.8 ± 1.2	4.2 ± 0.3	30.3 ± 0.8	23.3 ± 0.2	32.6 ± 0.4
TPR-PS	3.1 ± 0.6	0.0 ± 0.0	8.4 ± 1.6	7.2 ± 0.8	81.6 ± 0.6

Except for CR-PS, PS fermentation with PW produced ethanol concentrations in excess of 40 g/L (**Table 4-4**). Notably, 40 g/L has been set by industry as the target ethanol concentration to ensure economic distillation of ethanol (Kang *et al.*, 2011). In comparison to reported studies of fed-batch fermentation of various paper sludge, the fermentation with process water as make up stream performed fairly well regarding ethanol production. Assessment of **Table 4-6** indicate only fermentation of de-ashed virgin pulp paper sludge by Kang *et al.* (2011) outperformed VP-PS regarding ethanol production. Kang *et al.* (2011) produced an ethanol concentration of 60 g/L as compared to 49.6 g/L attained in SSF of VP-PS with PW. As a result of de-ashing pre-treatment employed, Kang *et al.* (2011) had a much higher glucan content and coupled with elevated solids loading could produce 17% more ethanol as compared to fermentation of VP-PS with PW in this study. Also, SSF of TPR-PS produced an ethanol concentration (44.6 g/L) that was at most 22% lower than results obtained by Robus *et al.* (2016) (47.3 to 57.1 g/L) (**Table 4-6**). Robus *et al.* (2016) obtained a higher ethanol concentration as a result of using de-ashed TPR-PS. The washing step employed by Robus *et al.* (2016) increased the glucan content to 58% (w/w), this significantly favoured higher ethanol production as compared to unwashed TPR-PS utilized in this study. CR-PS produced about 20% less ethanol in comparison to results obtained by Boshoff *et al.* (2016). This could be attributed to the higher glucan content of CR-PS (42.2% w/w obtained from Mpact Springs mill) utilized by Boshoff *et al.* (2016) (**Table 4-6**).

Table 4-6: Comparison of fermentation yield markers in this study to reported literature on fermentation of paper sludge

Paper sludge type	Solids loading (g/L)	Glucan content (%)	Cellulase activity (FPU/ml)	Enzyme dosage (FPU/g glucan)	Fermentation	Reactor type	Fermentative Micro-organism	Ethanol conc. (g/L)	Ethanol yield (Y_{Et}) %	Productivity (g/Lh)	References
Waste fibre and Paper sludge	300	38.3 - 40.8	-	20.0 - 50.0	Continuous SSF	300 L pilot fermenter	Red star yeast	35 - 40	-		Finnish study (Vehmaanpera *et al.*, 2012)
Kraft Virgin pulp*	180	58.2	84.7 (*Viscamyl Flow*)	38.4	Fed batch-SSF with PW	5 L bench scale bioreactor	*S. Cerevisiae* MH1000	49.6	92.6	0.459	This study
						150 L pilot scale bioreactor		35.0	67.9	0.470	
		55.7	130.0 (*Optiflow RC 2.0*)	35.9	Fed batch-SSF	5 L bench scale bioreactor		34.2	66.9	0.230	(Boshoff et al. 2016)
		58.2	140.0 (*Viscamyl Flow*)	38.4	Fed batch-SSF	20 L bench scale bioreactor		46.8	87.4	0.325	(Williams, 2017)
Kraft Virgin pulp	258	44.0	9.0 (PS origin Cellulase)	23.3	Fed batch-SSF	Shake flask	*S. Cerevisiae* TJ14	40.1	62.5	-	(Prasetyo *et al.*, 2011)
De-ashed Kraft	185	64.8			Fed batch-SSCF		*E. Coli* ATCC 55124	47.8	70.0	0.398	

Untreated Kraft	135	44.5	59.0 (*Spezyme CP*)	10.0	Fed batch-SSF	Shake flask	*S. Cerevisiae* ATCC 200062	45.0	-	-	(Kang *et al.*, 2011)
De-ashed Kraft	278	64.8			Fed batch-SSF		*S. Cerevisiae* ATCC 200062	60.0	70.0	0.500	
Kraft	121	62.0	100.0 (*Logen Cellulase (DP151)*)	20.0	Semi-continuous	800 mL fermenter	*S. Cerevisiae* D5A	35.0	91.1	-	(Fan *et al.*, 2003)
	194			15.0				50.0	81.5	-	
	132			20.0				42.1	91.2	-	
Corrugated recycle*	270	37.5	84.7 (*Viscamyl Flow*)	29.3	Fed batch-SSF with PW	5 L bench scale bioreactor	*S. Cerevisiae* MH1000	37.9	73.3	0.451	This study
						150 L pilot scale bioreactor		26.5	51.1	0.315	
		42.2	130.0 (*Optiflow RC 2.0*)	26.0	Fed batch-SSF	5 L bench scale bioreactor		45.5	78.2	0.448	(Boshoff et al. 2016)
		37.5	140.0 (*Viscamyl Flow*)	29.3	Fed batch-SSF	20 L bench scale bioreactor		39.4	65.7	0.235	(Williams, 2017)
		20.8	84.7	52.1	Fed batch-SSF with PW	5 L bench scale bioreactor		44.6	-	0.362	This study

Tissue printed recycle*	330		(*Viscamyl Flow*)			150 L pilot scale bioreactor	*S. Cerevisiae* MH1000	44.0	-	0.338	
De-ashed tissue printed recycle*	207	54.0	148.0	27.8	Fed batch-SSF	1.3L benchtop fermenter		47.38	88.23	0.40	(Robus 2013)
	217	58.0	(*Optiflow RC 2.0*)	24.5				57.06	90.98	0.48	

*Study research from our group

4.3.1.2 Scaled-up paper sludge fermentation with process water in 150 L bioreactor

In contrast to the bench scale fermentations, TPR-PS produced the highest ethanol concentration of 44.0 ± 0.6 g/L (**Figure 4-12**). Similar to observations made in bench scale, the ethanol concentration increased from 39.3 ± 0.5 g/L to 45.6 ± 0.7 g/L after the last feeding was added at 120 hours (**Figure 4-12**). VP-PS yielded the second best ethanol concentration of 36.3 ± 0.3 g/L at 60 hours (**Figure 4-10**). After the last feeding was added at 60 hours, there was no increase in ethanol concentration (**Figure 4-10**). This was dissimilar to bench scale fermentation of VP-PS and could be attributed to the attachment of substrate on fermenter wall (biofouling). Upon a visual inspection at the end of fermentation process, substrate (VP-PS) appeared to be attached to fermenter wall (i.e. biofouling). It is apparent that such accumulation of biomass prevents water from moving freely within the paper sludge thereby lowering the performance of the enzymatic process (Boshoff *et al.*, 2016). Furthermore, contamination was replicated in pilot scale fermentation of CR-PS and contributed to low ethanol yield (**Figure 4-11**). Similar to bench scale fermentation of CR-PS (**Figure 4-8**), lactic acid production started after 72 hours of fermentation and increased to a high of 26 g/L (**Figure 4-11**). Though Vehmaanpera *et al.* (2012) (Finish study) did not report lactic acid contamination in pilot scale fermentation of paper sludge and waste fiber, Isci *et al.* (2009) reported similar contamination in fermentation of pre-treated switchgrass with lactic acid concentration over 10 g/L. After 84 hours of fermentation, lactic acid concentration above 10 g/L appears to inhibit *S. Cerevisiae*, as this was consistent with observation made in bench scale fermentation of CR-PS (**Figure 4-11**). Also, similar to VP-PS, biofouling was observed with CR-PS in 150 L reactors and also contributed to low ethanol concentration attained for CR-PS.

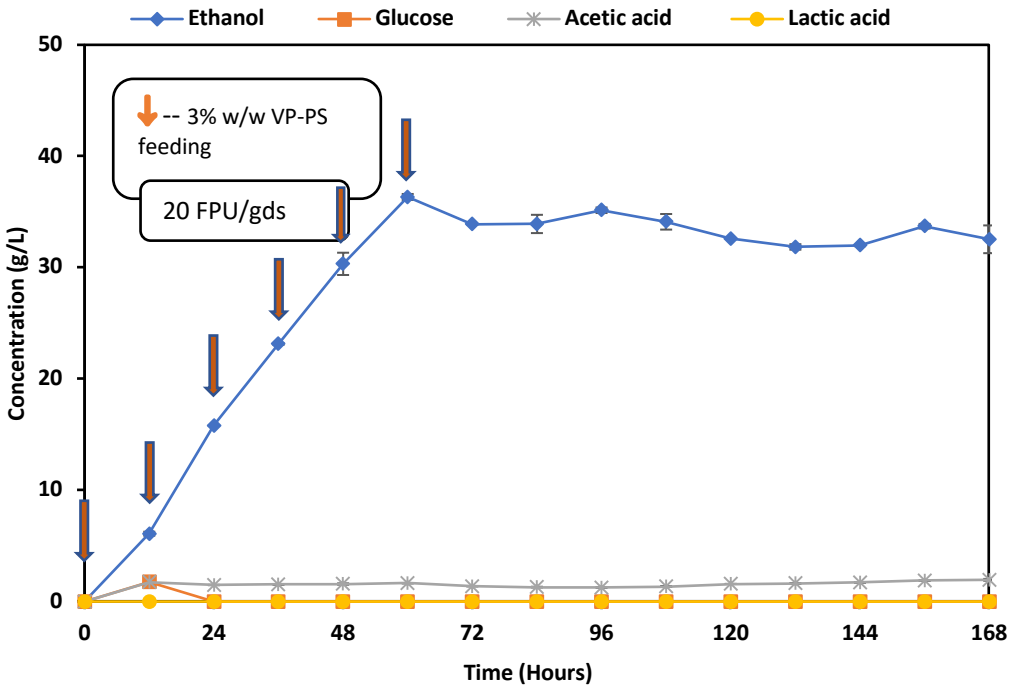


Figure 4-10: Ethanol concentration profile for 150 L fermentation of virgin pulp PS with PW; arrows ↓ represents feeding points

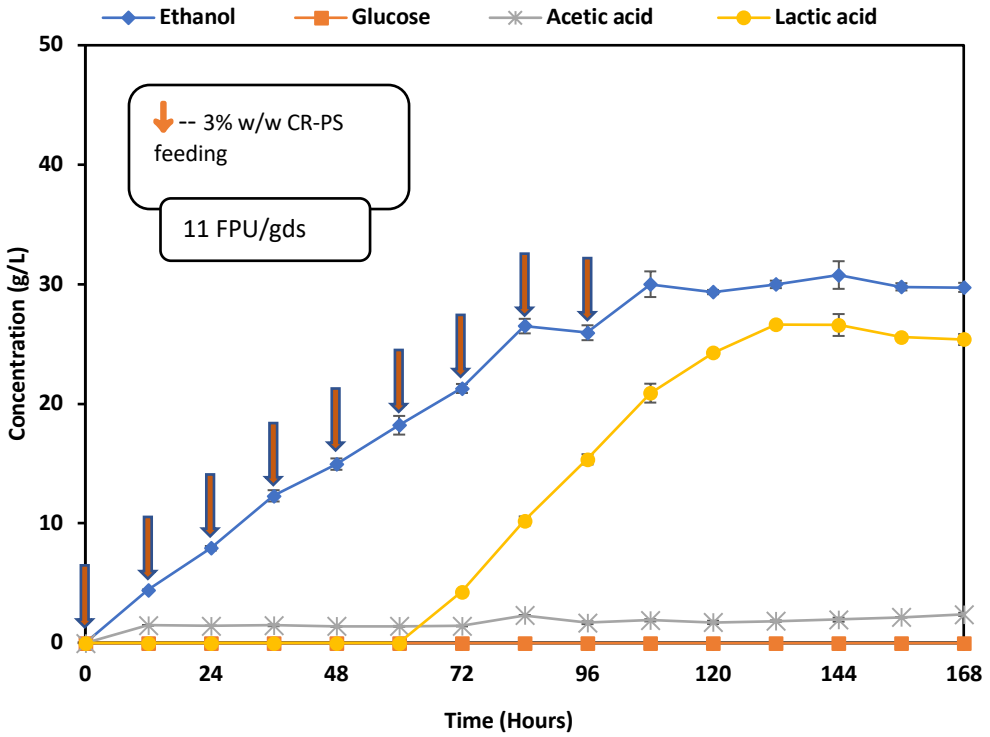


Figure 4-11: Ethanol concentration profile for 150 L fermentation of corrugated recycle PS with PW; arrows ↓ represents feeding points

