RECLAMATION OF INDUSTRIAL PROCESS WATER FROM SOLID AND LIQUID EFFLUENTS, THROUGH INTEGRATED BIO-ENERGY PRODUCTION

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Report to the
Water Research Commission

by

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EXECUTIVE SUMMARY

BACKGROUND

The present project addresses opportunities to reclaim industrial wastewater from solid and liquid effluents such as paper waste sludge through integrated bio-energy production. Sequential application of fermentation for ethanol production and anaerobic digestion for biogas production are applied as appropriate bio-energy technologies, both to produce valuable and clean energy sources, while at the same time to process and reclaim the wastewaters in which these solids wastes are produced. The intended outcome of the project is to maximise the potential to reclaim industrial wastewater for re-use, while also producing acceptable quantities of bio-energy sources. A critical component of the project is anaerobic digestion of the solid and liquid effluents produced by ethanol-fermentation, which should be performed at a scale commensurate with the pilot-scale ethanol production, which is performed in a 100 L fermenter.

AIMS

The following were the aims of the project:

1. To design and construct an experimental anaerobic biodigester module consisting of 8 X 50 L reactors. No such experimental system exists in SA, and will serve this project as well as several to follow, very well, leading to greater industrial roll-out of the technology.
2. To develop and scale up the optimised process developed (in a different investigation) for production of bioethanol from a range of paper sludge wastes to 100 L bioreactor scale.
3. Determine potential biogas yield from paper sludge wastes, and from fermentation residues remaining after bioethanol production from each of these feedstocks, using 50 L reactors.
4. Characterise the final residues (solid and liquid components) of anaerobic biodigestion and determine the suitability for, and extent of water reclamation, and suitability of water and solid residues from these biofuel production processes for industrial and/or agricultural application. Determine energy yield from combustion of solid residues of anaerobic biodigestion.
5. Stakeholder engagement in the fruit industry in the Western Cape, to determine the scope and level of buy-in for ethanol production from fruit wastes.

SUMMARY OF WORK TO DATE

The following project activities have been completed:

- Literature survey on integrated production of ethanol and biogas from paper waste sludge.
- Design and construction of experimental anaerobic biodigesters.

- Combined production of ethanol and biogas from paper sludge waste, together with a portion of wastewater recycling.
- Scale-up of integrated ethanol-biogas process to 100 L (bioethanol) and 30 L (biogas) reactors.
- Assessment of the sustainability of the proposed processes, through mass &energy balances and economic viability modelling.
- Stakeholder engagement in the fruit industry to determine scope and buy-in for ethanol production from fruit wastes.

CONCLUSIONS

- Eight biogas digesters were designed and procured. The purchase and modification of existing reactors was the most cost-effective solution, and could be achieved by combining the available capital budget in the present project with other sources of equipment funding.
- The literature review and initial experimental runs confirmed that integrated ethanol-biogas production, with process water is possible. Further development of the integrated fermentation-digestion process, with the use of processed wastewater in both of these processed, provided the following conclusions:
    - Processed wastewater from the clarifiers of the adjacent pulp mills can be used as make-up water in both the fermentation and anaerobic digestion processes, with limited impacts on process operation and performance.
    - Fermentation increased the COD of the reaction mixture and therefore produced a supernatant that has a much higher COD compared to that of the starting liquid, i.e. process water.
    - The higher COD, as obtained through fermentation, could not be eradicated through anaerobic digestion. However, anaerobic digestion did contribute to lowering the COD of the liquid.
    - Sequential fermentation and anaerobic digestion of paper sludge yielded more bio-energy compared to the individual processes.
    - Fruit pomace, a potential feedstock for ethanol production: An industrial and literature survey.
    - Citrus, apple and grape are the main fruits produced in the Western Cape.
    - Fruit Pomace is a by-product of fruit processing.
    - Fermentable – and structure sugars – constitute a large fraction of fruit pomace.
    - The chemical composition of pomace differs significantly between different fruits. The type of process technology used also influences the final result.
    - White grape pomace has been identified as the most promising feedstock for ethanol production. This in part is attributed to the extensive production of white grapes in the Western Cape but also to the large amount of fermentable sugars that remain in the pomace following juice extraction.
    - Industry have not considered ethanol production from pomace. Tradition dictates pomace to be used either as an animal feed, for the purpose of composting or landfilling in the case of discharge.

- Most industries identified electricity generation as the preferred choice and future investment. However, economy of scale and the low cost of fossil fuel have impeded progress.
- The economic viability of paper sludge to bio-fuels was investigated. Three different substrates namely virgin, corrugated recycled and tissue printed recycled waste sludge served as feedstocks. Bio-fuel technologies including fermentation (i.e. ethanol production), anaerobic digestion (i.e. biogas production) and the combination of these were investigated.
- Only virgin sludge has the potential to produce economically viable biofuels. A minimum ethanol selling price of 13.6 ZAR/L was estimated which means the project can break even. Alternatively, methane from virgin sludge is the economically more favourable process. The production cost of methane is 30% less than market price of natural gas at 1.41 ZAR/m^3 (STP).
- The economic viability of the other two feedstocks (i.e. corrugated recycled and printed tissue recycled waste sludge) could be enhanced by utilising both bio-fuel technologies in sequence. However, none of the feedstocks actually yielded a profitable process. Corrugated recycled sludge are produced in too little quantities to make economically sense whereas printed tissue recycled waste sludge contains large amounts of ash which negatively yield of biofuels.

RECOMMENDATIONS

- Use only the supernatant of the fermentation stillage for anaerobic digestion. No real chemical difference exist in the fermentation solids before and after anaerobic digestion.
- High COD generated by fermentation may require additional processing beyond anaerobic digestion.

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ACRONYMS & ABBREVIATIONS

AD	Anaerobic digesters
AOX	Adsorbable organic halides
BOD	Biological oxygen demand
C/N	Carbon to nitrogen ratio
CEPPWAWU	Chemical, energy, paper, printing, wood and allied workers' union
COD	Chemical oxygen demand
CR	Corrugated recycle
CSD	Continuously stirred digester
ECF	Elemental chlorine free
HMF	5- hydroxymethylfurfural
HRT	Hydraulic retention time
NREL	National renewable energy laboratory
NSSC	Neutral sulphite semi chemical
OLR	Organic loading rate
PAMSA	Paper Makers' Association of South Africa
PS	Paper/primary sludge
PW	Processed wastewater
PW/CW	Processed wastewater to clean water ratio
RCF/RPF	Recycle pulp fibre
SCFA	Short chain fatty acids
SHF	Separate (enzymatic) hydrolysis and fermentation
SS	Suspended solids
SSF	Simultaneous saccharification and fermentation
TAN	Total ammonia nitrogen
TCF	Total chlorine free
TPR	Tissue printed recycle
TS	Total solids
TSS	Total suspended solids
VP	Virgin pulp
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.

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.

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CHAPTER 1: BACKGROUND

1.1 INTRODUCTION

The present project addresses opportunities to reclaim industrial wastewater from solid and liquid effluents such as paper waste sludge and fruit wastes through integrated bio-energy production. Sequential application of fermentation for ethanol production and anaerobic digestion for biogas production are applied as appropriate bio-energy technologies, both to produce valuable and clean energy sources, while at the same time to process and reclaim the wastewaters in which these solids wastes are produced. The intended outcome of the project is to maximise the potential to reclaim industrial wastewater for re-use, while also producing acceptable quantities of bio-energy sources. A critical component of the project is anaerobic digestion of the solid and liquid effluents produced by ethanol-fermentation, which should be performed at a scale commensurate with the pilot-scale ethanol production, which is performed in a 100 L fermenter.

The present report addresses two key components of Deliverable 1 of the project, i.e. a literature review to show the potential of and select process conditions for optimisation of the integrated fermentation-digestion process, as well as reporting on the design and acquisition of biogas digesters of appropriate scale, for experimental test work.

1.2 PROJECT AIMS

The following were the aims of the project:

1. To design and construct an experimental anaerobic biodigester module consisting of 8 X 50 L reactors. No such experimental system exists in SA, and will serve this project as well as several to follow, very well, leading to greater industrial roll-out of the technology.
2. To develop and scale up the optimised process developed (in a different investigation) for production of bioethanol from a range of paper sludge wastes to 100 L bioreactor scale.
3. Determine potential biogas yield from paper sludge wastes, and from fermentation residues remaining after bioethanol production from each of these feedstocks, using 50 L reactors.
4. Characterise the final residues (solid and liquid components) of anaerobic biodigestion and determine the suitability for, and extent of water reclamation, and suitability of water and solid residues from these biofuel production processes for industrial and/or agricultural application. Determine energy yield from combustion of solid residues of anaerobic biodigestion.
5. Stakeholder engagement in the fruit industry in the Western Cape, to determine the scope and level of buy-in for ethanol production from fruit wastes.

1.3 SCOPE AND LIMITATIONS

The literature review component of the report is based on peer-reviewed articles in international scientific journals. The design and construction of the anaerobic digesters were based on experiences within the research group as well as local and international partners.

1.4 SUMMARY OF WORK TO DATE

Table 1-1 and **Table 1-2** summarise the work to date, revised due dates and deliverables submitted thus far.

Table 1-1: Work completed to date

No.	Task	Summary of work to date
1	Literature survey on integrated production of ethanol and biogas from paper waste sludge.	Completed on schedule.
2	Design and construction of experimental anaerobic biodigesters	Completed on schedule. 30 L reactors selected due to manufacturing and operational realities.
3	Combined production of ethanol and biogas from paper sludge waste, together with a portion of wastewater recycling	Completed
4	Scale-up of integrated ethanol-biogas process to 100 L (bioethanol) and 30 L (biogas) reactors.	Completed ahead of schedule.
5	Characterise residues (solid and liquid streams) of ethanol fermentation and anaerobic biodigestion, to quantify amounts of water reclamation, together with the potential for industrial and agricultural applications.	Completed.
6	Determine net energy yield from combustion of solid residues of anaerobic biodigestion of paper sludge waste with a bomb calorimeter.	Completed
7	Assessment of the sustainability of the proposed processes, through mass &energy balances and economic viability modelling.	Completed
8	Stakeholder engagement in the fruit industry to determine scope and buy-in for ethanol production from fruit wastes.	Completed

Table 1-2: Deliverable due dates and deliverables submitted to date.

No.	Deliverable	Status	Due date
No Items Listed			

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

A major source of landfilled waste from the pulp and paper industry which currently has no specific eco-friendly solution is primary sludge (PS). It is estimated that approximately 500 000 wet tons of PS is produced annually in South Africa by the members of the Paper Making Association of South Africa (PAMSA) (Boshoff, 2015). Considerable amounts of reclaimable water is lost in landfilling due to high moisture content of the primary sludge as referenced from section 2.3. Apart from the industry landfilling primary sludge and discharging substantial volumes of potentially reusable processed wastewater into the environment, there is a probability of concurrent wastewater treatment alongside bioenergy production from these two major waste streams. Various South African primary sludge tested by (Williams, 2017; Boshoff, 2015) showed a decrease in water holding capacity of up to 90% of the original primary sludge after bioethanol fermentation. Anaerobic digestion of primary sludge on the other hand showed a less prominent decrease in water holding capacity (up to 56% decrease) as compared to bioethanol fermentation.

Sequential ethanol fermentation of primary sludge and anaerobic digestion of paper sludge fermented residues will not only considerably reduce the amount of solid waste to be sent to landfill but further decrease the water holding capacity per unit mass as compared to separately employing either of the two biochemical processes. Moreover, water is added to solid waste to obtain a slurry suitable for fermentation and/or biogas production. The possibility of employing dirty process wastewater discharged from primary clarifiers as make-up water for both fermentation or biogas production and thus effectively clean-up the dirty processed water for recycling is an issue which needs to be investigated. Apart from production of bioenergy, this would potentially increase the quantities of available water for recycling and reduce the burden on wastewater treatment in the pulp and paper industry.