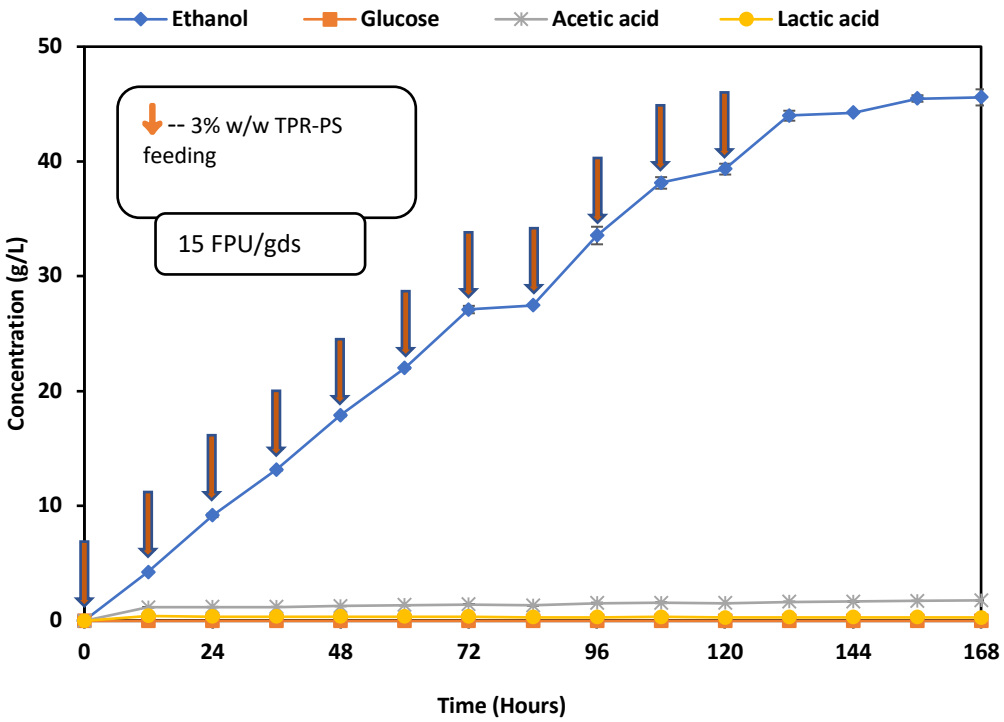


Figure 4-12: Ethanol concentration profile for 150 L fermentation of tissue printed recycle PS with PW; arrows ↓ represents feeding points

Similar to bench scale fermentations, an ANOVA analysis conducted on the produced ethanol concentration from the three paper sludge fermentations showed statistical significance (*p*-value (0.032) < 0.05). In comparison to bench scale, ethanol production was only replicated in TPR-PS (**Figure 4-12**). This could be attributed to lower WHC of TPR-PS (3.8 kg water/kg), this produced better mixing quality in fermenter and ensured water moving freely within substrate leading to improved enzymatic process (Boshoff *et al.*, 2016). In contrast, as previously discussed, biofouling in VP-PS and contamination issues in CR-PS caused ethanol production to be about 40% lower as compared to bench scale (**Table 4-7**). Unlike the 5 L bioreactors that employed a combination of an axial impeller with a Rushton impeller, the 150 L fermenter employed for this pilot study was fitted with a Rushton blade impeller, as this might not sufficiently overcome biofouling caused by the high water holding capacity of VP-PS and CR-PS (Boshoff *et al.*, 2016). As a result, low cellulose conversion were obtained in VP-PS and CR-PS (**Table 4-7**) as compared to bench scale fermentation (**Table 4-4**) and this resulted in more residual sugars in remaining solids (**Table 4-8**).

Vehmaanpera *et al.* (2012) from the VTT research center at Finland are the only research group to conduct a pilot scale fermentation of paper sludge and waste fiber (**Table 4-6**). Pilot scale fermentation of TPR-PS showed exceptional fermentation performance in comparison to Vehmaanpera *et al.* (2012) and the other

paper sludges in this study, even though it had the lowest glucan content (**Table 4-1**). TPR produced an ethanol concentration about 10% to 15% higher than that obtained by Vehmaanpera *et al.* (2012). Though VP-PS gave a lower ethanol concentration in comparison to TPR-PS, it yielded the highest productivity of 0.470 g/(L.hr) as result of the ethanol concentration peaking at 60 hours (**Table 4-7**). Despite the fact that biofouling adversely affected fermentation of VP-PS, ethanol production observed from VP-PS was at most 13% lower than that of Vehmaanpera *et al.* (2012) (**Table 4-6**). CR-PS gave the lowest ethanol production as a result of the negative effects of LAB contamination and biofouling. This resulted in significantly lower ethanol concentration of 26.5 g/L as compared to Vehmaanpera *et al.* (2012) (35 to 40 g/L) and to fermentation of TPR-PS (44.0 g/L) (**Table 4-6**). There is potential to produce higher ethanol concentrations in the CR-PS and VP-PS at pilot scale level, especially VP-PS, provided changes in impeller design are made to improve mixing quality to prevent biofouling in the fermenter.

Table 4-7: Mass balance from fermentation of PS in 150L fermenter

Operating conditions	Units	Virgin pulp	Corrugated recycle	Tissue printed recycle
Enzyme dosage	FPU/gds	20	11	15
	FPU/g-glucan	38.4	29.3	72.2
Mass of dry PS fed	g	12600	18900	23100
	g/L	180	270	330
Percentage dry PS fed	% (w/w)	18	27	33
Glucose fraction	%	58.2	37.5	20.8
Xylose fraction	%	12.2	13.1	4.9
Total glucose fed	g	7332	7088	4805
Glucose in residue	g	1415	3283	497
Soluble residual glucose	g	0	0	0
Total glucose consumed	g	5917	3805	4308
Conversion of total cellulose	%	78.7	53.7	89.7
Total xylose fed	g	1531	2467	1504
Xylose in residue	g	126	769	167
Soluble residual xylose	g	956	1556	1246
Lactic acid	g	0	714[2] (1771)[3]	20.3
Acetic acid	g	136.5	161[2] (170)[3]	124.6
Glycerol	g	223.7	236[2] (0)[3]	184.5
Ethanol concentration	g/L	34.0	26.5[2] (29.7)[3]	44.0
Theoretical ethanol yield/ Y_{Et}	%	67.9	51.0	< 100[1]
Productivity	g/(L.hr)	0.470	0.315	0.338
Ethanol yield	g ethanol/g glucose consumed	0.387	0.439	NA
Ethanol yield	g ethanol/g glucose fed	0.313	0.236	NA
Overall ethanol yield	kg ethanol/tonne dry PS	181.9	88.4	133.3

[1]High ash fraction in TPR-PS caused underestimation of glucan content by NREL method. This resulted in Y_{Et} being greater than 100% (Boshoff *et al.*, 2016)

[2]At 84 hours of fermentation

[3]At the end of fermentation

Table 4-8: Chemical composition of dried fermented residues from 150 L fermenter

Fermented solids	Glucan (% w/w)	Xylan (% w/w)	Lignin (% w/w)	Extractives (% w/w)	Ash (% w/w)
VP-PS	14.2 ± 0.2	3.1 ± 0.0	23.0 ± 2.0	19.7 ± 1.0	43.4 ± 0.4
CR-PS	19.5 ± 2.1	6.1 ± 1.2	27.0 ± 0.9	18.1 ± 0.7	30.9 ± 0.5
TPR-PS	2.7 ± 0.1	1.0 ± 0.0	8.1 ± 0.4	7.6 ± 0.5	80.7 ± 0.2

4.3.1.3 Water reclamation through fermentation

Approximately 80% to 90% of previously entrapped water in paper sludge (PS) could be reclaimed through fermentation (**Table 4-9**). Water reclamation was based on the principle that, the treated substrate retained a lower water holding capacity compared to that of the original substrate. Fermentation reduced the water holding capacity (WHC) of all paper sludge by more than 70% (w/w). This resulted in water reclamation of up to 223, 221 and 290 L per tonne of virgin pulp, corrugated recycle and tissue printed recycle PS, respectively. Process water was used in water reclamation instead of clean water. It was worth utilizing process water for reclamation because usage of clean water would not be financially sound. The financial cost of using clean water is avoided while there is no cost attached to using clean water for the fermentation process.

The amount of water reclaimed in this study was 30% and 60% higher for corrugated recycle and virgin pulp PS respectively, as compared to results obtained by Boshoff *et al.* (2016). This could be attributed to the high WHC of fermented VP solid residues (4.54 g water/g solids) and CR solid residues (2.55 g water/g solids) obtained by Boshoff *et al.* (2016) as compared to this study (**Table 4-9**). In addition, Boshoff *et al.* (2016) utilized paper sludges from different mills with different chemical composition and properties. Hydrolysis and fermentation performance are significantly affected by the nature and composition of the paper sludge (Boshoff *et al.*, 2016). As established by Boshoff *et al.* (2016) ,this in turn affects the WHC and amount of water reclaimed.

Table 4-9: Water reclaimed and water holding capacity of paper sludge before and after fermentation

Paper Sludge	Before		After		Water reclaimed (%)	Water reclaimed (L/tonne PS)
	Amount Fed (g)[1]	Water Holding Capacity (g_{water}/g_{solid})[1]	Amount Recovered (g)[1]	Water Holding Capacity (g_{water}/g_{solid})[1]		
Virgin pulp	450	7.969	238	2.031	87	223
Tissue Printed Recycled	825	3.806	645	0.696	82	290
Corrugated Recycled	675	6.745	361	1.842	85	221

[1]Based on dry solids

4.3.1.4 Water quality subsequent to fermentation

The Chemical Oxygen Demand (COD) of the stillage after paper sludge fermentation was substantially higher than that of the recycled process water used as input to the process. A more than ten-fold increase in COD was observed in stillage (**Table 4-10**). This observation is similar to reported studies on fermentation stillage derived from other substrates (**Table 4-10**). Vehmaanpera *et al.* (2012) from VTT research center at Finland reported a COD increase of up to 21 960 mg/L for stillage derived from fermentation of paper sludge and waste fiber. This was about 75 to 85% lesser than COD of stillages obtained from this study. The lower COD stillage attained by Vehmaanpera *et al.* (2012) could be attributed the operation of a continuous fermentation strategy. Wilkie, Riedesel and Owens (2000) indicated a continuous fermentation approach reduces stillage COD as a result of increased ethanol yield, as this in turn lowers the organic strength of final stillage after removal of ethanol. Except for CR-PS stillage, the COD increase noticed in this study were not beyond what is normally observed with other stillages obtained from fermentation of molasses, starch and lignocellulosic feedstocks (**Table 4-10**). The higher COD stillage of CR-PS could be as a result of contamination occurrence that led to high lactic acid production (Wilkie, Riedesel and Owens, 2000). The lactic acid produced remains in the final stillage and increases the stillage COD. As a result of the high COD stillage produced from fermentation of various feedstocks, some form of treatment is required to reduce the COD. One such treatment method that shows potential for COD reduction while producing bioenergy from stillage is

anaerobic digestion. The potential COD reduction of stillage by anaerobic digestion is examined in detail in section 4.3.3.3.

Table 4-10: Chemical oxygen demand of process water and stillage after fermentation

Substrate	Chemical Oxygen Demand (mg/L)		Reference
	Before	After (Stillage)	
Virgin pulp PS	4 780	86 800	This study
Tissue Printed Recycled PS	2 620	128 800	
Corrugated Recycled PS	4 780	138 200	
Waste fiber	-	79 680	(Vehmaanpera *et al.*, 2012)
Paper sludge and waste fibrer	-	21 960	
Beet molasses	-	65 000 - 115 800	(Wilkie, Riedesel and Owens, 2000)
Cane juice	-	22 000 - 45 000	
Cane molasses	-	22 500 -130 000	
Starch feedstocks[1]	-	14 000 - 97 000	
Pretreated lignocellulosic feedstocks[2]	-	19 100 - 119 000	

[1]Starch feedstocks including apple, banana, barley, potato, cassava, cherry, corn, grapes, raisins, wheat, sorghum, whey, raspberry and rice spirits
[2]Lignocellulosic feedstocks such as hardwood (willow), softwood (spruce and pine), grass and mixed biomass pre-treated using techniques like steam explosion, ammonia freeze explosion (AFEX) and acid (dilute and concentrated) hydrolysis

4.3.2 Anaerobic digestion of paper sludge

Biomethane potential test of paper sludge and process water (section 4.2.3) were up scaled to 30 L anaerobic digesters. Batch anaerobic digestion was conducted at 37°C and solids loading of 10% (w/w). However, digestion of VP-PS was conducted at 6% (w/w) solids loading to avoid high viscosity in digesters (Williams, 2017). Digesters were inoculated with inoculum obtained from South African Breweries. Biogas and methane production were determined as illustrated in section 3.2.4.

4.3.2.1 Biogas and methane production by anaerobic digestion

The biogas production per total solids fed over a period of 30 days is shown in **Figure 4-13**. The results presented are averages of duplicate runs. TPR-PS showed the longest lag phase of about 10 days, afterwards there was an increase in biogas production (**Figure 4-13**). This could be attributed to the temporal effect of

process water, as microbial community acclimatize to its environment (Liver and Hall, 1996; Meyer and Edwards, 2014). Alternatively, CR-PS within the first 5 days showed a high biogas production rate (5-7 Lkg $TS_{fed}^{-1}day^{-1}$) but dropped to a steady state production rate of about 4.2 Lkg $TS_{fed}^{-1}day^{-1}$. The decrease in production rate could be due the effect of corrugated recycle process water, as this adverse effect was highlighted in section 4.2.3 (**Figure 4-5**). VP-PS showed no distinct lag phase and outperformed the other paper sludges over the digestion period of 30 days (**Figure 4-13**). The better biogas production in VP-PS could be due to the reduced initial solids loading of 6% w/w as compared to the other paper sludges of 10% w/w (Williams, 2017). The lower solids loading used for VP-PS leads to an increased amount of free water for mixing, as this improves bacterial growth and biogas production (Serrano, 2011; Liao and Li, 2015).

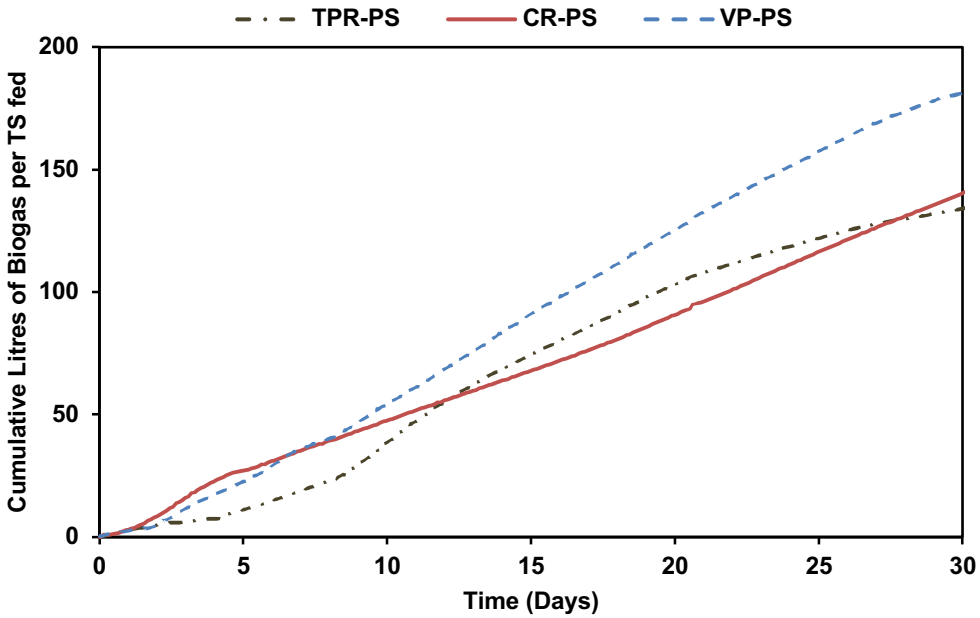


Figure 4-13: Cumulative biogas production of PS with PW in 30L bench scale digesters

The cumulative biogas production on the basis of total solids fed were 182.8 ± 27.6, 142.9 ± 12.6 and 134.9 ± 16.3 L/kg TS_{fed} for VP-PS, CR-PS and TPR-PS, respectively (**Table 4-11**). These values were statistically insignificant because an ANOVA analysis yielded a *p*-value (0.062) greater than 0.05. However, an ANOVA analysis conducted on the cumulative methane production on total solids fed showed statistical significance (*p*-value (0.031) < 0.05). VP-PS yielded the highest methane production of 99.5 ± 15.1 L CH_4/kg TS_{fed} and could be due to the high cellulose content of VP-PS as compared to the other paper sludges (**Table 4-1**). Additionally, virgin pulp process water had a favorable effect on biogas production from VP-PS as highlighted in section 4.2.3 (**Figure 4-4**). Another contributing factor stated in the previous paragraph could be the lower solids loading use for VP-PS, as this improved biogas production due to increased levels of free

water that enhances bacterial growth (Serrano, 2011; Liao and Li, 2015; Williams, 2017). Alternatively, TPR-PS gave the lowest methane yield of 65.0 ± 8.1 CH_4/kg TS_{fed}. Tissue printed recycle process water had no significant effect on methane production from TPR-PS (section 4.2.3, **Figure 4-6**), thus the lower methane yield observed could be attributed to the lower cellulose content of TPR-PS as compared to the other paper sludges (**Table 4-1**). It was worth mentioning that biogas and methane produced in this study surpassed (35 to 55% higher yield) previous result obtained by Williams (2017) in anaerobic digestion of PS with clean water. This may be the result of a better experimental design. Mass transfer was improved by replacing Rushton impeller with axial impellers, while digester leakage issues were significantly reduced due to improved lid design. Furthermore, compared to reported literature on AD of paper sludge (**Table 2-13)**, methane production in this study was only bested by Bayr and Rintala (2012) and Dalwai (2012) due to extended retention time (40 to 60 days).

Table 4-11: Anaerobic digestion of paper sludge with corresponding biogas production and methane concentration values

PS type	Cumulative biogas/TS (L/Kg)	Cumulative biogas/VS (L/Kg)	Methane %					Cumulative CH_4/TS (L/Kg)	Cumulative CH_4/VS (L/Kg)
			1st week	2nd week	3rd week	4th week	Average		
Virgin pulp	182.8 ± 27.6	243.2 ± 36.8	44.6 ± 3.0	45.1 ± 1.2	49.3 ± 7.3	49.2 ± 2.7	47.1 ± 2.5	99.5 ± 15.1	132.4 ± 20.0
Corrugated recycle	142.9 ± 12.6	192.9 ± 17.0	42.3 ± 2.3	48.4 ± 1.5	53.0 ± 2.2	54.4 ± 0.5	49.5 ± 5.5	77.8 ± 7.5	105.1 ± 10.2
Tissue printed recycle	134.9 ± 16.3	363.4 ± 44.0	47.1 ± 0.7	48.0 ± 0.9	46.3 ± 1.8	48.4 ± 0.4	47.5 ± 0.9	65.0 ± 8.1	175.1 ± 21.9

4.3.2.2 Bioenergy production from anaerobic digestion of paper sludge in comparison to fermentation

Anaerobic digestion (AD) gave about 35% to 55% less bioenergy as compared to standalone fermentation of paper sludge (**Table 4-12**). This could be due to the higher ethanol yield from fermentation than that of methane yield from anaerobic digestion of paper sludge (**Table 4-16**). These results were consistent with results obtained by Williams (2017), who also obtained about 50% to 80% more bioenergy from fermentation as compared to anaerobic digestion of paper sludge. Alternatively, the methane produced from anaerobic digestion has a better heat value (55.53 MJ/kg) than that of ethanol (29.85 MJ/kg) derived from

fermentation. Also, methane is comparatively a cleaner fuel as compared to ethanol, because it produces the least amount of CO_2 in its combustion process (Chynoweth, Owens and Legrand, 2001).

Although bioenergy yields might not be enough to determine the best bioprocessing route, the low biofuel energy from anaerobic digestion seems to suggest fermentation might be a better bioprocessing technology. In order to conclusively determine the best bioprocessing route a techno-economic analysis on both processing technologies is required.

Table 4-12: The bioenergy production from standalone anaerobic digestion and fermentation of paper sludge with process water

Process type	Paper sludge type	Product Yield (Kg/tonne PS)	Product Energy (MJ/tonne PS)
Fermentation only (Section 4.3.1)	VP	275.4[1]	8 221
	CR	152.2[1]	4 543
	TPR	135.1[1]	4 033
Anaerobic digestion only (Section 4.3.2)	VP	67.7[2]	3 759
	CR	52.9[2]	2 938
	TPR	44.2[2]	2 454

[1]Ethanol production from fermentation of paper sludge
[2]Methane production from anaerobic digestion of raw paper sludge

4.3.2.3 Water reclamation through anaerobic digestion

About 40% to 60% of water was reclaimed through anaerobic digestion (AD) of paper sludge (**Table 4-13**). AD reduced the water holding capacity (WHC) of all paper sludge about 20% to 50%. This resulted in water reclamation of up to 92, 51 and 127 L per tonne of virgin pulp, corrugated recycle and tissue printed recycle PS, respectively. However, this is about 50% to 75% less than what was achieved through fermentation of paper sludge (section 4.3.1.2). An indirect correlation exists between water retention and cellulase activity. In the case of fermentation, the broth is supplemented with industrially produced cellulase, this actively reduces the cellulose quantity. Anaerobic digestion, on the other hand, make use of hydrolytic bacteria for cellulase production, a process described to be notoriously slow (Vertes *et al.*, 2010). Although cellulase activity in anaerobic digestion was not measured, it is assumed that the enzyme reactivity level obtained with AD was significantly lower compared to fermentation. Hence, different levels of cellulase activity may therefore be

responsible for the different water holding capacity outcome, as water retention is a function of cellulose quantity and structure, this has been reported for paper sludge before by Boshoff *et al.* (2016).

Table 4-13 Water reclaimed and water holding capacity of paper sludge before and after anaerobic digestion

Paper Sludge	Before		After		Water reclaimed (%)	Water reclaimed (L/tonne PS)
	Amount Fed (g)[1]	Water Holding Capacity (g_{water}/g_{solid})[1]	Amount Recovered (g)[1]	Water Holding Capacity (g_{water}/g_{solid})[1]		
Virgin pulp	1 100	7.969	771	5.035	56	92
Tissue Printed Recycled	1 800	3.806	1510	2.017	56	127
Corrugated Recycled	1 800	6.745	1 324	5.470	40	51

[1]Based on dry solids

4.3.2.4 Water quality subsequent to anaerobic digestion

The Chemical Oxygen Demand (COD) of the supernatant water after paper sludge anaerobic digestion was about 20% to 40% lower than that of the recycled process water used as input to the process (**Table 4-14**). Since anaerobic digestion did not contribute to a higher COD, the resulting supernatant water could be redirected into process water stream of the plant without further treatment. It is important to note that anaerobic digestion was not only performed on paper sludge but also on process wastewater. Anaerobic digestion is well known to reduce the COD of wastewater over 50% (Singh and Thakur, 2006; Meyer and Edwards, 2014; Kamali and Khodaparast, 2015), as this is well above the reduction levels obtained in this section (about 20 to 35% COD reduction). In the anaerobic microbial community, part of the microbial community (hydrolytic bacteria) increases COD by conversion of lignocellulosic content of paper sludge while another guild of bacteria reduces COD by producing biogas from soluble organic compounds (Vertes *et al.*, 2010). Due to the absence of solid substrate, anaerobic digestion of wastewater are able to attain high COD reduction levels as a result of the consumption of the soluble organic matter. Alternatively, the steady state hydrolysis of paper sludge and consumption of solubilized organic matter could accumulate residual COD content in final supernatant water after anaerobic digestion. Thus, the low COD reduction after anaerobic digestion of paper sludge with recycled process water could be attributed to the residual accumulation of soluble organic matter in subsequent supernatant water.