The purpose of this study is to optimise the sequential fermentation and anaerobic digestion of primary sludge and dirty processed wastewater, to maximise the potential for water reclamation, treatment and recycling. All solid and liquid products from these processes are to be characterised carefully, together with measurement of energy product yields. The purpose of the literature review below is to:

(i) determine types and quantities of solid and liquid wastes from paper and pulp industry in SA,

(ii) propose operating conditions and expected yields to maximise bio-energy production, and

(iii) to consider the potential of sequential ethanol-biogas production processes for water reclamation and treatment for recycling

2.2 THE PULP AND PAPER INDUSTRY PROCESS AND WASTE GENERATED

With the introduction of new and progressive technologies, the pulp and paper industry has grown tremendously in production and demand since its industrial conception in the middle of the 19th century. Paper and cellulosic fibres can be produced from pulp, recycled waste paper and cotton linen rags with the former being dominant (Koplan et al., 2002). Cotton and linen rags are used in making expensive fine-grade papers such as letterheads, resume papers, bank notes and security certificates. Globally half of the fibre used for pulp manufacture comes from harvested wood supplemented with sawmill dust and some non-wood agricultural matter such as sugarcane bagasse, reeds and straw (Sixta, 2008). Furthermore bamboo, flax, hemp and jute fibres are also minor sources of raw material for paper pulp. Wood pulp is made from the mechanical, thermal and chemical processing of wood chips. In the year 2000, the entire virgin fibre (wood) pulp production totalled 187 million metric tonnes and at the moment more than 90% of pulp (virgin pulp fibre) produced worldwide is from wood pulp (Sixta, 2008).

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). . The raw material supply for the South African pulp and paper industry is indicated **Table 2-1** below. Recycled fibre is another important source of raw material for which 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

2.2.1 Industry Waste Generated at each stage of the Manufacturing Process

Different types of waste ranging from gaseous, solid and liquid components are produced from the paper and pulp making process which starts with preparation of wood or recycled waste paper to pulp and final finished product rolled from the paper machine. Presently the effluent stream consisting of several components are separated into respective liquid and solid waste streams by physiochemical treatments such as sedimentation and filtration clarifiers. Depending on the quality of wastewater from

primary clarifiers, secondary and tertiary treatments such anaerobic digestion wastewater systems are applied to further treat the liquid stream before being discharged into the environment. **Figure 2-1** gives a general description of the paper and pulp production and the kind of waste generated at each process.

2.2.1.1 Wood Preparation

Wood yard operations purpose is to collect logs, wood residues and chips. Debarking and chipping machinery debark, chip the logs and later stockpile the bark and woodchips for further processing (Sixta, 2008). As seen from **Figure 2-1** this stage usually gives solid waste in the form of bark refuse, wood particles and sawdust. Generally, this solid waste is low in moisture content are mostly converted to energy in boiler and furnace equipment (Robertson, 1990).

2.2.1.2 Pulping/Cooking

The pulping process encapsulate the reduction of lignocellulosic material into cellulose fibres known as pulp. Pulp can be achieved through mechanical and chemical treatment of lignocellulose or combination of both. Mechanical pulp is made by applying mechanical force to wood causing separation of fibres into pulp while chemical pulping involves chemicals for dissolving of lignin (polymer that binds fibres in wood) to yield pulp (Sixta, 2008). Chemical pulping techniques commercially employed are the Soda-AQ, Kraft/sulphate and sulphite methods that roughly accounts for 80% of the world's pulp production (Suhr et al., 2015). Malodourous gases such as hydrogen sulphide and oxides of sulphur are emitted from pulping and their recovery processes. Often secondary control technologies such as scrubber systems are employed to capture these toxic gases to avoid air pollution. Likewise, inorganic sludge (dregs and lime mud) and spent sulphite liquor are typical waste generated, since chemical pulping uses mixtures of Na_2S, NaOH and other compounds (Suhr et al., 2015; Monte et al., 2009).

2.2.1.3 Pulp washing and Screening

Pulp is washed with clean water to mainly recover valuable chemicals used in cooking process to chemical recovery systems. Afterwards the washed pulp is screened remove uncooked fibre bundles and knots as depicted in **Figure 2-1**. Efficient washing generates a number of benefits, which includes reduction in cooking chemical losses and reduction of black liquor carry-over with the pulp into the bleach plant (Suhr et al., 2015). This is done to prevent dissolved chemicals and knots from interfering with downstream process. This stage produces considerable quantities of wash wastewater and weak liquor with suspended knot fibre bundles. The suspended knot fibre bundles after primary clarification usually end up in landfills since incineration of this solid waste is unattractive due their high water holding capacity.

2.2.1.4 Pulp Bleaching

Unbleached pulp still has some amount of residual lignin, which gives it a brownish colour. Bleaching is a delignification process done to give pulp its whitish colour. Initially the pulp is treated with highly alkaline solution of NaOH and oxygen. And final bleaching is carried out afterwards in series of reactors consisting of bleaching agents such as H_2O_2, ClO_2, H_2SO_4, etc. (Suhr et al., 2015). As indicated in **Figure 2-1** bleach water consisting of organic and chlorinated organic compounds is the main source of waste production. Bleach wastewater are usually treated by biological technologies such as aerobic and anaerobic systems or in combination with physiochemical technologies (Singhal & Thakur, 2009).

2.2.1.5 Paper Making

The final product is produced at this stage through mechanical treatment of fibres. Fibres bond to each other while chemicals are added to give some special properties. White water, fibres and fillers are the main wastes generated at this final stage. White water is recycled multiple times in modern paper and pulp mills, until it is enriched with fibres and fillers (Hubbe, 2007). Afterwards enriched white water undergoes various primary wastewater treatment to concentrates fibres and fines into the solid waste which mostly ends up in landfills.

2.2.2 Pulping of Recovered Paper

The usage of recovered fibre as a replacement feedstock for producing paper products has increased globally since the 1960s. First, the recovered paper is re-pulped with hot water or white water (water from the paper machine) in a mechanical or hydraulic agitation machine to obtain fragmented fibres. Afterwards, a cleaning and screening process is applied to the pulped fibre to separate contaminants from fibre. As indicated in **Figure 2-1**, the pulped, screened and cleaned fibre can be optionally upgraded by de-inking and furthermore if required bleached before it is finally converted into products in the paper machine.

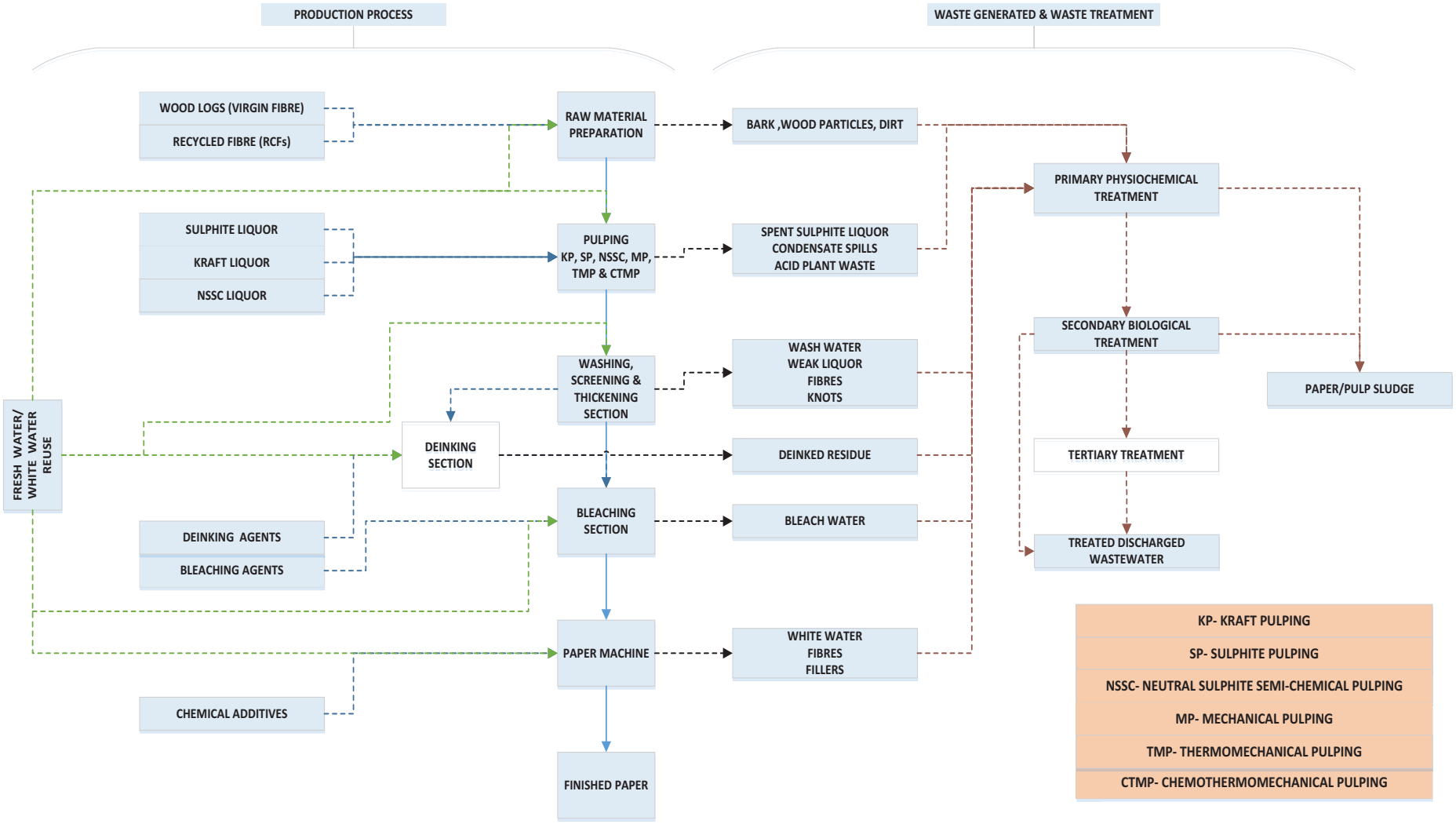


Figure 2-1: Paper and pulp making process and produced organic waste schematic representation.

2.2.3 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
	Felixton	Unbleached Bagasse pulp	70
	Merebank	Thermomechanical pulp	220
		Groundwood Pulp	6
	SilvaCel	Hardwood Pulp	*
Sappi	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
	Saiccor	Dissolving pulp	500
Total			**2602***

*1.9 million green metric tons 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	Bellville	Crepe tissue	25
	Klipriver	Crepe tissue	23
	River view	Crepe tissue	10
	Rosslyn	Fluting and test liner	50
Sappi	Ngodwana	White top and Kraft linerboard	240
		Newsprint	140
	Tugela	Kraft linerboard, fluting and other Kraft	390
	Cape Kraft	Test liner, 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	Test liner	80
Other	Approximately 12 other smaller mills often dealing with recycled paper		77*
Total			**2648***

(*Estimate)

2.2.4 Water Use in the Industry

It can be inferred from **Figure 2-1** that the pulp and paper industry is largely dependent on water in their production operations. All the major processes along the production line requires substantial amounts of water, which is 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, which can sometimes be detrimental to the environment if not treated properly. Lately stricter regulations have forced many paper and pulp mills to recycle as much as processed 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, which demand less water intake and discharge mildly polluted wastewater, 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 SOLID WASTE AND LIQUID EFFLUENT

Inferred from **Figure 2-1**, solid waste generated by the industry is in the form paper sludge which originates from primary wastewater clarifiers. On the other hand, the liquid effluent discharged to the environment are in the form treated processed wastewater. In this section, the relative proportions of different constituents in both waste streams will be highlighted, with processed wastewater particularly having an extensive diversity of compounds. The constituents of processed wastewater and composition of paper sludge from primary clarifiers depends on the type of pulping method, kind of the

wood or fibre material, management practices and the quantity of water to be used in the particular process. The type of wood considerably affect the kind of waste generated in the production of pulp and paper since the proportion of lignin and hemicellulose differ in softwood and hardwood. Also, if the main raw material is supplemented with plant fibres such as bagasse, it tends to change the nature of the solid and liquid waste produced. Furthermore, the kind of pulping and bleaching method significantly affects not only the quality of pulp or paper produced, but also the quantity, composition and toxicity of waste generated. Similarly, effluents of chlorine dioxide bleaching in Kraft pulp mills commonly contain of chlorinated organic compounds, while total chlorine free (TCF) bleaching yields no chlorinated organics and low Chemical Oxygen Demand (COD) in processed wastewater (Ali & Sreekrishnan, 2001). Due to stricter environmental regulations being enforced, various treatment technologies are employed on site and off site on waste streams from the paper and pulp industry. Consequently, the kind of treatment technologies undertaken by pulp and paper mills do affect the colour, composition and toxicity levels of discharge waste streams.

2.3.1 Solid waste (Paper Sludge) Characterization

Primary paper sludge is the solid waste collected from primary clarifiers which is presently mostly disposed of in landfills. As indicated in **Figure 2-1**, waste streams consisting of effluents from various stages of the production process are treated in primary clarifiers (physical treatment) which results in residual waste paper sludge and clarified wastewater. Primary paper sludge usually comprises of organic and inorganic matter (unusable degraded short fibres from the paper making process as well as inks, glues, clay, residues and chemicals used in the recovery process) which originates from the reprocessing unit that recycles paper and a waste stream coming from the thermo-mechanical or chemical pulping plant. 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). The water holding capacity (WHC) is the amount of water that a particular material can saturate. The water holding capacity of primary sludge is high (within 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 2.1 show the variation in the feed, process types and amount of primary sludge emanating from different milling operations in South Africa.

Table 2-6: The kind of feed, process, products and primary clarifier sludge production by 11 South African Paper and Pulp Mills1 (Redrawn from Boshoff, 2015)

Company: Mill	Sample numbers	Feed[2]	Process[3]	Products[4]	Production (dry ton/year)	Moisture content (%)
Kimberly-Clark: Enstra	1, 2, 3, 4	RF, NPW, VP	RP, DI	TP	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 = V irgin wood, E = Eucalyptus, P = Pine, BP = Bagasse pulp.

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

4 TP = Tissue paper, B = Baycel pulp, KL = Kraft linerboard, CB = Containerboard, OP = Office paper, S P =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 solid waste from pulp and paper mills is difficult to determine due to several interfering factors. Primary 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.

Primary sludge consists mostly of cellulose with lower amounts of hemicellulose and lignin as indicated in **Table 2-8**. The carbohydrate content of primary sludge varies between 20 to 70% (Fan & Lynd, 2007). Cellulose is a glucose polymer with crystalline structure connected by β-(1→4)-glycosydic 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 which 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.

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 Constituents on liquid waste (processed Wastewater) from primary clarifier

Each stage within the pulp and paper production process consumes large amounts of water, which finally re-emerges as processed wastewater. Individual stages of the pulp and paper production process generates processed wastewater of different composition as indicated in **Table 2-10** below. Feed material such as recycled fibre (RCFs), and wood (virgin fibre) contains compounds like lignin, carbohydrate and extractives, which undergo considerable chemical changes due to the processes employed in the pulp and paper operations. For example, the use of softwood yields greater quantities of phenolic by-products than hardwood (Environment Canada & Health Canada, 1991). Additionally, the kind of pulping and bleaching technologies applied on the sort of feed material can generate several toxic substances like unsaturated fatty acids, diterpentene alcohols, resin acids, chlorinated resin acids, juvaniones and many other chemicals which is expected in clarified processed wastewater (Pokhrel & Viraraghavan, 2004). 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 processed wastewater, as it employs chemicals like chlorine dioxide and hydrogen peroxide for pulp brightening (Pokhrel & Viraraghavan, 2004).

Processed wastewater is anticipated to contain oxygen-consuming organic compounds originating from Kraft and sulphite pulping process with COD loads much higher in the sulphite pulping (Suhr et al., 2015). Similarly, carbohydrates, lignin derivatives, methanol, organic acids, furfural and reduced-sulphur compounds with resin, fatty acids, terpenes and ethanol in lower concentrations are expected to be present in processed wastewater due to chemical and mechanical pulping of different types of raw material utilized in pulp and paper mills (Blackwell et al., 1979; Rexfelt & Samuelson, 1970; Rintala & Puhakka, 1994).

Furthermore, chlorinated organic compounds are also expected to be identified in clarified processed wastewater, if the pulp is bleached using chemical agent like chlorine dioxide. Bleach wastewater mainly comprises of degradation compounds of residual lignin in pulp after chemical pulping (Rintala & Puhakka, 1994). The application of elemental chlorine free bleaching (ECF-ClO_2), which has substituted elemental chlorine bleaching, generates an enormous variety of chlorinated organic compounds, unlike totally chlorine free bleaching (TCF- ozone, O_2, H_2O_2, etc.). About 70 to 80% of the chlorinated organic compounds expected in clarified processed wastewater has been reported to be high molecular mass material (MW> 1000), which primarily consist of cross-linked aliphatic compounds (Environment Canada & Health Canada, 1991). The low-molar-mass chlorinated organic compounds (MW< 1000) entails chlorinated phenols and chlorinated acids.

Table 2-10: Characteristics of wastewater generated in various pulp and paper processes (Kamali et al., 2016; Pokhrel & Viraraghavan, 2004)

Fibrous raw material	Operation	Main processes	Main additives	Main rejects	Typical effluents parameters						
					Process	pH	COD (mg/L)	BOD (mg/L)	TSS (mg/L)	Other parameters	
										Type	Quantity
Virgin fibre	Raw materials operation	Debarking, chipping and conveying	-	Bark, tannin, lignin, hemicelluloses and some large amounts of organic compounds such as resin acids as well as soil and dirt	Wood yard and chipping	7	1275	556	7150	-	-
	Pulping (paper grade or dissolving grade)	Kraft pulping and Sulphite pulping, Thermomechanical Pulping (TMP) Chemical thermomechanical Pulping (CTMP)MPCMP	Sodium hydroxide (NaOH), sodium sulphide (NaS_2) (Kraft pulping), and some other inorganic compounds, such as Na_2CO_3, $NaHCO_3$, Na_2SO_4, Na_2SO_3, and $Na_2S_2O_3$ (sulphite process), etc.	Knots, uncooked woods, bark particles, soluble wood materials, colour, methanol, resin acids (Including Isopimaric, sandacopimaric, levopimaric, abietic, dehydroabietic, neoabietic and palustric acids), fatty acids, carbohydrate, lignosulfonates, acetic acid, BOD, COD, and dissolved inorganics, lignin, and hemicelluloses, also some chemical additives such as soluble silicates ($3SiO_2$, Na_2O)	Kraft Cooking	13.5	1669.7	460	40	-	-
					Sulphite pulping	2.5	4000-8000	2000-4000	-	*Methanol*	250 (mg/L)
										TS[α]	800-850 (mg/L)
					NSSC pulping	-	39800	13300	-	*Carbohydrate*	6210 (mg/L)
										Acetic acid	3200 (mg/L)
										TS[α]	868 (mg/L)
					TMP	4.0-4.2	3343-4250	-	330-510	*TN*[a]	0.01-0.02 (mg/L)
										TP[b]	1.31-1.47 (mg/L)
					APMP[c]	7.43	7521	3000	350	*Lignin*	516 (mg/L)
										MTBE extractives	147 (mg/L)
					CTMP	12	25000	6800	-	*Colour*	42000 (C.U)
					MP[d]	6.1-8.1	91-1150	6-69	3-45	-	-

	Bleaching	Chemical or mechanical pulp Bleaching	Elemental chlorine free (ECF): ClO_2 and H_2SO_4 (in an acidic environment) or NaOH, O_2, and H_2O_2 (in an alkaline phase). Total chlorine free (TCF): H_2SO_4, ozone, chelating agents, and/ or H_2O_2	Chlorophenols, AOX, EOXs, polychlorinated biphenyls, polychlorinated dibenzodioxines, dioxins, furans, chlorinated lignosulfonic acids, chlorinated resin acids, residual lignin, colour, COD, carbohydrate, inorganic chlorines, VOCs, and halogenated hydrocarbons	Kraft pulp bleaching	8.5	426	25.5	-	*Lignin* *Phenol* *AOX*	50.00 (mg/L) 0.535 (mg/L) 2.82 (mg/L)
					Bleaching[e]	8.2	3680	352	950	*Residual chlorine* Chloride content	9040 (mg/L) 1340 (mg/L)
	Washing	Bleached pulp washing	Solutions containing chemicals like an alkali caustic soda	Residual lignin, bleaching agents and some hardly biodegradable organic compounds	-	-	-	-	-	-	-
	Papermaking	Dewatering, pressing and drying, finishing	Dyes, resins, fillers[f], sizing agents[g], binders, coating aids, strength agent[h], biocides, optical brighteners, colorants, pigments, etc.	Mineral additives, AOXs, resins, BOD, COD, resin acids, particulate wastes, etc.	Paper machine	6.5	1116	641	645	-	-
RCFs (Recycled fibres)	Pulping/ deinking	RCFs pulping	Caustic soda, sodium silicate, hydrogen peroxide, soap, etc.	Metallic components, sand, glass, plastic, coatings, fillers, organic compounds from the paints and printing inks such as 2,4,7,9-Tetramethyl-5-decyne-4,7-diol, pulping additive chemicals, compounds like Si and Ca, higher amounts of organics[i],	Newsprint mill	-	3500	-	250	Colour	1000 (Pt-Co)
					Recycled paper mill	6.2-7.8	3380-4930	1650-2565	1900-3138	-	-
					Recycled paper mill	6.36	4328	669	645	VFA[k] VSS[l]	501 (mg/L) 850 (mg/L)

			thermoplastic resins[j], TCMTB, chlorophols, etc.							
RCFs	Deinking	Substances like H_2O_2, NaOH, Na_2SiO_3, Na_2CO_3, and other compounds like Surfactants	Deinking additives and ink particles, fibres, fines, fillers, ash, etc.	De-inking effluents	7-8	-	-	-	Moisture Ash[m]	98.7 (%) 0.54 (%)

α- Total Sulphur; **a**- Total nitrogen; **b**- Total phosphorus; **c**- Alkaline peroxide MP; **d**- Effluents from seven Canadian mills; **e**- Chlorination and alkaline extraction stages.
f- e.g. clay, titanium dioxide, calcium carbonate; **g**- e.g. rosin, starch and styrene copolymers.
h- Wet strength additives like synthetic resins (such as urea formaldehyde and melamine formaldehyde) and Epichlorohydrin (ECH)-derived compounds (such as polyamine-epichlorohydrine resin (PAE)) as wet strength additives.
i- From light-weight coated paper, because of the coating binders.
j- In case of laser printing papers.
k- Volatile fatty acids.
l- Volatile suspended solids.
m- Ash (after ignition at 900°C) consisted of SiO_2 (16.70%), Al_2O_3 (16.53%), CaO (22.46%), TiO_2 (32.39%), BaO (5.43%), CuO (2.59%), S_2O_3 (1.17%), Fe_2O_3 (0.92%), Na_2O (0.33%

2.4 PRODUCTION OF BIOETHANOL AND BIOGAS FROM PAPER SLUDGE

Globally, countries are encouraging bioethanol production for usage as biofuel with bioenergy mandates and directives such as the 2003 Renewable Fuels Directive of the EU, producer incentives in Canada and the mandates of Argentina and Brazil for 5% biodiesel blend by 2010 (Vertes et al., 2010). Biogas Furthermore, biogas production via anaerobic digestion have lately gathered rekindled attention due to its renewable energy capability. Ahring, 2003 estimated that anaerobic digestion of bioethanol wastes and wastewaters can roughly add 30% more value to bioethanol production from corn. Likewise, this advantageous scenario can possibly be applied to paper and pulp sludge biochemical processing, in particular the primary clarifier sludge, which has additional negative cost feedback. As such biofuel production from waste biomass preferably paper sludge is not only a source of renewable energy but also an environmentally friendly technology, which can reduce vast amounts of paper sludge produced worldwide annually.

2.4.1 Advantages of paper sludge as a bioenergy feedstock

Unlike conventional biomass feedstock, the cultivation and harvesting cost are eliminated when paper and pulp sludge is employed as feedstock with transport cost also being potentially circumvented if the processing facility is built on site (Aden & Foust, 2009; Kumar & Murthy, 2011). Moreover, fibres in paper sludge are more accessible to enzymes and microbes during biological processes due to the pulping stage in papermaking. Hence there slight or no impediment from lignin as seen in other biomass feedstocks, which will result in improved digestibility. Zhu et al., 2012 and Lark et al., 1997 concluded sludge from chemical pulping had better digestibility than mechanical pulping operations, since lignin and carbohydrates are dissolved through high pressure and temperature cooking. Boshoff, 2015 from our research group also corroborated this detail when the digestibility of virgin pulp PS was found to be superior to corrugated recycled PS, at solid loadings between 3-9% (w/w).