Table 4-14: Chemical oxygen demand of process water before and after anaerobic digestion

Paper Sludge	Chemical Oxygen Demand (mg/L)	
	Before	After
Virgin	4 780	3 720
Tissue Printed Recycled	2 620	1 670
Corrugated Recycled	4 780	3 220

4.3.3 Sequential fermentation and anaerobic digestion of paper sludge

Sequential biochemical processing was achieved by fermenting paper sludge, with subsequent ethanol removal by evaporation, and immediately transferring the resulting fermentation stillage into 30 L anaerobic digesters for biogas production. The fermentation process was completed for the three types of paper sludge as per section 4.3.1, while the fermentation stillage for anaerobic digestion were prepared in 150 L fermenters.

Biogas production came from COD reduction of stillage. This was established as the chemical composition of the fermentation solids before and after anaerobic digestion remained approximately the same (**Table 4-15**). This would suggest that the enzymes added in fermentation converted most of the “reasonable accessible” organic content of paper sludge and that the cellulases present in the AD process were not able to perform any meaningful further hydrolysis of the residual solids. This kind of outcome was not reported by the Finnish study on AD of stillage derived from fermentation of paper sludge (Vehmaanpera *et al.*, 2012). Hydrolysis-fermentation performance in this study was able to convert digestible material from paper sludge into ethanol and soluble byproducts. Thus, soluble byproducts such as residual sugars (mostly pentose sugars), glycerol, organic acids (lactate and acetate), proteins and yeast cell debris could be responsible for the high COD observed with stillage derived from paper sludge fermentation. Residual sugar concentration of fermentation stillage range between 5 to 15 g/L. Wilkie, Riedesel and Owens (2000) indicated for every 10 g/L of residual sugar, there is a 16 g/L COD increment. Another important but undesirable byproduct is lactic acid that results from contamination of fermentation process. High lactic acid concentration were observed in corrugated recycle stillage and had significant effect on biogas production. This is further discussed in the next section.

Table 4-15: Chemical composition of fermentation solids and solids following anaerobic digestion

Constituents	Paper Sludge					
	Virgin Pulp		Corrugated recycle		Tissue printed recycle	
	Before	After	Before	After	Before	After
Cellulose (% w/w)	14.2	14.2	19.5	19.5	2.7	2.0
Xylan (% w/w)	3.1	3.0	6.1	4.8	0.9	0.0
Lignin (% w/w)	25.8	29.0	27.0	31.3	8.1	7.0
Extractives (% w/w)	17.1	19.8	18.1	14.2	7.6	6.5
Ash (% w/w)	39.8	34.0	30.9	30.2	80.7	84.6

4.3.3.1 Biogas and methane production through anaerobic digestion of fermentation stillage

A high daily production of biogas was recorded for the first 10 days of digestion (**Figure 4-14**). This led to about 90% of the accumulative biogas yield being produced in the first 10 days of digestion (**Figure 4-14**). This was probably due to high soluble organic content caused by enzymatic hydrolysis of PS in the fermentation process (Liu *et al.*, 2015). Though Vehmaanpera *et al.* (2012) did not report on the daily biogas production profile from PS stillage, Liu *et al*. (2015) observed similar maximum daily productions within the first 7 days of anaerobic digestion of fermented stillage of sugarcane bagasse. However, after this period of high productivity, biogas production fell sharply with decreasing methane quality (**Figure 4-14**). This correlated with a decrease in pH (below pH 6 after week 2) and an increase in volatile fatty acid (VFA) content, especially acetic acid concentration above 5 g/L (example illustrated in **Figure 4-15,** remaining examples in **Appendix Figure 5-3**, **Figure 5-4** and **Figure 5-5**). Xiao *et al.* (2013) reported that below pH 6, acetic acid concentrations above 3 g/L inhibited methanogens (biomethanation failure). Biomethanation failure was probably caused by over organic loading of high soluble organic matter associated with batch operating process (Nielsen, Uellendahl and Ahring, 2007). The Finnish study on AD of fermented paper sludge stillage by Vehmaanpera *et al.* (2012) avoided this harmful inhibition of methanogens by operating a continuous AD system with intermittent feeding of stillage. In a continuous AD system with intermittent feeding of stillage, organic acids such as acetic acid concentrations are kept at low concentrations to prevent the inhibition of methanogens (McCarty, 1964). As a result, Vehmaanpera *et al.* (2012) produced biogas and methane yields 2 to 3 times more than what was reported in AD of VP, CR and TPR stillage.

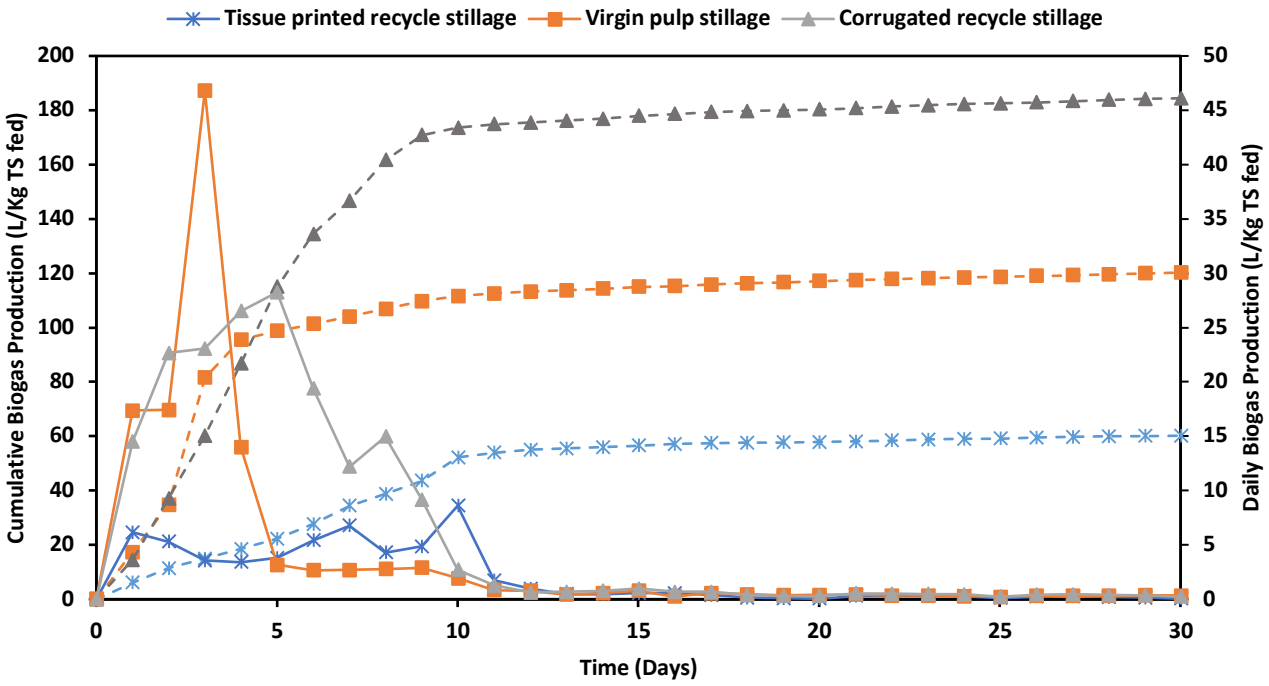


Figure 4-14: Daily and cumulative biogas production from fermented stillage in 30L digesters

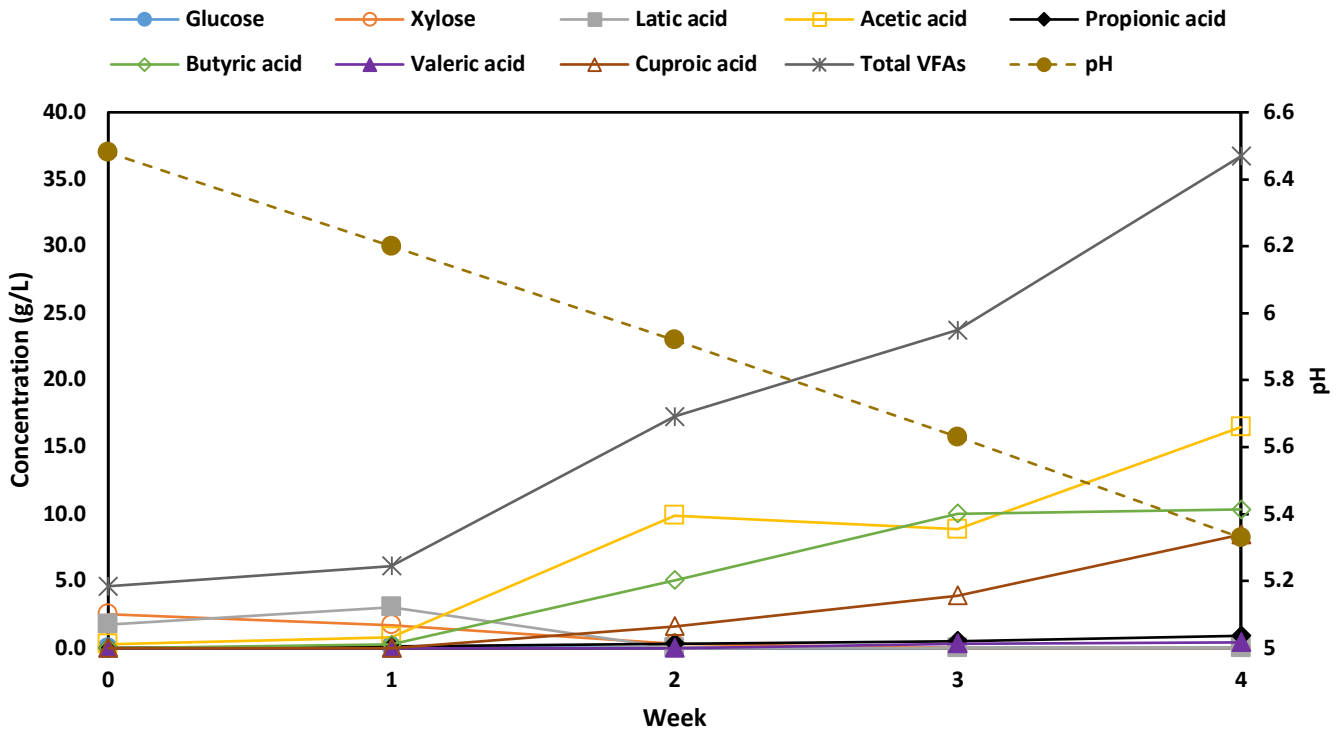


Figure 4-15: VFAs concentration profile for 30 L digestion of Tissue printed recycle PS stillage

An ANOVA analysis conducted on both cumulative biogas and methane production on total solids fed showed statistical significance (p-value (0.014) < 0.05). CR stillage yielded the highest biogas and methane production of 184.4 ± 2.3 L/kg TS_{fed} and 126.6 ± 1.2 L CH_4/kg TS_{fed}, respectively (**Table 4-16**). The produced

biogas and methane yields from CR stillage were about 40 to 65% higher than VP and TPR stillages (**Table 4-16**). This could be attributed to the high lactic acid concentration present in corrugated recycle stillage after fermentation (**Figure 4-8**). Lactic acid is an essential intermediate chemical for biogas production (Vertes *et al.*, 2010). Satpathy *et al.* (2017) established high lactic acid concentration in anaerobic reactors significantly increased biogas yield.

Compared to anaerobic digestion of raw paper sludge, stillage produced about 30% to 70% less biogas and methane per unit of total solids fed, except for the stillage derived from CR-PS, which produced 184 L/kg TS_{fed} compared to 143 L/kg TS_{fed} for untreated paper sludge (**Table 4-16**). The biogas and methane production CR stillage was 20 % and 40% higher than AD of raw CR-PS respectively. As stated in the previous paragraph, this could be as a result of the high lactic acid concentration present in stillage after fermentation of CR-PS (**Figure 4-8**).