Additionally, combining the utilization of paper sludge and clarified processed wastewater 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). Although the combination of fermentation of paper sludge followed by anaerobic digestion of fermented residue is likely significantly reduce the amount of landfill waste, there is uncertainty about the quality of the potential reclaimable water. This study seeks to investigate this uncertainty and ascertain if wastewater treatment was correspondingly achieved through biochemical processing of paper sludge and clarified processed wastewater. Pulp and paper industry on-site waste treatment facilities already employ anaerobic and aerobic digestion basins to reduce the COD and BOD content of effluent streams, before being discharged to the environment. It can be potentially more ecological and economically sensible to recycle the processed wastewater from the primary clarifiers into biological energy production in combination with paper sludge, while simultaneously reducing the COD and BOD content of processed wastewater.

2.4.2 Ethanol production from paper and pulp sludge

Bioethanol production from lignocellulosic raw material involves a sequence of bioprocesses described in **Figure 2-2** below.

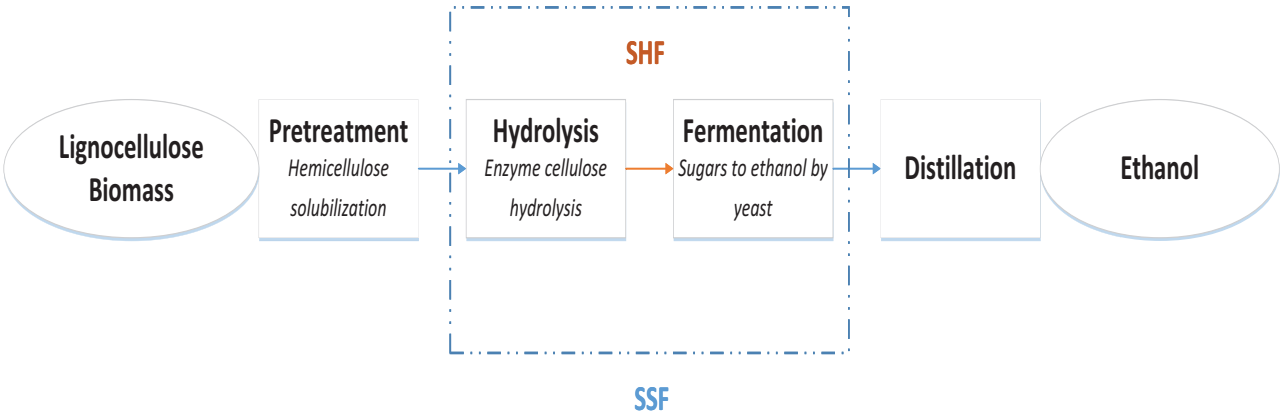


Figure 2-2: Schematic representation of ethanol production from lignocellulose biomass (Vertes et al., 2010)

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. 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). Separate (enzymatic) Hydrolysis and Fermentation (SHF) 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 glucose into ethanol also within optimum temperature of 30°C to 35°C (Vertes et al., 2010). Simultaneous Saccharification and Fermentation (SSF) contrariwise incorporates the enzymatic hydrolysis of cellulose and the successive fermentation of the cellulose hydrolysate into a single step. The fermenting microorganism is introduced into the reactor to convert the produced glucose to ethanol, as soon as cellulose conversion to glucose is instigated. 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). Contrariwise, 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°C (Lark et al., 1997).

2.4.3 Process Parameters on paper sludge fermentation

Although SSF doesn't operate at optima temperature and pH for paper sludge fermentation, (Prasetyo et al., 2011) reported ethanol concentration for SSF was almost twice as much as that of SHF under the same conditions. For SSF process of paper and pulp sludge to be economically viable, it is essential to produce ethanol concentrations in excess of 40 g/l, as distillation at lower concentrations

would be too energy intensive, making such process not financially sensible. 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, 2013 and Boshoff, 2015 investigated the fermentability of various South African pulp and paper mill sludge samples and indicated the economic enzyme dosages ranging from 10 FPU g^{-1} to 20 FPU g^{1} employing Optiflow RC 2.0 with cellulase activity of 130 FPU/ml.

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 with the exception of pentose sugars utilization. Robus, 2013 and Boshoff, 2015 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 will tested in this study.

2.4.3.3 Solids loading, Feeding and Agitation

High solids loading in paper sludge fermentation resultantly yields higher ethanol concentrations. However, this is hard to achieve due to the high water holding capacity of paper sludge (>60). 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 in order to improve ethanol concentration and yield which can in turn intensify mixing power requirements (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. 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. **Table 2-12** below shows SSF runs for various substrate loadings and enzyme dosages by (Ballesteros et al., 2002). Ethanol concentration increased as substrate loading also increased for both enzyme dosages of 15 and 45 FPU/g substrate. A fed-batch system with three feeding rates of 5%, 3% and 2% experimented at 15 FPU/g substrate lead to ethanol concentration and yield greater than 10% (w/v) batch culture at a notably higher enzyme dosage of 45 FPU/g substrate.

Table 2-12: SSF runs at different solids loading and enzyme dosages (Ballesteros et al., 2002):

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

2.4.3.4 Viscosity and Water holding capacity

Although a high degree of digestibility is crucial for achieving desired concentration, yield and productivity, the water holding capacity and viscosity of the sludge, which are intrinsic characteristics of the material, limits the solids loading and hence, production performance of a run. Water is bound as intracellular water or by a surrounding matrix of highly hydrated extracellular polymers in paper sludge (Hagelqvist, 2013b). The water holding capacity of primary sludge depends on the amount of cellulose present and the length of the cellulose fibres (Boshoff, 2015). This consequently contributes to the high viscosity of paper sludge. Boshoff, 2015 indicated high viscosity negatively influences digestibility through physical constraints for enzyme access. Additionally, higher agitation rates to counter high viscosity levels leads to reduction in enzyme stability due to high shear stress of the blades on the cellulase (Boshoff, 2015; Fan & Lynd, 2007). **Table 2-13** gives a summary of bioethanol production from paper and pulp sludge fermentation varying critical parameters such as solids loading.

Table 2-13: Bioethanol production from Kraft and recycled sludge

	Solids loading (g/L)	Ash Content %	Fermentation	Fermentative Micro-organism	Ethanol conc. (g/L)	Conversion %	Productivity (g/Lh)	References
Kraft	40.8	10.7	SHF	*S. cerevisiae* GIM-2	9.5	56.3	0.59	(Peng & Chen, 2011)
Kraft	30		SSF	*S. cerevisiae* ATCC	13.6	79.5	0.14	
	60	36		200062	25.5	74.5	0.27	
	30		SSCF	*E. coli* ATCC 55124	15	80.1	0.16	(Kang et al.,
	60				32.5	78.8	0.34	2010)
Kraft	135	36	SSF	*S. cerevisiae* ATCC	22.5	66.1	0.19	
				200062				(Kang et al.,
			SSCF	*E. coli* ATCC 55124	29.1	69.8	0.24	2011)
De-ashed	139	10.7	SSF	*S. cerevisiae* ATCC	24.1	71	0.20	
Kraft				200062				
			SSCF	*E. coli* ATCC 55124	29.2	70.8	0.24	(Kang et al.,
	231		Fed batch-SSF	*S. cerevisiae* ATCC	47.8	70	0.40	2011)
				200062				
	154		Fed batch-SSCF	*E. coli* ATCC 55124	60	70	0.50	(Boshoff et al.,
Kraft*	180	9	Fed batch-SSF	*S. cerevisiae* MH1000	34.2	66.9	0.23	2016)
Corrugated recycle*	270	20	Fed batch-SSF	*S. cerevisiae* MH1000	45.5	78.2	0.448	
Recycled	190	NA	SSF	*K. marxianus* ATCC 36907	34	73	0.52	(Lark et al., 1997)
Recycled	75	29.3	SHF		19.6	54	0..33	

			SSCF	*Pichia stipitis* CBS 5773	18.6	51	0.39	(Marques et al., 2008)
De-ashed recycle*	207	14	Fed batch-SSF	*S. cerevisiae* MH1000	47.72	88.33	0.40	(Robus, 2013)
	217	10	Fed batch-SSF	*S. cerevisiae* MH1000	57.31	90.75	0.48	
No indication	170	32.6	SSCF- 30°C	*Z. mobilis* 8b	46.32	95	0.39	
			SSCF- 37°C		36.80	76	0.31	(Zhang & Lynd, 2010)
			SSCF- 30°C	*S. cerevisiae* RWB222	40.14	89	0.33	
			SSCF- 37°C		45.23	94	0.38	

* Study research from our group

2.5 BIOGAS PRODUCTION FROM PAPER SLUDGE AND FERMENTATION RESIDUE

Anaerobic digestion involves the degradation and stabilization 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, 2014a; Kamali et al., 2016). However, it can also be applied to solid wastes from paper and pulp processes, as discussed below, while 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. Williams, 2016 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 2-14** 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, primary sludge had a bio-methane potential 2 to 3 times greater than secondary sludge, which point toward primary sludge as the more suitable for biogas production (Bayr & Rintala, 2012a; Gottumukkala et al., 2016).

Table 2-14: 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	280 ml mini-digester[a]	30	33	-	90.6	173.60 ± 5.87	(Huiliñir et al., 2014)
Secondary PPS	500 ml Erlenmeyer-flask digester	38	76	-	70	53 ± 26	(Hagelqvist, 2013a)
Primary PS	5 L 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	
Tissue printed recycle PS	30 L digester[c]	37	28	-	37.13	169.3 ± 29.2	(Williams, 2016)
Corrugated recycle PS					74.07	111.7 ± 3.8	
Virgin pulp PS					75.17	127.4 ± 11.3	
Primary PS	1 L BMP assay	55	42	-	84	230 ± 20	(Bayr & Rintala, 2012b)
Secondary PS					81.5	100 ± 10	
Primary PS	1 L BMP assay	35	42	-	84	210 ± 40	(Bayr & Rintala, 2012b)
Secondary PS					81.5	50 ± 0	
Virgin PS	100 ml BMP assay	37	60	-	67-97	382	(Dalwai, 2012)
Recycled PS					31-40	226	
Corrugated cardboard	BMP assay	35	-	-	97.7	278 ± 12	(Owens & Chynoweth, 1993)
Printed newspaper					97.6	100 ± 3	
Unprinted newspaper					97.9	84 ± 3	
Office paper					92.7	369 ± 14	
Magazine					78.1	203 ± 8	

PS – Paper sludge; ***PPS*** – Pulp and 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: Key stages in Biomethanation process highlights the biomethanation process with unique functional group of microbes performing specific tasks in relation to each other. The first phase, which is 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). Cellulose and xylan often entrenched in recalcitrant molecules such as lignin are relatively slow to hydrolyze often times making this stage the rate-determining step of the biomethanation process (Yadvika et al., 2004). This makes fermentation residues well suited for biogas production as compared to untreated primary sludge, because of the hydrolytic enzymes added during Simultaneous Saccharification and Fermentation (SSF). Monomers and oligomers resulting from the hydrolysis step is further fermented into short chain fatty acids, CO_2 and H_2 by another guild of anaerobic bacteria which comprises of *Bacteroides, Clostridium, Butyribacterium, Propionibacterium, Pseudomonas*, and *Ruminococcus* (Ahring, 2003). This phase often referred to as the acidogenesis stage generally occurs rapidly, which can result in short chain fatty acids (SCFA) accumulation and digester failure when feedstock fed contains large amounts of readily fermentable carbohydrates (Ahring, 2003). Next, acetogenesis proceeds by another special guild of anaerobes referred to as syntrophic acetogens, which convert various types of SCFA into acetate, CO_2 and H_2 (Ahring, 2003). Lastly, methanogens, which are different from bacteria and belong 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).