Table 4-16: Biogas and methane production with paper sludge and paper sludge stillage

Fermentation Stillage	Paper Sludge		Fermentation Stillage	
	Cumulative biogas (L/Kg TS_{fed})	Cumulative CH_4 (L/Kg TS_{fed})	Cumulative biogas (L/Kg TS_{fed})	Cumulative CH_4 (L/Kg TS_{fed})
Virgin pulp	182.8 ± 27.6	99.5 ± 15.1	120.3 ± 0.9	64.8 ± 0.8
Corrugated recycle	142.9 ± 12.6	77.8 ± 7.5	184.4 ± 2.3	126.6 ± 1.2
Tissue printed recycle	134.9 ± 16.3	65.0 ± 8.1	60.3 ± 4.1	22.3 ± 1.0

4.3.3.2 Bioenergy production from sequential as compared to standalone fermentation and anaerobic digestion of paper sludge

The sequential operated biochemical process produced about 20% to 50% more energy than individual fermentation of paper sludge (**Table 4-17**). For example, a total of 10 650 MJ per kilogram dry VP-PS was produced by using the individual processes in sequence. At least 80% of the energy was produced from fermentation while the remainder came from anaerobic digestion of the stillage. Alternatively, a total of 9 288 MJ per kilogram dry PS was produced from sequential processing of CR-PS. Anaerobic digestion of stillage contributed about 50% the bioenergy in the sequential process. Additionally, the combined fermentation of paper sludge and anaerobic digestion of stillage yielded about 50% to 60% more bioenergy as compared to AD of paper sludge (**Table 4-17**). A noteworthy observation is the higher methane yield and

bioenergy production from corrugated recycle stillage as compared to AD of CR-PS (**Table 4-16**). Corrugated recycle stillage produced 38% more methane and bioenergy than AD of raw paper sludge (**Table 4-16**). As previously discussed in section 4.3.3.1, this could be attributed to the high COD of stillage and the influence of the high lactic acid concentration of CR stillage.

The energy generated from stillage came from COD reduction of stillage. This was established as the chemical composition of the fermentation solids before and after anaerobic digestion remained approximately the same (**Table 4-15**). The HHV of the VP, CR and TPR solid residues were 12.5, 11.3 and 3.5 MJ/kg respectively. Combustion of these solid residues could serve as energy for the ethanol distillation process in the fermentation or sequential bioprocessing of paper sludge. The combustion of the solid residues contributed about 40% more energy to the fermentation or sequential processes.

Table 4-17: The heat values and energy conversion efficiencies for standalone and sequential biochemical processes

Process type	Paper sludge type	Product Yield (Kg/tonne PS)		Bioenergy (MJ/tonne PS)
Fermentation only (Section 4.3.1)	VP	275.4[1]		8 221
	CR	152.2[1]		4 543
	TPR	135.1[1]		4 033
Anaerobic digestion only (Section 4.3.2)	VP	67.7[2]		3 759
	CR	52.9[2]		2 938
	TPR	44.2[2]		2 454
Sequential treatment (Section 4.3.3)	VP	275.4[1]	43.7[3]	10 650
	CR	152.2[1]	85.5[3]	9 288
	TPR	135.1[1]	15.1[3]	4 869
Combustion of solid residues	VP	528[4]		6 600
	CR	534[4]		6 034
	TPR	732[4]		2 562

[1]Ethanol production through fermentation
[2]Methane production through anaerobic digestion of raw paper sludge
[3]Methane production through anaerobic digestion of stillage
[4]Amount of solid residues produced after the sequential process

4.3.3.3 Water quality subsequent to sequential fermentation and anaerobic digestion

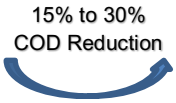
The COD of final stillage after sequential fermentation and anaerobic digestion was considerably higher than that of the recycled process water used as input to the process (**Table 4-18**). COD of final stillage was at least 15 times higher than that of recycled process water used as input into the sequential process

(**Table 4-18**). Anaerobic digestion of fermented stillage was able to only reduce the COD of final stillage by at most 30% (**Table 4-18**). In comparison to the Finnish study of anaerobic digestion of fermented paper and fiber waste stillage, Vehmaanpera *et al.* (2012) achieved higher COD reductions of 54% to 66%. The superior COD reduction by Vehmaanpera *et al.* (2012) could be attributed to the usage of a continuous AD system with intermittent feeding of stillage, as this was able to produce more biogas by utilizing more of soluble organic matter that contribute to COD.

Table 4-18: COD of effluent streams in different steps of the sequential fermentation and anaerobic digestion process

Paper Sludge	Unit	Process Water[1]	Supernatant following Fermentation[2]	Supernatant following Anaerobic Digestion[3]
VP	mg/L	4 775	86 750	72 500
CR	mg/L	4 775	138 217	95 394
TRP	mg/L	2 618	128 765	90 275

[1]Collected at Pulp and Paper Plant
[2]Fermentation of paper sludge
[3]Anaerobic digestion of fermentation stillage



Due to the significant increase in COD as a result of fermentation and the inability of anaerobic digestion process to rehabilitate the effluent stream to a quality equal to that of the starting liquid i.e. process water, industry may need to consider further techniques in order to reduce the COD to required levels. One option that could significantly reduce the COD is the dilution of the stillage with clarifier process water (**Table 4-3**). Compared to the quantity of clarifier process water generated, stillage from this process would constitute about 1% of the amount of clarifier process water generated. For example, about 50 to 150 kiloliters per day of stillage would be produced in an industrial scale simulation of this process as compared to about 3 000 to 15 000 kiloliters per day of process water generated by pulp mills (Personal communication, 2016). Mixing of both streams would significantly reduce the COD of the resulting stream to below 10 000 mg/L, as this could be handled by conventional treatment systems in the pulp and paper industry.

4.3.4 Perspectives on sequential and standalone bioprocessing technique based on water reclamation, water quality and bioenergy production

Sequential bioprocessing is the preferred process for water reclamation and bioenergy production from paper sludge as compared to individual fermentation or anaerobic digestion. Despite its impacts on water quality, the sequential process was able to produce about 20% to 60% more bioenergy than individual

technologies (**Table 4-17**). Except for CR stillage, anaerobic digestion of stillage contributed at most 20% bioenergy to the sequential process (**Table 4-17**). This was about 20% lower than the bioenergy gained from combustion of solid residues (**Table 4-17**). Subsequent anaerobic digestion of fermented mixture did not make use of solid residues (**Table 4-15**). Hence, a better modification of the sequential process would be the separation of solid residues from fermented mixture. Combustion of the solid residues would add 40% bioenergy to the process while anaerobic digestion of supernatant stillage would generate the additional 20% bioenergy.

Despite the significant increase in COD, fermentation bested anaerobic digestion in terms of water reclamation and produced similar water reclamation to that of sequential process (about 80% to 90% water reclamation was achieved for the sequential process which similar to **Table 4-9**). Though anaerobic digestion of paper sludge with recycled process water reduced the COD of subsequent supernatant water (**Table 4-14**), the bioenergy benefits in combination with the amount of water reclaimed were considerably lower as compared to the sequential process or fermentation process (**Table 4-13** and **Table 4-17**). Furthermore, anaerobic digestion was able to reduce the COD of fermentation stillage up to 30% (**Table 4-18**). Though this COD reduction was comparatively lower as compared to reported study by Vehmaanpera *et al.* (2012), subsequent dilution of final stillage with clarifier process water would significantly reduce the COD of resulting stream as compared to stillage. The resulting diluted stream could be handled by a centralized waste-water-treatment in a pulp mill. This proposal nullifies the negative impact of the sequential process and maintains the water reclamation and bioenergy benefits which is significantly better than individual technologies.

4.4 CHARACTERISTICS AND POTENTIAL USES OF SOLID RESIDUES GENERTED FROM SEQUENTIAL BIOPROCESSING OF PAPER SLUDGE

4.4.1 Characteristics of solid residues

As discussed in section 4.3.3 (**Table 4-15**), anaerobic digestion did not change the chemical composition of fermented solid residues. This was also true for the elemental and metalloid composition, as this also did not show any significant changes before and after anaerobic digestion of stillage containing fermented solids (**Table 4-20**). However compared to the starting substrate i.e. paper sludge, fermentation resulted in about 50% to 90% reduction in glucan content (**Table 4-19**). Alternatively, the lignin content increased significant (about 30% to 85% increase) as result of failure by cellulase to degrade it. Additionally, the ash content increased about 20% to 50% as a result of reduction in cellulose and hemicellulose content of paper sludge in the fermentation process (**Table 4-19**). The ash content of both paper sludge and solid residues largely consisted of calcium (about 70% to 90% of ash). The major difference noticed between the metallic content of paper sludge and solid residues was the increase in the concentration of most of the metallic elements measured (**Table 4-20**). Depending on the metal component, an increase of up to about 85% in concentration was observed. This could be attributed to reduction in organic content of paper sludge as a result of fermentation process (**Table 4-19**). Take sodium and phosphorous for example, TPR PS had a sodium and phosphorous concentrations of 238 and 181 mg/kg and increased to 1 603 and 475 mg/kg after fermentation, respectively (**Table 4-20**). Furthermore, the fermentation process reduced the starting amount of paper sludge by about 47%, except for TPR PS which showed the least reduction of about 26% due to its high ash content. The residual solids, as a result of its components showed some promising applications in other areas associated with the agricultural and industrial sector, these are discussed in the next section. The utilization of solid residues in other areas could create a zero waste processing route for paper sludge bioprocessing.

Table 4-19: Chemical composition of raw paper sludge and solid residues after bioprocessing

		Glucan (% w/w)	Xylan (% w/w)	Lignin (% w/w)	Extractives (% w/w)	Ash (% w/w)
Virgin Pulp	Raw PS	58.2 ± 0.4	12.2 ± 0.1	4.1 ± 0.1	5.4 ± 0.1	20.8 ± 0.1
	Residues[1]	14.2 ± 0.3	3.0 ± 0.3	29.0 ± 0.2	19.8 ± 0.5	34.0 ± 0.5
	Residues[2]	7.8 ± 0.2	2.2 ± 0.0	23.0 ± 2.0	23.7 ± 1.0	43.4 ± 0.4
Corrugated recycle	Raw PS	37.5 ± 0.4	13.1 ± 1.1	13.1 ± 0.1	10.4 ± 0.1	25.9 ± 0.3
	Residues[1]	19.5 ± 0.9	6.1 ± 0.2	27.0 ± 0.5	18.1 ± 0.2	30.9 ± 0.4
	Residues[2]	11.8 ± 1.2	4.2 ± 0.3	30.3 ± 0.8	23.3 ± 0.2	32.6 ± 0.4
Tissue printed recycle	Raw PS	20.8 ± 0.1	4.9 ± 0.2	6.4 ± 0.1	5.1 ± 0.1	62.9 ± 0.4
	Residues[1]	2.7 ± 0.1	0.9 ± 0.0	8.1 ± 0.4	7.6 ± 0.5	80.7 ± 0.2
	Residues[2]	3.1 ± 0.6	0.0 ± 0.0	8.4 ± 1.6	7.2 ± 0.8	81.6 ± 0.6

[1]Residues from 150 L pilot scale bioprocessing
[2]Residues from 5 L bench scale bioprocessing

Table 4-20: Quantity and metalloid composition of solid residues after sequential bioprocessing of paper sludge with recycled process water