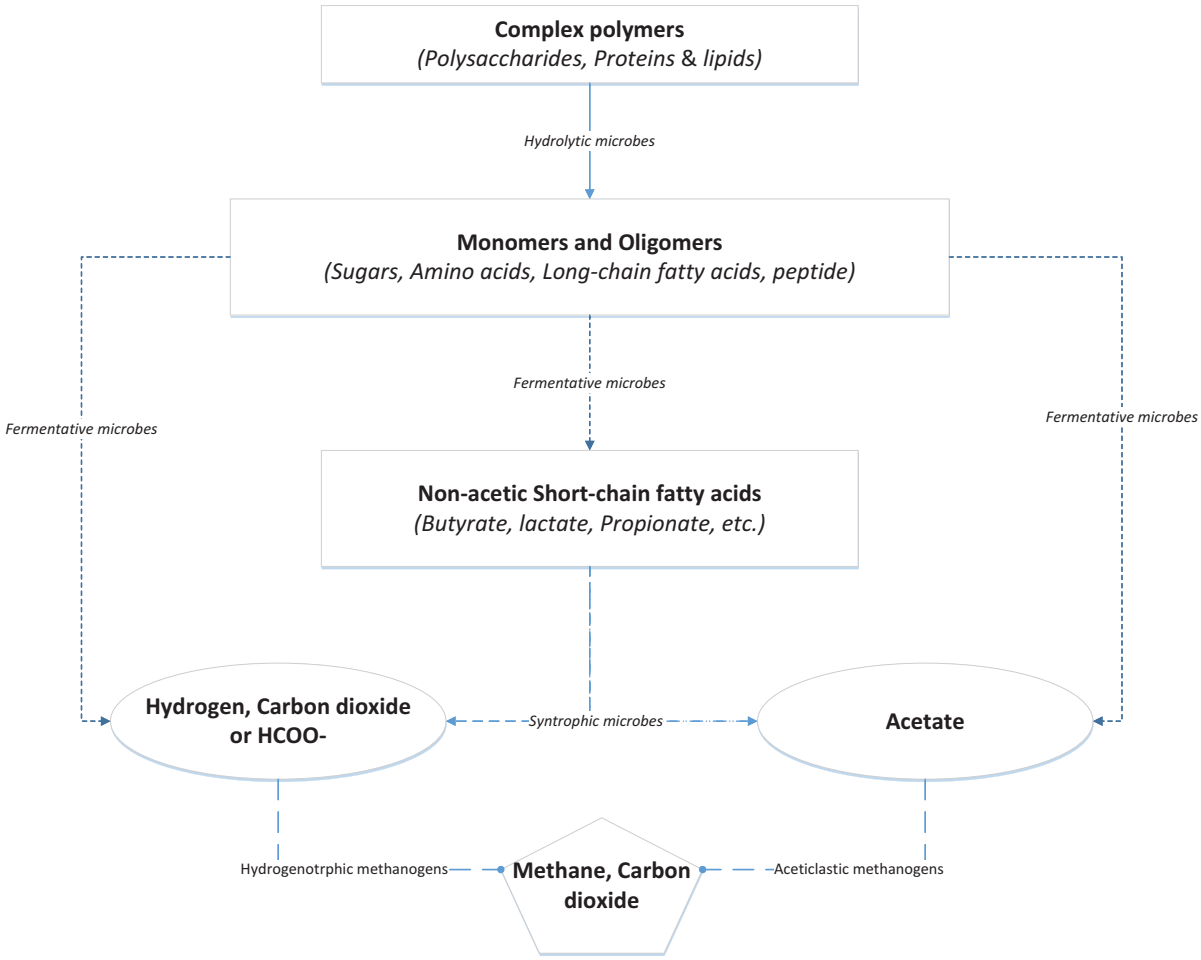


Figure 2-3: Key stages in Biomethanation process

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 particular feedstock in order to achieve optimum performance (Chen et al., 2008; Meyer & Edwards, 2014b).

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 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)*. However, anaerobes are most active in the mesophilic and thermophilic temperature range (Singh et al., 1993; Takizawa et al., 1994; Zennaki-Bensouda et al., 1996). 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 Organic loading rate (OLR)

Organic loading rate is an important parameter that indicates the amount of volatile solids fed into an anaerobic digester per day under continuous feeding (Mao et al., 2015). Biogas production generally surges up to some extent with increasing OLR, although stability and continuous productivity of the digestion processes can potentially be disrupted. Bacterial inhibition can occur pertaining to extremely high OLR, which results in higher acidogenic activity than methanogenic bacterial activity. This eventually leads to irreversible acidification due to extreme increase in volatile fatty acids production. Gas yield increased by 67% when a plant operating on manure as feedstock increased OLR from 346 kg VS/day to 1030 kg VS/day (Yadvika et al., 2004). As such there is an optimal feed rate in relation to the type of feedstock and reactor size beyond which further increase in substrate quantity will rather have inhibitory effect on the digestion process. Sundrarajan et al., 1997 reported a maximum yield of

0.36 m^3/kg VS for at an OLR of 2.91 kg VS/m^3/day for a lab-scale digester utilizing municipal solid waste as feedstock.

2.5.2.5 Retention time

Microbial growth is linked with retention time. Hydraulic retention time (HRT) is the average time period that an input substrate spends inside an anaerobic digester before its removal. Acquiring an efficient HRT hinges on other parameters such as OLR, substrate composition and temperature and can vary from 30-60 days (Yadvika et al., 2004; Meyer & Edwards, 2014a). 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).

2.5.2.6 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; Karim et al., 2005) strongly suggested that agitation averts the formation of floating solid layers, which 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 with regard to 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 COMPLICATIONS IN UTILIZATION OF CLARIFIED PROCESSED WASTEWATER IN PAPER SLUDGE FERMENTATION AND ANAEROBIC DIGESTION

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 processed wastewater will be mill-specific. This is of particular importance since some compounds as mentioned earlier will have considerable effect on processed wastewater utilization in bioethanol and biomethane production. Suntio et al., 1988 presented a compilation of some 250 chemicals identified in pulp mill effluents with HMF (5- hydroxymethylfurfural) and furfural, which are regarded to be the representative inhibitors for yeast and bacterial growth included in this catalogue. Further potential toxic class of compounds included are fatty acids, phenolic compounds (tannins), sulphur compounds, inorganics (ash) and heavy metals which either singly or synergistically can possibly inhibitor biological processes.

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 inhibitory towards both microorganisms (Leonard & Hajny, 1945; Mussatto & Roberto, 2004). Subsequent research studies conducted by (Larsson et al., 1999; 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; Palmqvist & Hahn-Hägerdal, 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 processed wastewater can inhibits 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), which can be troublesome in processed wastewater utilization in anaerobic digestion. Additionally, resin acids and terpenes also affect bacterial activity in anaerobic digestion in concentration indicated in **Table 2-15**.

Given that most 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, 2014a). Sulphur reducing bacteria (SRB) competes with methane producing bacteria (MPB) for organic and inorganic substrate in order to reduce sulphate to sulphide. Consequent inhibition results from the sulphide production, which is toxic to various methanogenic bacteria groups (Chen et al., 2008). Although sulphur compounds have inhibitory effects on MPBs, SRBs ability to partially or completely degrade branched and long chain fatty acids, organic acids, alcohols and aromatic compounds is a desirable attribute, which will be beneficial towards COD and BOD reduction from the processed water clarification and reclamation perspective. Additionally, due to peroxide reinforced alkaline bleaching or APMP pulping, hydrogen peroxide and AOX can be present in pulp mill effluents at higher concentrations, which can worsen anaerobic digestion performance (Habets & de Vegt, 1991). Also, possible high ash content in processed wastewater and paper sludge due to re-pulping 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 processed wastewater inhibitors for pulp and paper sludge biochemical processing

	Fermentation		Anaerobic digestion		References
Compound	**Critical concentration (mg/L)**				
Phenolic compounds	1000 *(4-hydroxybenzoic acid)*[a]		350-3000 *Tannins*		(Meyer & Edwards, 2014a; Ando et al., 1986)
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, 2014a)
Sulphite			50		(Meyer & Edwards, 2014a; 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)			Aluminium	>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

2.6.2 Mitigation Strategies to combat negative effect of inhibitors

It must be noted that due to the myriad and mill-specific concentration of compound classes in pulp and paper mill effluents, it is difficult to predict the effect clarified processed wastewater utilization in bioenergy production from paper sludge will transpire especially with bioethanol fermentation given that anaerobic wastewater treatment of pulp and paper mill effluent is well reported and already applicable. Individually, various chemical compounds in both processed wastewater and paper sludge might not be potent enough to inhibit biological processes, nonetheless synergism amongst these compounds is the greatest threat to fermentation and anaerobic digestion. Thus, mitigation strategies may possibly have to be adopted to lessen and negate the potential inhibitory effect of processed wastewater utilization in bioenergy processes.

One such mitigation step which can be effective is co-feeding processed wastewater with fresh water to decrease toxicants below critical concentrations for satisfactory fermentation and anaerobic digestion to transpire. Another established mitigation strategy mostly applied in anaerobic digestion is acclimation and adaptation of inoculum to adverse conditions triggered by toxicants. Acclimation techniques are rarely permanent and can be induced relatively rapidly with microbial community seemly tolerant to several chemical and physical stressors (Meyer & Edwards, 2014b). Adaptation happens simultaneously with acclimation when individuals within a microbial community acquiring some genetic mutations which impart an advantage under the imposed conditions and these individuals are then chosen over others over several generations (Morgan-Kiss et al., 2006). Microbial acclimation/adaptation can vary from one week to several months with the concentrations of inhibitors in the substrate slowly and progressively increased over time (Benjamin et al., 1984; Liver & Hall, 1996; Wu et al.,1993; Nilsson & Strand, 1994).

2.7 CONCLUSION

There is large potential in reuse of clarified processed wastewater from pulp and paper mills in biogas and bioethanol production from paper sludge, both from economic and environmental point of view. The present project will specifically investigate the application of bioethanol and biogas processing as methods to reclaim and treat process waters, rather than disposal, to eventually reduce the fresh water intake of pulp mills.

2.7.1 Gap in literature.

The prospect of reclaiming water from paper sludge and treating process wastewater, by application of anaerobic digestion and fermentation for bioenergy production, needs to be explored. Earlier research studies from our research group have demonstrated the feasibility of small scale bioethanol and biogas production from paper sludge (Robus, 2013; Anne-Marie, 2015; Boshoff, 2016; Williams, 2017). Independently, anaerobic digestion of wastewater is a well-established treatment technology which is employed in some pulp and paper mills.

Although biological treatment significantly decreases toxicity of processed wastewater, there are no energy benefits that can potentially offset cost. Hence, it is vital that processes are developed that will be able to yield the treatment benefits, while concurrently producing bioenergy. Likewise, employing processed wastewater in co-production of ethanol and biogas from paper sludge will make more economical sense and prevent the generation of another liquid waste stream which may need further treatment. This proposed research study aims not to only investigate the feasibility of processed wastewater utilization in biochemical processes, but also to further decrease the water holding capacity of paper sludge through the sequential application of fermentation and anaerobic digestion. Likewise, wastewater treatment (COD, BOD, AOX and SS reduction) is simultaneously achieved through sequential application of biochemical processing at potentially moderate cost. The effect of processed wastewater on yeast, enzymatic and bacterial biochemical processes needs to be primarily investigated. This will in effect determine optimal conditions (enzyme and inoculum dosages) for successful biochemical processing of paper sludge to biofuels.

2.7.2 Potential expected outcome for water recycling and bioenergy through this study

About 69% of paper sludge is landfilled with about 60-80% composed of water (Hagelqvist, 2013b). Financially paper and pulp sludge management accounts for 60% of waste treatment cost, hence it imperative for the industry reduce sludge production with increasing and looming strict environmental regulations (Mahmood & Elliott, 2006).

- An expected potential outcome of this process will be the recycling processed wastewater into pulp and paper mills, the possibility of a closed loop water cycle system for the pulp and paper industry can realistically be investigated. The aim is to achieve this simultaneously with significant bio-energy production, to improve process economics.
- Another important potential outcome of the proposed process will the significant reduction of landfill waste which has ecological and financial benefits to the environment and the pulp and paper industry. Boshoff, 2015 and Williams, 2016 showed that fermentation and anaerobic digestion of paper sludge significantly reduce the water holding capacity for three paper and pulp sludge as seen in **Table 2-16**. Their studies demonstrated viability of reducing landfill waste through biochemical processes.
- In a study by (Liu et al., 2015) more biofuel energy was extracted for a given amount of sugarcane bagasse when both bioethanol and biogas production were combined compared to the two-separate individual biochemical processes alone. Likewise, co-production of ethanol and biogas from paper sludge and processed wastewater will not only maximize the treatment of pulp and paper mill effluents but equally augment the amount of bioenergy that can be sourced from this proposed concept.

Table 2-16: Water Holding Capacity (WHC) after biochemical processing

Paper and Pulp Sludge (PPS) type	WHC for Raw PPS (g water/g PPS)	WHC for Fermented PPS (g water/g PPS)	WHC for Digested PPS (g water/g PPS)	References
Virgin pulp	8.61	4.54	-	(Boshoff, 2015)
	12.6	1.00	8.6	(Williams, 2016)
Corrugated recycle	6.62	2.55	-	(Boshoff, 2015)
	10.0	2.00	8.0	(Williams, 2016)
Tissue printed recycle	4.80	0.50	2.1	(Williams, 2016)

CHAPTER 3: RESEARCH PROJECT SCOPE

3.1 PRIMARY RESEARCH QUESTIONS

1. Can the quality of clarified processed wastewater from the pulp and paper industry be improved through sequential fermentation of primary sludge and anaerobic digestion of fermented residues?
2. How much water can potentially be reclaimed from sequential fermentation and anaerobic digestion of primary sludge with processed wastewater at optimized conditions?

3.2 SECONDARY RESEARCH QUESTIONS

1. How does the use of clarified processed wastewater as make-up water affect biological microorganisms and processes such as fermentation (with yeast) and anaerobic digestion (microbial consortia)?
2. How does the ethanol and biogas yield from this process at different reactor levels compare to previous studies by (Boshoff, 2015; Robus, 2013; Williams, 2017) from our research group?
3. After sequential fermentation and anaerobic digestion of primary sludge with clarified processed wastewater, is the treated water recyclable for the pulp and papermaking process?

3.3 PROBLEM STATEMENT

Apart from landfilling paper sludge, the pulp and paper industry utilizes substantial amounts of fresh water and in this manner, disposes of sizable quantities of processed wastewater into the environment. Although previous research studies by (Anne-Marie, 2015; Boshoff, 2015; Robus, 2013; Williams, 2017) have established small and scale-up bioethanol and biogas production from primary sludge, the potential of sequential biochemical processing while reclaiming and qualitatively treating dirty processed wastewater needs to be investigated at different reactor levels. (Anne-Marie, 2015; Boshoff, 2015; Robus, 2013) established optimized solids loading, inoculum and enzyme dosages for various paper sludge samples from different South African pulp and paper mills while (Williams, 2017) scaled-up the optimized parameters in 20 L fermenters and 30 L digesters. Similarly, influence of clarified processed wastewater on fermentation and anaerobic digestion important process parameters such as enzyme and inoculum dosages is paramount for large scale successful implementation of this proposed study. Hence, inoculum and enzyme screening protocols are proposed and together with optimal conditions from earlier studies, valuable data can be obtained on a variety of processed wastewater and primary sludge feedstock at different reactor levels.

3.4 RESEARCH OBJECTIVES

i. Investigate the influence of processed wastewater, as replacement for fresh water, on yeast, a bacterial consortium and cellulases to determine optimum inoculum and enzyme dosages.

ii. Determine whether a biochemical process solely utilizing processed wastewater is achievable and if not which co-feeding with fresh water will permit successful fermentation and anaerobic digestion of primary sludge.

iii. Determine the amount of reclaimable water through sequential fermentation of primary sludge and anaerobic digestion of fermented residues.

iv. Complete characterization of primary sludge from the three pulp and paper mills and solid residues after sequential fermentation of primary sludge and anaerobic digestion of fermented residues. Possible application of this residue as compost is to be considered.

v. Water quality analysis of clarified processed wastewater before and after sequential fermentation of primary sludge and anaerobic digestion of fermented residues.

vi. At optimum conditions, compare ethanol yield from processed wastewater fermentation of paper sludge in 150 L fermenter to 20 L and 5 L bioreactors performed by (Williams, 2017) and (Boshoff, 2015) respectively (Scale-up).

vii. Compare biogas yields from anaerobic digestion of primary sludge and fermented residues with processed wastewater at different reactor levels (BMP and 30 L digesters) to ascertain the effects of scale up and also compare to scale up study performed by (Williams, 2017) with fresh water.

CHAPTER 4: RESEARCH DESIGN AND METHODOLOGY

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

4.1 FEEDSTOCK PREPARATION

4.1.1 Paper sludge preparation

Three different primary sludge samples were collected by (Williams, 2016) from three notable mills across South Africa. Virgin pulp, corrugated recycle and tissue and printing recycle paper sludge were respectively acquired from Mondi Richards Bay, Mpact Felixton and Twincare Bellville. 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 2 mm screen. Afterwards dried milled paper sludge samples were stored in sealed plastic bags at room temperature for later use in outlined biochemical processes.

4.1.2 Processed wastewater preparation

Clarified processed wastewater were obtained from the same paper and pulp mills as the paper sludge samples, with the only exception being that of tissue and printing recycle processed wastewater which 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 processed wastewater samples were stored in a cold storage room at -8°C in order to thwart the development of any microbial activity. At the onset of fermentation and digestion experiments, the pH of the three types of processed wastewater were tested to determine if they were within the required range for successful biochemical processing. An optimum pH between 3-6 and 6.8-7.4 is essential for fermentation and anaerobic digestion respectively. With the exception of Kraft virgin pulp processed wastewater from Mondi Richards Bay (pH of 2), the other two types of processed wastewater were all within biochemical processing pH range. Subsequently, the pH of virgin pulp processed wastewater was adjusted accordingly with 25% ammonia solution (Merck) to the required pH range. Thereafter, the various processed wastewater were centrifuged at 8000 rcf for 15 minutes. Only Felixton corrugated recycle processed wastewater had settleable solids.

4.2 SAMPLE CHARACTERIZATION AND PRODUCT STREAM ANALYSIS

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

i. Sample preparation (NREL/TP-510-42620)

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

iii. Ash content (NREL/TP-510-42622)

iv. Extractives (NREL/TP-510-42619)

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

4.2.1 Proximate analysis

Proximate analysis will be performed on samples to determine volatile matter, fixed carbon and the ash content using Mettler Toledo STAR TGA in accordance with ASTM E1131 standard procedure.

4.2.2 Ultimate analysis

Ultimate analysis will be performed to determine the elemental composition samples using LECO TruSpec Micro Elemental Analyser.

4.2.3 HPLC analysis

Sugar and ethanol concentrations will be 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).

4.2.4 Biogas Analysis

Biogas gases will be collected in Tedlar bags and analysed using compact GC4.0 Gas Chromatography (GC) equipment which displays the different gases and their respective fractions.

4.2.5 Water quality analysis

Analysis of clarified wastewater before and after sequential fermentation and anaerobic digestion is fundamental to this research study. The water quality analysis listed below will determine whether comparative purification of clarified processed wastewater is being achieved through sequential biochemical processing.

i. Heavy metals

ii. Sulphates

iii. Total dissolved solids

iv. Total suspended solids

v. COD

vi. AOX

4.3 EXPERIMENTAL APPROACH

This research study follows the experimental approach as indicated in **Figure 4-1**. The experimental work begins with the collection of three primary sludge samples from three major mill operators across South Africa. This collection task was done previously by (Williams, 2016) from our research group. The corresponding processed wastewater samples were also collected recently from the same mills with the exception of one type of processed wastewater gotten from another different mill. At the outset, the effect of processed wastewater on yeast, enzyme and bacteria performance was investigated. This is designated by the yellow shaded section in **Figure 4-1**. The screening stage which is of fundamental importance to this research study was undertaken to see whether solely employing processed wastewater in biochemical processes is applicable and if not, which co-feeding ratio of processed wastewater and fresh water will be suitable for both fermentation and anaerobic digestion. Bio-methane potential (BMP) test and yeast adaptation screening were performed using five distinct processed water to clean water ratios (0, 25, 50, 75, 100% processed wastewater mixed with fresh water). The optimal inoculum dosage based on (Boshoff, 2015; Williams, 2016) previous work. Subsequently, the enzyme screening was also executed, based on which PW/CW ratio performed the best in the yeast adaptation screening. Based on the optimum conditions gathered from both enzyme and yeast adaptation screening, optimization batch SSF runs in shake flask for paper sludge was implemented.

To develop and scale up the optimised processes, processed wastewater fermentation of paper sludge at optimal conditions were performed in 150 L and/or 20 L bioreactors. Likewise, processed wastewater anaerobic digestion of paper sludge and fermented paper sludge residue based on optimal conditions gathered from BMP test were carried out in 30 L anaerobic digester. The blue shaded regions in **Figure 4-1** indicates the scale up stage.

Concurrent treatment of processed wastewater via biochemical processes is of prime importance to this study. Hence water quality test analysis such as COD, AOX and WHC, as indicated in brown shaded region in **Figure 4-1,** will be performed before and after both fermentation and anaerobic digestion to ascertain water reclamation and quality improvements are being achieved. Finally, the data obtained from the experimental work will be used to update an Aspen Plus® model of the SSF developed by (Chen et al., 2014; Robus, 2013; Williams, 2016).

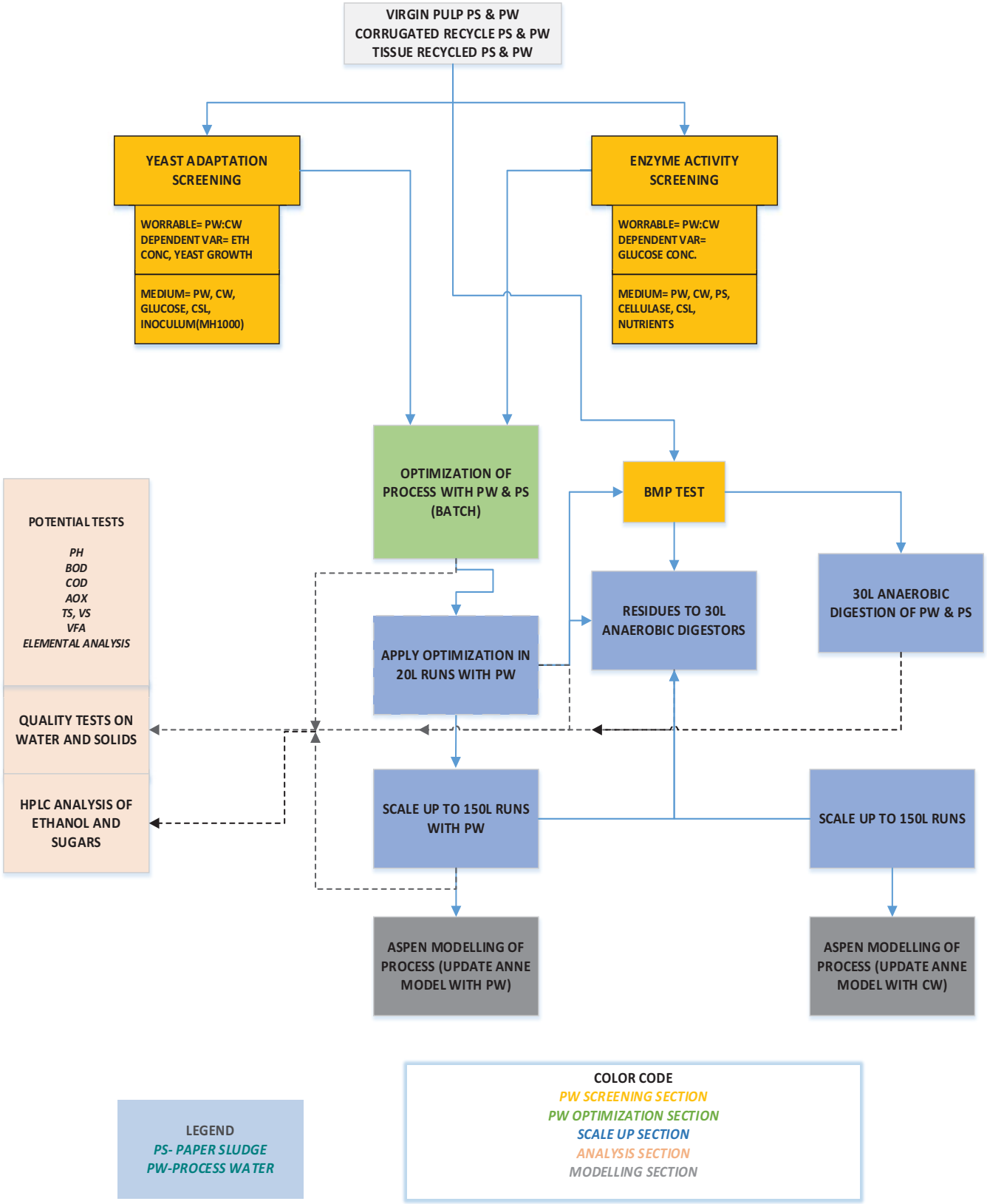


Figure 4-1: Experimental approach to study

4.3.1 Processed wastewater yeast adaptation screening

Yeast adaptation screening of processed wastewater samples for ethanol concentration were performed in batch cultures using 250 ml cotton plugged Erlenmeyer shake flasks. Batch media comprised of five distinct PW/CW ranging from 100% clean water (CW) to 100% processed wastewater (PW) for each type of processed wastewater. The 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) 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. Finally, the prepared media were inoculated with 5 mL 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 (O.D) and experimental ethanol concentration.