	Virgin pulp		Tissue printed recycle		Corrugated recycle	
Solid residues (kg/kg dry TS_{fed})	0.528		0.732		0.534	
Volatile solids (kg/kg dry solid residues)	0.690		0.154		0.698	
Ash content (kg/kg dry solid residues)	0.310		0.846		0.302	
Higher heating value (MJ/ kg dry solid residues)	12.5		3.5		11.3	
	Residues	PS	Residues	PS	Residues	PS
Boron (µg/kg)	23274	39513	7890	7026	15161	17352
Vanadium (µg/kg)	10628	3049	4986	4029	13942	10246
Chromium (µg/kg)	175686	61715	14070	9882	93175	35762
Cobalt (µg/kg)	2270	590	1977	1935	3848	2887
Nickel (µg/kg)	29224	4254	47813	51210	11603	10318
Copper (µg/kg)	84224	8907	108472	97919	37492	44285
Arsenic (µg/kg)	5567	972	924	793	2827.0	1204
Selenium (µg/kg)	556	99	157	130	299	149
Strontium (µg/kg)	36219	34285	196206	148520	1824	45076
Molybdenum (µg/kg)	8723	608	2430	2189	1910	1529
Cadmium (µg/kg)	588	267	151	129	532.0	201
Antimony (µg/kg)	504	100	38	46	57	86
Barium (µg/kg)	158981	75884	50861	40638	183943	129619
Mercury (µg/kg)	459	84	2310	1635	663	190
Lead (µg/kg)	34014	5541	8797	6776	21280	12183
Uranium (µg/kg)	362	122	844	727	1204	283
Zinc (mg/kg)	2178	56	387	357	629	102
Aluminium (mg/kg)	6258	2202	21374	15385	18954	14186
Manganese (mg/kg)	886	738	86	63	401	180
Iron (mg/kg)	3836	1850	2816	2078	6725	5615
Calcium (mg/kg)	42513	73245	342796	258471	129033	48268
Potassium (mg/kg)	995	541	983	638	1107	888
Magnesium (mg/kg)	1775	1973	4606	3455	3745	2840
Sodium (mg/kg)	5495	6886	1603	238	5254	4231
Phosphorous (mg/kg)	4283	972	475	181	2583	738
Silicon (mg/kg)	1636	2106	2634	2563	1339	1704

4.4.2 Potential applications of solid residues

The potential applications considered for utilization of solid residues were; combustion to produce energy, fertilizer production and nutrient supplementation to poor soil environment and clinker production. These applications were considered in relation to the composition of the solid residues (**Table 4-20**).

4.4.2.1 Combustion of solid residues to produce energy for distillation purposes

One potential application is to incinerate the solid residues in a boiler to generate in house steam, as this could serve as an energy source for distillation system after fermentation. Except for TPR solid residues, VP and CR solid residues contained about 70% (w/w) volatile solids (**Table 4-20**). The volatile content of CR and VP residual solids consist of lignin, residual sugars and extractives and upon combustion contributes to the higher heating value (HHV) (**Table 4-15**) (Demirbas *et al*., 2004). The heating values of VP and CR solid residues are 12.5 and 11.3 MJ/kg, respectively (**Table 4-20**). As indicated in section 4.3.3.2, combustion of these solid residues added about 40% more bioenergy to the fermentation or sequential process (**Table 4-17**). The incineration of solid residues added about 20% more bioenergy than AD of fermentation stillage (section 4.3.3.2). On the other hand, the high ash content and low HHV of TPR residues made it an unattractive option to generate in house steam through combustion (**Table 4-20**). This was consistent with results obtained by Robus *et al.* (2016) who also established the high ash content and low HHV of TPR fermentation solids were not a viable boiler feedstock.

4.4.2.2 Nutrient supplement for poor soil environments and fertilizer production from urine

Solid residues after sequential biochemical processing of PS have the potential to be used as nutrient supplements for plantation and natural forest soils (Demeyer *et al*., 2001; Patterson, 2001; Goodwin and Burrow, 2006; Pitman, 2006). Primary, secondary and trace elements for plant growth could be identified in the solid residues (**Table 4-20**) (Scheepers, 2014). Take phosphorous and potassium as examples. Both these components are considered primary nutrients for plant growth and are readily applied to soil in the form of mineral fertilizer (Demeyer *et al*, 2001). With their concentrations exceeding 4000 mg/kg (**Table 4-20**) in some instances, one cannot deny that these residual solids may serve as a biological fertilizer. Likewise, residuals also contain boron, copper, iron, magnesium, molybdenum, sodium and zinc which are considered essential trace elements for plant growth (**Table 4-20**) (Patterson, 2001). Lopsided development of fertilizers has led to a steady reduction of these trace elements in farm land soil (Patterson, 2001). Employment of these residual solids may serve as a means re-introduce these elements back into the soil. However, the mechanics

associated with bioavailability of these elemental nutrient for plant uptake are affected by pH, metalloid concentrations, concentrations of organic and inorganic molecules, nutrients and microbial activity (Violante *et al*., 2010). Consequently, the application of solid residues in this study on soil environments should further be investigated to completely ascertain its effects, both on tree growth and long term soil impact.

Another potential novel application of calcium rich TPR residue, is in water recovery and fertilizer production from collected urine (WISA conference, Randall, 2018). Fresh urine contains nutrient rich phosphorous and nitrogen compound which are important fertilizing agents (Demeyer *et al*, 2001). Using calcium, Randall (2018) reported 99% of phosphorous in urine could be captured as calcium phosphate solids for fertilizer production. Apart from phosphate production, the addition of calcium prevented the degradation of urea which could recovered through reverse osmosis for struvite fertilizer production alongside of treated water from urine (Randall, 2018). Apart from the already present macro and micronutrients, the high calcium content of TPR residue (about 90% w/w of ash content) renders it an excellent capturing agent in this innovative process (**Table 4-20**).

4.4.2.3 Partial usage of solid residues in clinker production

Solid residues have the potential to be used in clinker production. Previous studies by Lin *et al.* (2012) and Buruberri *et al.* (2015) have established the partial use of organic sludge and biomass ash in clinker production. The organic content of the sludge contributed to heat generation in the clinkering process while the biomass ash, rich in elements such as calcium, aluminum, silicon and iron contributed to the quality of the produced clinker (Buruberri *et al.*, 2015). Solid residues generated in this study have the characteristics to be used as partial substitutes for clinker production due to its organic content and ash content (**Table 4-20**). In particular TPR residues, because of its high ash content (85% w/w) could be directly be utilized in clinker production (Likon and Trebše, 2005). About 98% of the ash content of TPR residue is made up of required elements (Ca (342796 mg/kg), Fe (2816 mg/kg), Al (21374 mg/kg) and Si (2634 mg/kg)) essential for clinker production (Buruberri *et al.*, 2015) (**Table 4-20**). Additionally, the organic content of CR and VP solid residues was about 70% (w/w) (**Table 4-20**). A combination of CR and VP residues with high ash TPR residues could have chemical characteristics similar to mixed substrate utilized by Buruberri *et al.* (2015) in the production of clinker. The high organic content of CR and VP residues (about 70% w/w) could serve as the heat generation part while the high ash content of TPR (85% w/w)) residues contribute to the strength and quality of the produced clinker.

CHAPTER 5: CONCLUSIONS & RECOMMENDATIONS

5.1 CONCLUSIONS

The main aim of this study was to determine how much water could be reclaimed from paper sludge through fermentation, anaerobic digestion and sequential bioprocessing. This was achieved by performing sequential bioprocessing and standalone fermentation and anaerobic digestion on paper sludge obtained from three different South Africa pulp mills. The conclusions from this study are given below with reference to the aims and objectives given in section 2.8.

i) Influence of recycled process water on fermentation and anaerobic digestion of paper sludge (section 4.2)

Recycled process had an adverse effect on yeast growth and resulted in 20% to 35% reduction in final biomass concentration as compared to the utilization of clean water in fermentation (section 4.2.1.1). Alternatively, ethanol production in recycled process water fermentative batch cultures were similar to clean water control cultures at identical cellulase dosage (sections 4.2.1.2 and 4.2.2). This allowed for the exclusive usage of process water in fermentation of paper sludge at various reactor levels.

Anaerobic digestion of paper sludge was either adversely or favourably affected by the type of recycled process water utilized (section 4.2.3). BMPs of virgin pulp recycled process water (VP-PW) and paper sludge produced at least 37% more biogas yield as compared to clean water control assay (section 4.2.3.1). This allowed for the exclusive utilization of VP-PW in scaled up anaerobic digestion of VP-PS. Alternatively, corrugated recycle process water, as a result of its toxicity, had a negative effect on microbial community resulting in decreased biogas production (10 to 23% less) as compared to clean water control assays (section 4.2.3.1). Tissue printed recycle process water had no effect on biogas production from paper sludge and subsequent scale up anaerobic digestion of TPR-PS were conducted with TPR-PW.

ii) Water reclamation after sequential bioprocessing and individual fermentation and anaerobic digestion of paper sludge with recycled process water (section 4.3)

Fermentation was able to reclaim more water than anaerobic digestion of paper sludge. Fermentation reclaimed about 50% to 75% more water from paper sludge (section 4.3.2.3). The better water reclamation experienced in fermentation could be attributed to action cellulase on the lignocellulose structure. In contrast

to anaerobic digestion, cellulase employed in the fermentation process was able break down more of the lignocellulose structure in paper sludge thereby releasing more of the entrapped water molecules. Furthermore, there was no significant difference in water reclaimed from the fermentation process only and the sequential bioprocessing, as there was no significant difference in composition of solid residues before and after anaerobic digestion of fermented mixture (section 4.3.3 and **Table 4-15**).

iii) Water quality of process water after sequential bioprocessing and individual fermentation and anaerobic digestion of paper sludge (section 4.3)

The COD of subsequent stillage after fermentation of paper sludge was considerably higher than that of recycled process water used as input to the process. Fermentation increased the COD of subsequent stillage more than ten-fold as compared to recycled process water (**Table 4-10**). In contrast to fermentation, anaerobic digestion decreased the COD of subsequent process water by about 20% to 40% (**Table 4-14**). The hydrolysis of cellulose and hemicellulose by commercial cellulase in paper sludge fermentation released soluble organic products such as glycerol, organic acids (lactate and acetate) and residual sugars (mostly unutilized pentose sugars) into stillage. These soluble organic products together with proteins from cellulase and yeast cell debris contributed to the substantial COD observed in stillage after fermentation. Subsequent anaerobic digestion of fermentation stillage was able to only reduce the COD by about 15% to 30%. The insufficient reduction in COD by anaerobic digestion left the final stillage with COD over 70 000 mg/L (**Table 4-18**).

iv) Biofuel and bioenergy from sequential bioprocessing and standalone fermentation and anaerobic digestion of paper sludge with recycled process water (section 4.3)

a. Ethanol production from paper sludge with recycled process water in bench and pilot scale fermenters (section 4.3.1)

Fed-batch simultaneous saccharification and fermentation of paper sludge with recycled process water produced higher ethanol concentrations in 5 L bioreactors than 150 L fermenter (section 4.3.1). Virgin pulp produced the highest ethanol concentration of 49.6 g/L in 5 L bioreactors as a result of its superior glucan content (section 4.3.1.1). On the other hand, tissue printed recycle yielded highest ethanol concentration of 44.0 g/L in 150 L fermenter due to its low water holding capacity, as this ensured enough free water movement in fermenter leading to improved hydrolysis of substrate (section 4.3.1.2). Biofouling was observed with fed-batch SSF of virgin pulp PS and corrugated recycle PS in 150 L fermenter, this prevented ethanol concentration

going above 40 g/L (section 4.3.1.2). Also, bacterial contamination and lactic acid production were observed with fermentation of CR-PS, both in 5 L and 150 L fermenters (section 4.3.1.1). This possibly led to inhibition of *S. Cerevisiae* and contributed to the low ethanol yield attained for CR-PS. Pilot scale fermentation of TPR PS produced an ethanol concentration 10% to 15% higher than pilot scale fermentation of paper sludge and waste fiber by Vehmaanpera *et al.* (2012) (Finnish study). While ethanol production observed for pilot scale VP-PS was at most 13% lower to that obtained by Vehmaanpera *et al.* (2012).

b. Biogas and methane production from paper sludge and fermentation stillage in 30 L digesters (sections 4.3.2 and 4.3.3)

Corrugated recycle stillage produced the highest biogas yield of 184.4 ± 2.3 L/kg TS_{fed}, this was about 40% to 60% higher than biogas yields obtained from virgin pulp and tissue printed recycle stillages (section 4.3.3.1). The superior biogas production from CR stillage was as a result of the high lactic acid concentration in stillage after fermentation. Except for CR stillage, anaerobic digestion VP and TPR stillages produced 30% to 70% less biogas and methane per unit of total solids fed as compared to anaerobic digestion of VP-PS and TPR-PS (section 4.3.3.1). Biomethanation failure was experienced in anaerobic digestion of stillages, this led to the production of biogas and methane yields 2 to 3 times lesser than that of Vehmaanpera *et al.* (2012).