4.3.2 Processed wastewater enzymatic hydrolysis of paper sludge

Enzymatic hydrolysis screening was carried out dependent on the processed wastewater performance at section 4.3.1. Paper sludge solids loading of 6% (w/w) was investigated for the three kinds of processed wastewater. Media consisted of the best performed PW/CW ratio from the yeast adaptation screening with the same nutrient source as described in section 4.3.1 and subsequently autoclaved at 121°C for 15 minutes. Filter sterilized Viscamyl Flow enzyme was introduced at a dosage of 5, 10 and 15 FPU/gds and the shake flasks incubated at 37°C at 150 rpm for 72 hrs in an orbital shaker. Likewise, sampling was undertaken at regular intervals to determine the released glucose which was computed as a percentage of original added cellulose.

4.3.3 Processed wastewater batch and feed batch SSF at different reactor levels at optimum conditions

Firstly, batch SSF will be carried out in shake flask at optimum conditions derived from the screening processes. Afterwards, fermentation experiments are to be carried out in 150 L and/or 5 L bioreactors to determine the effect of scaling up the process. The fermentation media consists of same nutrient source as described in screening processes. Optimum conditions (solids loading, processed wastewater and fresh water co-feeding ratio) is based on screening processes and previous studies reported by (Boshoff, 2015; Robus, 2013). Similarly, samples will be taken every 24 hours and prepared for HPLC analysis to determine ethanol and sugar concentration.

4.3.4 Bio-methane potential (BMP) tests for processed wastewater and paper sludge

Bio-methane potential test is used to determine the maximum methane potential of a particular substrate. The set of BMP tests conducted for this research study followed the protocol defined by (Angelidaki et al., 2009). The intended amount of dry paper sludge was added to each serum bottle and filled with the required proportion of processed wastewater and clean water similar. The PW/CW ratios employed in the BMP tests were identical to section 4.3.1. Thereafter, each 100 mL serum bottle with substrate and water was loaded with necessary quantity of inoculum to achieve a working volume of 70 mL. The serum bottles were then

plugged with thick butyl rubber stoppers and sealed with aluminium crimps. 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 with one connected to a nitrogen gas pipeline and other serving as a gas outlet.

Parameters and Conditions

- A total solid loading of 6% was investigated for each corresponding paper sludge and processed wastewater. An inoculum concentration of 6.25% (v/v) was used for 6% solids loading, which corresponds 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 each BMP set for Virgin pulp paper sludge and its processed wastewater since they had an initial pH of 2. The other set of BMPs for the other two substrates and their corresponding processed didn't require any pH adjustment since their initial pH was already perfect for anaerobic digestion.
- The BMP tests were conducted was at mesophilic temperature ranges, i.e. 37°C. The temperature of the BMP tests was maintained by using an oven incubator.
- Incubation of 30-45 days for all the BMP tests.

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

The laboratory scale-up batch anaerobic digestions were conducted in eight custom built 30 L continuous stirred digesters (CSD) manufactured by Thermodynamics and Fluid Design (TFD) Ltd in South Africa. The digesters, detailed in **CHAPTER 5**: are an upgraded version of the similar equipment utilized by (Williams, 2016) from our research group. The CSD is made of rectangular shaped jacketed stainless steel vessel with working volume 21 L. Placed on top of each digester lid is 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. Jacketed vessels of the digester have water circulating in the jacket for temperature control. Liquid sampling and drain valves for the digester are 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.

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 residues will run in parallel, hence there will be no pressing, drying and preparation of fermented residue. Instead, fermented residue with a certain amount of moisture content after evaporation will be transferred carefully into digesters to start the digestion process.

Evaporation will be conducted at the end of the fermentation at 60-70°C to get rid of ethanol with 5-10% water loss expected when compared to industrial distillation. This 5-10% water loss will be compensated for with appropriate amount sterilized wastewater and/or fresh water added to the digesters. The exact the same conditions of temperature and pH as in the BMP tests were also applied to CSD digesters with intermittent agitation for 30 days.

4.3.6 Replicates, Blanks, and Controls

All shake flask experiments and BMP experiments were conducted in triplicates which allowed for statistical analysis of the data collected and also guaranteed the reproducibility of the assays. To determine the gas production of substrate and water, control BMP assays which constituted paper sludge, processed wastewater and/or clean water without inoculum were performed.

CHAPTER 5: DESIGN OF BIOGAS DIGESTORS

5.1 INTRODUCTION

Completion of the experimental process development, to achieve both acceptable bio-energy production together with significant waste-water clean-up and recycling through integrated fermentation and anaerobic digestion, required access to suitable process equipment. Considering the intention of the project to deliver industrially-relevant research results, a key requirement for this process equipment is the ability to process kilogram-scale samples of the paper waste sludge and industrial wastewaters, to allow process development and performance measurement at a scale that is relevant to industrial practise.

With these outputs in mind, the project deliverables included the design and construction of eight (8) anaerobic biodigesters, to allow completion of experimental work at kilogram-scale, which is required in the scale-up deliverable of the project. No such experimental system exists in SA, and will serve this project as well as several to follow, very well, leading to greater industrial roll-out of the technology. The present Chapter3 of the report covers the design, construction and commissioning of these 8 reactors.

The design of the digestors was based on both the experience in the research group and consultation with research and industrial partners. During the design process, a preferred working volume of 30 L was selected for these digestors, based on space requirements for a large number of reactors. Of particular concern in the design were the requirements for mixing of anaerobic digestion (AD) process that utilise paper waste sludge, a feedstock well-known to have high viscosity and therefore difficulty in mixing.

Based on the completed design of the equipment, as reported in Section 3.2 below, various options for procurement of the reactors were considered. One option was to contract a manufacturer to build completely new reactors, which was investigated thoroughly, resulting in quotes that were well-beyond the budget available for equipment in the present project. A more cost-effective opportunity arose from discussions with local industrial partners, from which 8 existing reactors of 30 L each, already constructed and partially commissioned, were sourced. These under-utilised and brand-new reactors could be purchased as a reduce cost, and subsequently be modified to achieve the design requirements for the present project. The required modifications to these reactors are reported in Section 3.3 below, which are to be completed by 31 January 2017.

With regards to the total cost of supply of the 8 digestors: The combined cost of initial purchase of the reactors (R342 400 excl. VAT) and the subsequent modifications to the reactors to achieve the equipment performance required for AD of paper waste sludge (R317 282 excl. VAT) resulted in a total capital expense of R659 682 (excl. VAT). This was substantially cheaper than the option to purchase 8 completely new reactors (best quote of R1 067 471 from local suppliers), but still beyond the budget of R221 903.48 (excl. VAT) available in the present project. The total cost of R659 682 (excl. VAT) for the 8 reactors were therefore

covered by a combination of the available funding in the present project with supplementary funding from NRF projects and university sources, to provide all of the reactors required for completion of this project and future research work. Sharing of the reactors between different projects will still allow on-time completion of the deliverables of this project.

5.2 DESIGN SPECIFICATIONS FOR ANAEROBIC DIGESTORS

5.2.1 Purpose of Design

To manufacture, install and commission eight (8) working anaerobic digesters with control system and reliable data logging functionality that can satisfy the process and specified design parameters. Equipment is intended to anaerobically digest different organic industry wastes, food wastes and agriculture wastes for biogas (methane) production, in particular paper waste sludge.

5.2.2 Method of Operation

Biowastes will be loaded into the digester with the inoculum and will be operated at 37 °C to 70 °C. Mixing in the digester will be intermittent or continuous within the range of 50-500 RPM by using overhead motor driven Rushton impeller. The gas produced will be logged by gas flow meter or U-tube based manometer and gas samples are collected at regular intervals to analyze the biogas quality. Digestate will also be sampled at regular intervals for the analysis. After the set hydraulic retention time, the digestate in the digester will be partially or fully drained.

5.2.3 Main Items of Equipment and Their Specifications

Equipment consists of two main components

1. Eight (8) digesters
2. Master control system

Specifications for each digester

1. 30 L volume stainless steel (316 grade) vessel. Components not in contact with the slurry of feed materials can be 304 stainless steel.
2. Sight glasses on one or more digestors preferred.
3. Head plate of the vessel should be easily removable for cleaning.
4. Each digester should be as compact as possible.
5. Maximum operating pressure of 0.5 mbar, controlled with a glass manometer.
6. Operating temperature: 37°C, but should be able to work in broad range 30 to 70°C

7. Heating system should be a water jacket and should cover the sides of the reactor; double wall digester with external thermostat.
8. Overhead stirrer shaft drive with anchor type impellers. Shaft is to have a bed in the bottom of the reactor, to minimise vibration and movement.
9. Motor of at least 140W and 200-400 N.cm torque. Agitation speed 50 to 500 RPM and frequency control with start and stop time options.
10. Digester level indication from 1 L to 30 L.
11. Screw-on, screw-off funnel for feeding; replaced with screw-on cap when funnel is not in use. Feed port should be on the head plate and port extension down into the reactor should be as short as possible, to avoid blockages.
12. Plunger to push the solids from the feeding port to the digester.
13. Dip pipe to be installed as per photos below. Should be close to stirrer shaft, and be removable from the lid with a screw thread. Dip pipe should be 2-3cm into the liquid slurry inside the digestor.
14. Sample collection port to be able to sample slurry and a drain port that can drain the whole slurry from the reactor. These two ports should be with stainless steel ball valves at the bottom of the digester; the sampling port and valve is to have a diameter of 25 mm (1”), while the drain part and valve should have a diameter of 60 mm. The alignment of sample port and ball valve to be such that a metal rod can be inserted to disperse blockages before sampling.
15. Digester should be mounted on a frame and the sampling port and drain ports should be 0.35 metres above the ground, and include pulley-system for lifting of lids.
16. Gas flow measurement of ±10 ml accuracy (U-tube manometer based or gas flow meter). Range of flow rates that can be measured with the proposed equipment must be included in the quotation.
17. One gas sample collection port and one gas release ports with ball valves. One gas release port is to release the gas for gas flow measurement and the second port to release the gas directly from the digester under pressurized conditions.
18. Additional space for two probes (pH and ORP) to future upgrade the digesters.
19. A base addition port for controlled pH operation.
20. A hole in the top plate closed with screw cap.
21. Round dish bottom
22. Rubber seal between digester and lid must be greased. Number of bolts to be used to secure the lid in place must be specified in the tender documents.