Regarding anaerobic digestion of paper sludge, VP-PS produced the highest amount of methane (99.5 L CH_4/kg TS_{fed}) in anaerobic digestion of paper sludge, this was followed by CR-PS (77.8 CH_4/kg TS_{fed}) and TPR-PS (65.0 CH_4/kg $TS_{fed.}$). The high methane production from VP-PS was attributed to the lower solids loading (6% w/w) as compared to 10% (w/w) employed for the other paper sludges. Also, the favorable effect of virgin pulp process water also contributed to the high methane yield obtain from VP-PS (section 4.2.3).

c. Bioenergy production from sequential bioprocess and standalone fermentation and anaerobic digestion of paper sludge (section 4.3.4)

Fermentation of paper sludge gave 35% to 55% more bioenergy as compared to standalone anaerobic digestion of paper sludge (**Table 4-12**Table 4-16). Additionally, sequential bioprocessing of paper sludge also produced about 20% to 60% more energy than individual fermentation of paper sludge (**Table 4-17**). The additional bioenergy derived from anaerobic digestion of stillage was more prominent in CR stillage than VP and TPR stillages. CR stillage contributed about 50% more bioenergy to the sequential process (**Table 4-17**). Furthermore, combustion of solid residues added about 40% bioenergy to the fermentation or sequential

process (**Table 4-17**). Based on bioenergy yields from paper sludge, anaerobic digestion does not seem to be an attractive option for industrial bioprocessing of paper sludge even though it reduced the COD of process water.

v) Potential industrial and/or agricultural use of solid residues after sequential bioprocessing of paper sludge (section 4.4)

Except for TPR residues, solid residues from VP and CR showed potential to generate in house steam that could be used in distillation system after fermentation. The HHV of VP and CR solid residues were 12.5 and 11.3 MJ/kg, respectively. This added about 40% more bioenergy to the fermentation or sequential process. Additionally, solid residues have the potential to be used as nutrient supplements for plantation and natural forest soils, as they contained primary, secondary and trace elements such as phosphorous, potassium, magnesium and molybdenum required for plant growth. Also, solid residues showed potential to be partially used in clinker production. Solid residues contained the required elements such as calcium, aluminum, iron and silicon essential for clinker production. Especially, TPR residues because of its high ash content (85% w/w) showed the best potential for clinker production.

In conclusion, all objectives of this research project were met regarding water reclamation and biofuel production through integrated biochemical processing of paper sludge.

5.2 RECOMMENDATIONS

i) Impeller upgrade in pilot scale fermenter

In pilot scale fed-batch fermentation of paper sludge with recycled process water, mixing difficulties caused biofouling in VP-PS and CR-PS. This led to poor mass transfer and reduced the rate of hydrolysis in fermenter (Boshoff *et al.*, 2016). Apparently, rushton impellers employed in pilot scale fermenter were not sufficient enough to overcome the biofouling effect. Rushton impellers unlike axial flow impellers, are not suited to high viscous solid and liquid mixtures (Myers *et al.*, 1996). A better modification will be the usage of multiple impellers consisting of both rushton and axial flow impellers.

ii) Continuous anaerobic digestion of fermented stillage

Batch digestion of fermented stillage yielded biogas and methane yields far below what was obtained by Vehmaanpera *et al.* (2012). Anaerobic digestion in batch digesters couldn't handle the high soluble organic loading of fermented stillage. Thus, it is recommended that future anaerobic digestion of fermented stillage is conducted in continuous digesters with intermittent feeding. McCarty (1964) indicated continuous AD systems prevent biomethanation failure caused by high organic loading. A continuous AD system could significantly decrease the COD of stillage while also producing methane yields similar to that obtained by Vehmaanpera *et al.* (2012) in pilot scale continuous AD of stillage obtained from fermentation of paper sludge and waste fiber.

iii) Aspen modelling and techno-economic evaluation of sequential fermentation and anaerobic digestion of paper sludge with recycled process water

It is recommended that a techno economic analysis be conducted on the sequential fermentation and anaerobic digestion and standalone processes, as additional economic factors play a role in determining the most feasible biochemical processing route on an industrial implementation scale. Factors such as the elevated COD of fermentation and sequential processing stillages would affect the techno economic analysis.

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APPENDIX

ADDITIONAL EXPERIMENTAL RESULTS

Table 5-1: Summary for Yeast screening at solids loading of 50 g/L to determine the effect of PW on microbial yeast

PW type	%PW	Ethanol Concentration (g/L)	Yield (g ethanol/g glucose fed)	% Theoretical concentration	Productivity (g/Lhr)	Yeast growth rate (hr^{-1})
Virgin pulp	0	22.827	0.457	89.343	0.951	0.077
	25	22.690	0.454	88.807	0.945	0.073
	50	22.056	0.441	86.326	1.838	0.072
	75	23.085	0.462	90.354	1.924	0.076
	100	22.937	0.459	89.773	1.911	0.070
Corrugated recycle	0□	20.657	0.413	80.849	0.861	0.089
	25□	21.887	0.438	85.663	0.912	0.090
	50□	22.023	0.440	86.196	0.918	0.085
	75□	21.407	0.428	83.785	0.892	0.081
	100□	21.083	0.422	82.517	0.878	0.082
	25*	21.588	0.432	84.493	0.900	N/A
	50*	21.765	0.435	85.186	0.907	N/A
	75*	21.779	0.436	85.240	0.907	N/A
	100*	21.786	0.436	85.267	0.908	N/A
Tissue printed recycle	0	22.201	0.444	86.893	0.925	0.096
	25	21.222	0.424	83.060	0.884	0.095
	50	22.615	0.452	88.514	0.942	0.089
	75	22.349	0.447	87.472	0.931	0.090

	100	22.925	0.458	89.725	0.955

(*)- Crude Corrugated recycle PW ; **(D)**- Centrifuged Corrugated recycle PW; **(N/A)**- Not applicable

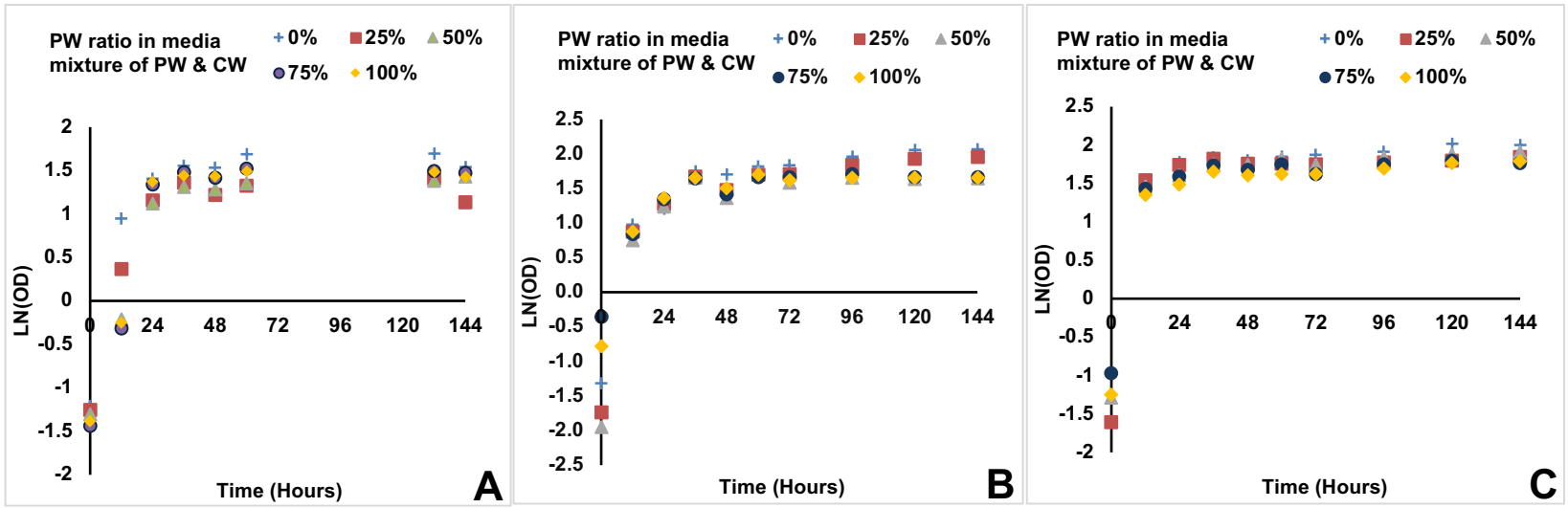

Figure 5-1: Effect of process water on yeast growth; A-*Virgin pulp PW,* B- *Corrugated recycle PW,* C- *Tissue printed recycle PW*

Table 5-2: Summary of yields for BMP test of paper sludge with process water

PS type	Volatile solids fed (% TS)	%PW	Cumulative biogas/TS (L/Kg)	Cumulative biogas/VS (L/Kg)	Methane %					Cumulative CH_4/TS (L/Kg)	Cumulative CH_4/VS (L/Kg)	BD_{CH4} (%)	HRT (Days)
					Week 1	Week 2	Week 3	Week 4	Week 5				
Virgin pulp	75.17	0	193.3 ± 55.8	257.2 ± 74.3	46.0	47.9	48.0	47.5	47.4	90.5 ± 26.0	120.3 ± 34.5	40.0 ± 10.9	45
		25	385.1 ± 26.9	512.2 ± 35.8	46.9	48.5	47.2	50.9	48.3	182.9 ± 13.3	243.3 ± 17.7	76.8 ± 5.6	
		50	392.2 ± 5.1	521.8 ± 6.8	50.3	48.8	48.3	50.3	49.8	189.6 ± 2.7	252.3 ± 3.5	79.6 ± 1.1	
		75	331.7 ± 18.6	441.2 ± 24.8	40.8	42.5	53.3	52.6	48.1	156.5 ± 7.7	208.2 ± 10.2	65.7 ± 3.2	
		100	303.2 ± 38.6	403.4 ± 51.3	34.6	32.8	28.4	70.5	49.0	143.4 ± 17.8	190.7 ± 23.7	60.2 ± 7.5	
		100#	319.1 ± 27.1	424.5 ± 36.1	28.3	35.5	48.9	64.7	51.2	157.5 ± 11.1	209.6 ± 14.8	66.2 ± 5.2	
Corrugated recycle	74.07	0	181.5 ± 19.5	245.0 ± 26.2	36.3	43.5	46.6	57.3	-	87.4 ± 9.7	118.0 ± 13.0	47.6 ± 5.3	30
		25*	183.0 ± 13.0	247.0 ± 17.6	22.5	44.9	53.9	58.3	-	95.5 ± 7.3	131.1 ± 10.0	52.9 ± 4.0	
		50*	163.8 ± 9.6	221.2 ± 13.0	21.6	40.3	54.1	52.4	-	79.0 ± 4.9	108.4 ± 6.7	43.8 ± 2.7	
		75*	155.7 ± 9.9	210.2 ± 13.4	20.0	40.3	50.0	52.8	-	73.5 ± 5.2	100.9 ± 7.1	40.7 ± 2.8	
		100*	147.3 ± 2.5	198.8 ± 3.3	20.5	37.2	47.7	61.5	-	72.3 ± 0.3	99.3 ± 0.5	40.1 ± 0.2	
		0	181.5 ± 19.5	245.0 ± 26.2	36.3	43.5	46.6	57.3	-	87.4 ± 9.7	118.0 ± 13.0	47.6 ± 5.3	
		25□	181.5 ± 4.5	245.1 ± 6.1	38.7	49.2	55.1	56.2	-	93.1 ± 2.6	125.7 ± 3.5	50.7 ± 1.4	
		50□	204.3 ± 1.5	275.8 ± 2.0	38.1	51.3	55.5	56.6	-	107.0 ± 0.8	144.4 ± 1.1	58.3 ± 0.4	
		75□	207.8 ± 9.5	280.5 ± 12.8	37.5	54.1	58.5	60.4	-	114.7 ± 5.8	154.8 ± 7.8	62.5 ± 3.2	
		100□	219.0 ± 7.1	295.6 ± 9.6	36.3	54.4	60.5	63.1	-	124.6 ± 3.9	168.2 ± 5.3	67.9 ± 2.1	
Tissue printed recycle	37.13	0	191.3 ± 5.7	515.3 ± 15.5	48.9	53.5	49.4	40.5	-	95.7 ± 3.1	257.6 ± 8.4		30
		25	206.7 ± 4.4	556.6 ± 11.9	49.2	54.1	49.0	41.2	-	105.2 ± 2.5	283.3 ± 6.6		
		50	196.2 ± 12.6	528.3 ± 34.0	50.1	54.6	49.4	40.7	-	100.7 ± 6.7	271.2 ± 18.0		

		75	195.4 ± 20.3	526.2 ± 54.7	49.7	54.4	49.7	43.6	-	100.3 ± 10.6	270.0 ± 28.6
		100	205.9 ± 15.1	554.4 ± 40.7	51.5	56.1	50.4	42.7	-	108.1 ± 8.2	291.2 ± 22.1

(*)- Crude Corrugated recycle PW ; **(□)-** Centrifuged Corrugated recycle PW; (BD_{CH4})- Biodegradability based in methane yield

(#)- BMP test of VP-PS conducted with 100% CR-PW

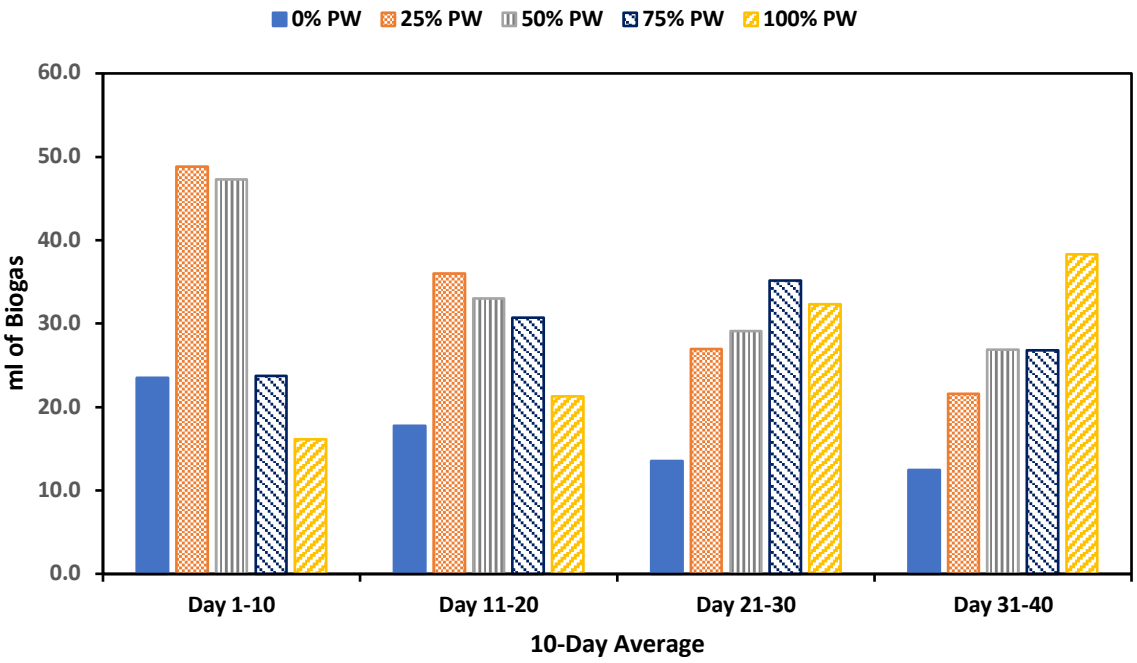

Figure 5-2: 10-day average biogas production during incubation period

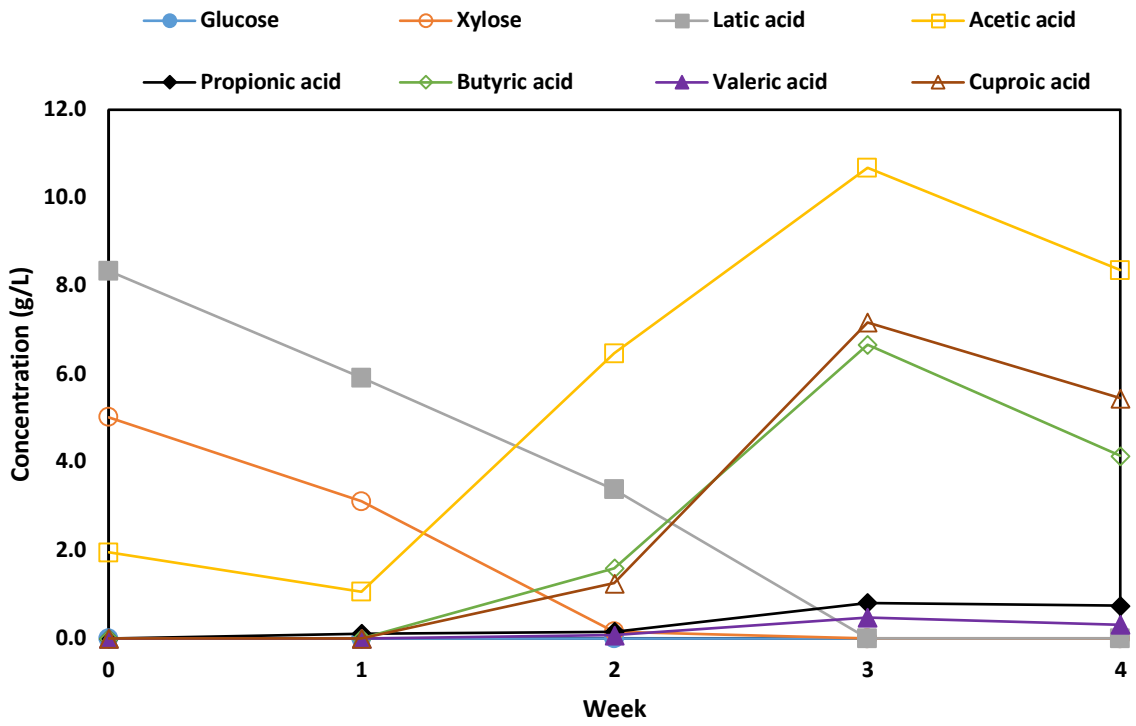

Figure 5-3: VFAs concentration profile for 30L digestion of Virgin pulp PS fermented stillage

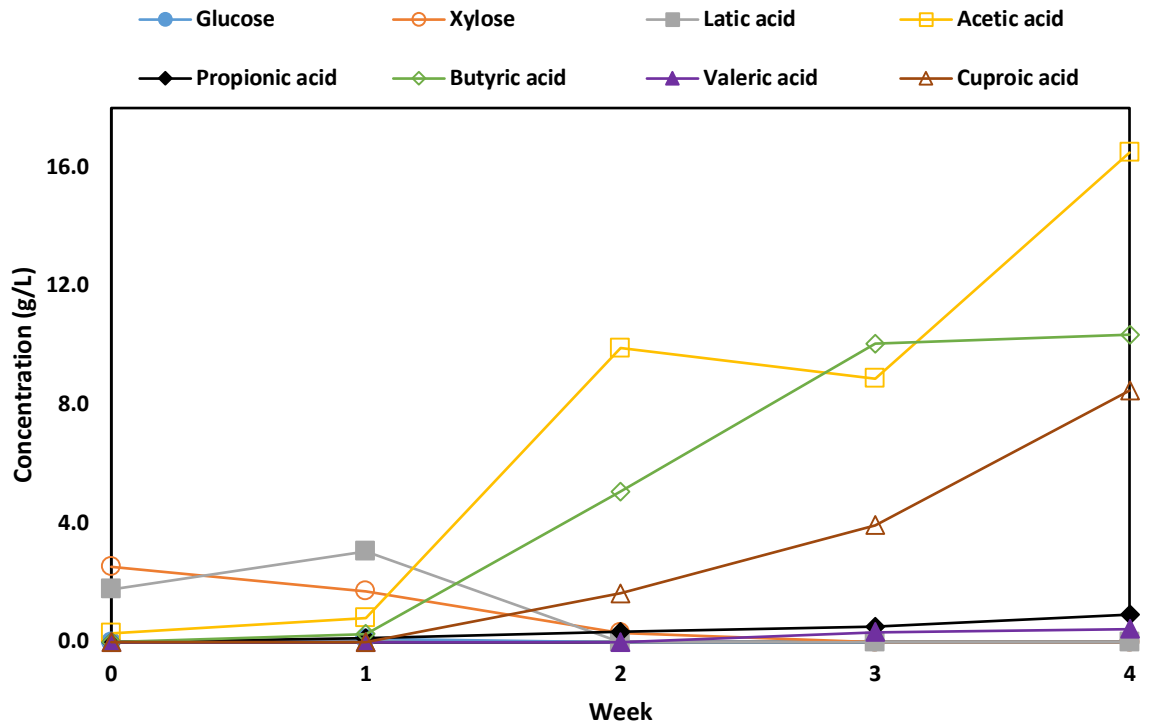

Figure 5-4: VFAs concentration profile for 30L digestion of Tissue printed recycle PS fermented stillage

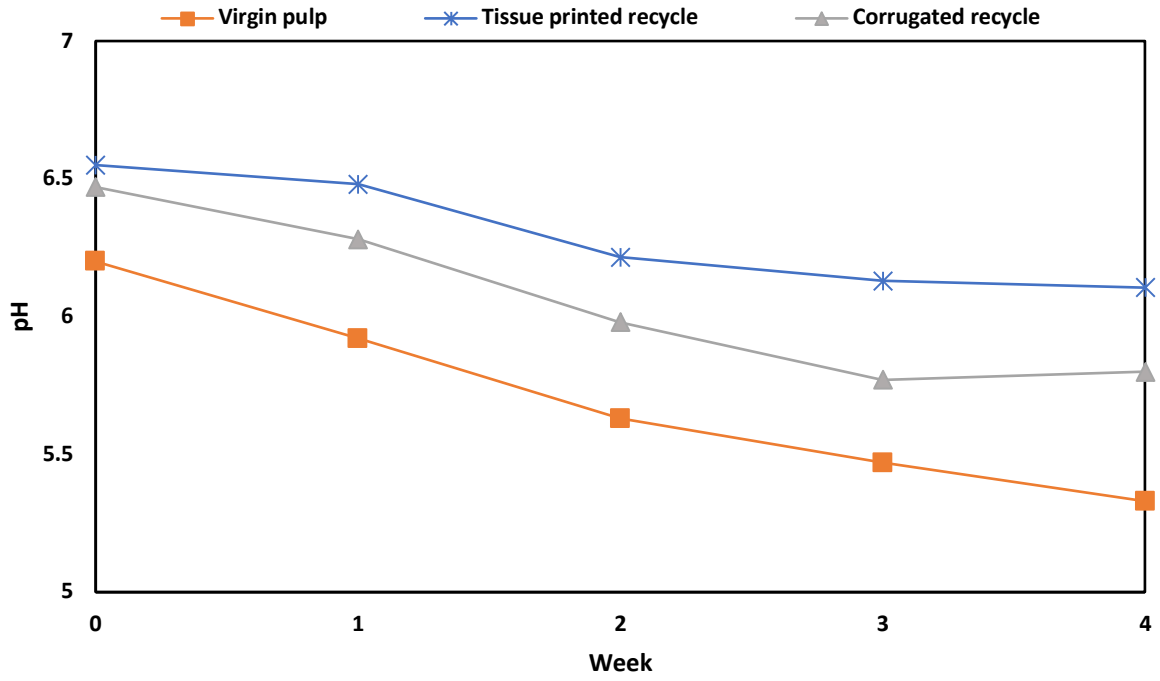

Figure 5-5: pH profile for 30L digestion of fermented stillage