Control system and data logging

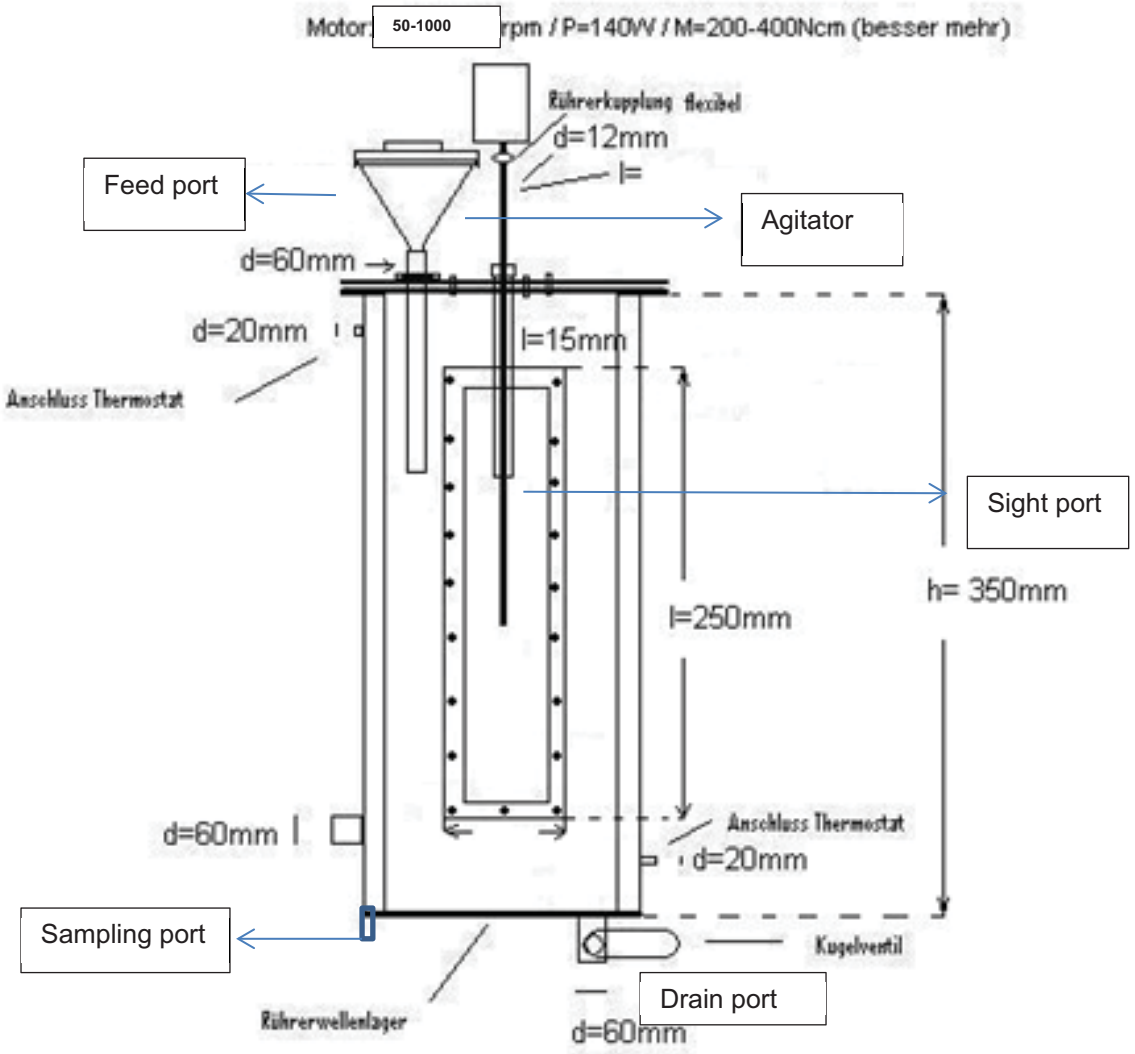
1. Instrument control: Eight digesters should be connected to one master control system, but controlled and operated independently.

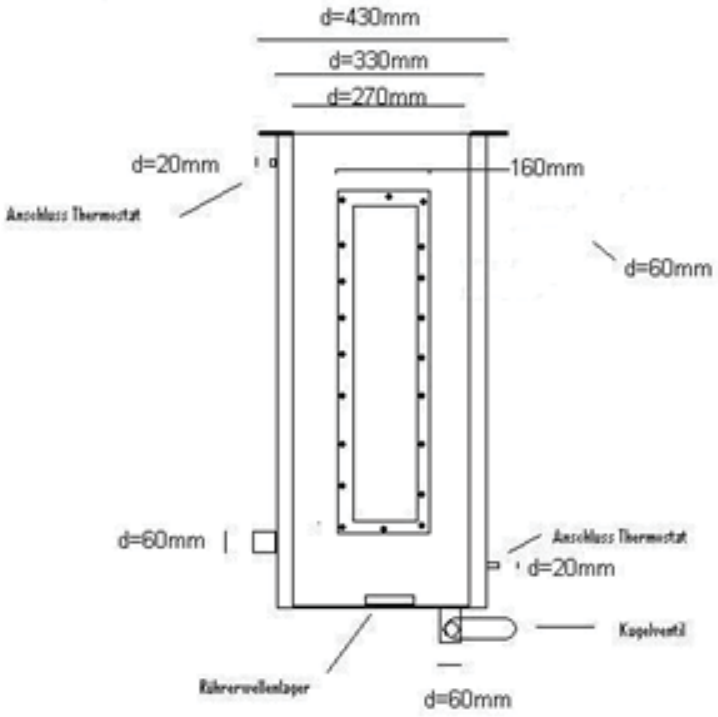
2. Input options for temperature, agitation speed and agitation frequency (with start and stop intervals).
3. Data logging for temperature, agitation speed and biogas flow, with options for pH and ORP monitoring and logging for future upgrade.
4. Data storage with data exportable to MS Excel.

Other specifications

1. All materials of construction, parts and components should be free of gas leaks and liquid leaks.
2. All parts and components of the system must be unconditionally guaranteed to be free of defect in workmanship and materials for twelve months from the date of installation.
3. All equipment to be manufactured according to the provisions of the Occupational Health and Safety Act, 1993.
4. Full training in equipment operation must be provided to the satisfaction of Stellenbosch University.

5.2.4 Examples of Suitable Designs Indicated in Drawings and Photos

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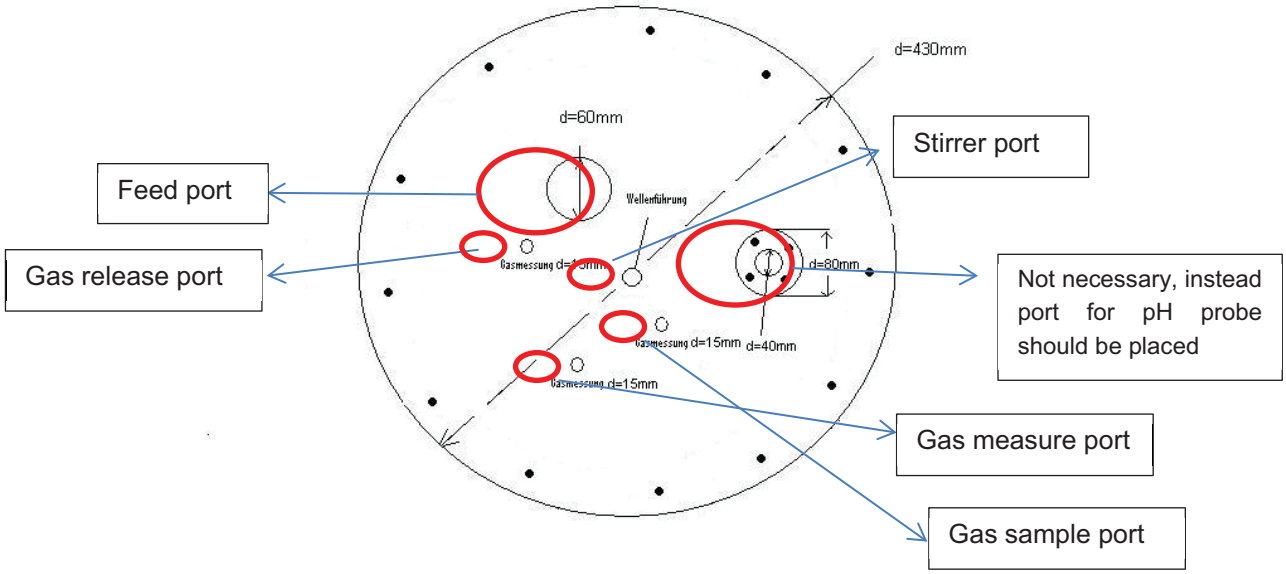
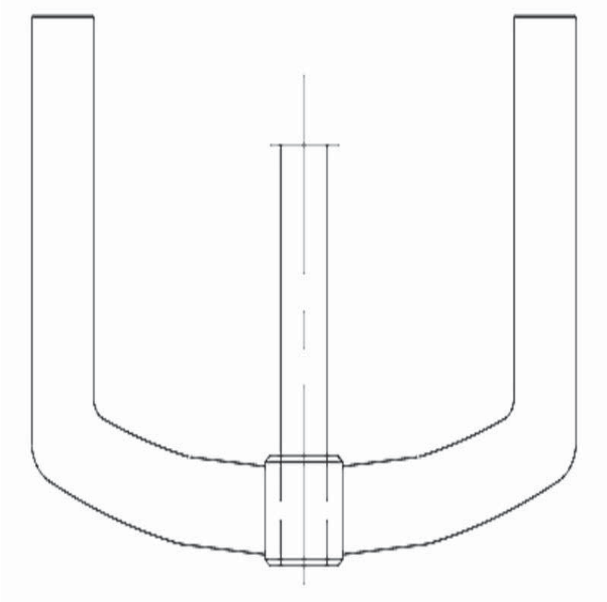
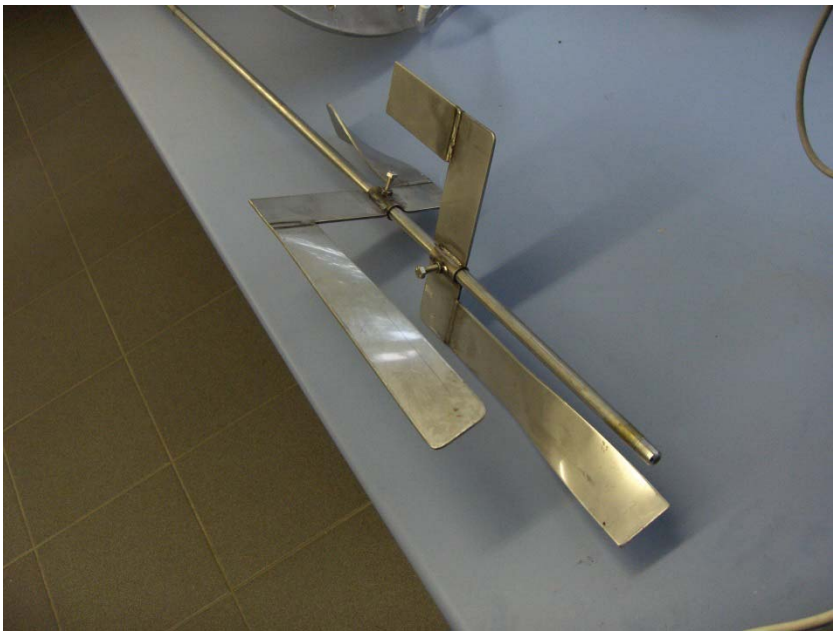
d=330mm
d=270mm
d=20mm
160mm
d=60mm
d=60mm
d=20mm
d=60mm


d=430mm
d=60mm
Feed port
Gas release port
Wellenführung
d=80mm
d=40mm
Stirrer port
Not necessary, instead port for pH probe should be placed
Gas measure port
Gas sample port

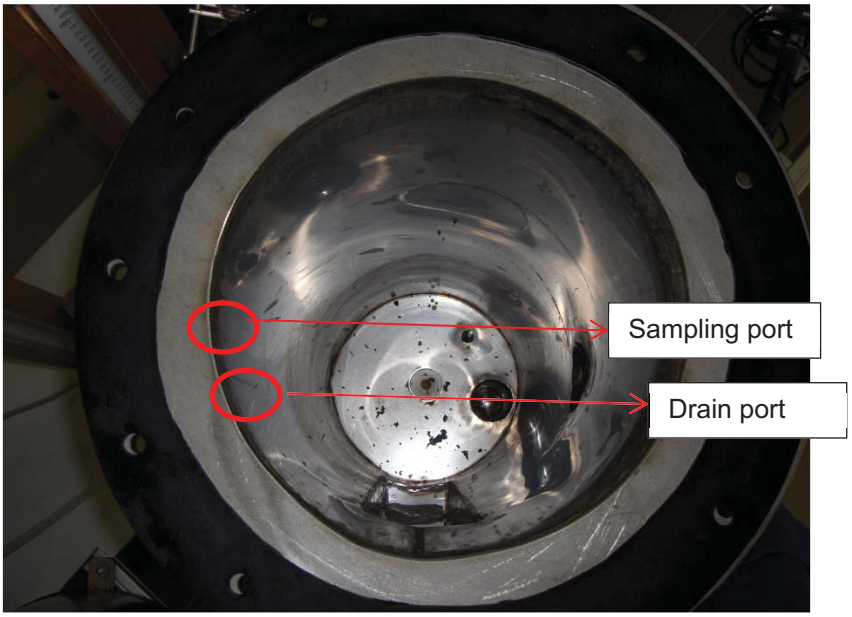
Suitable impeller types

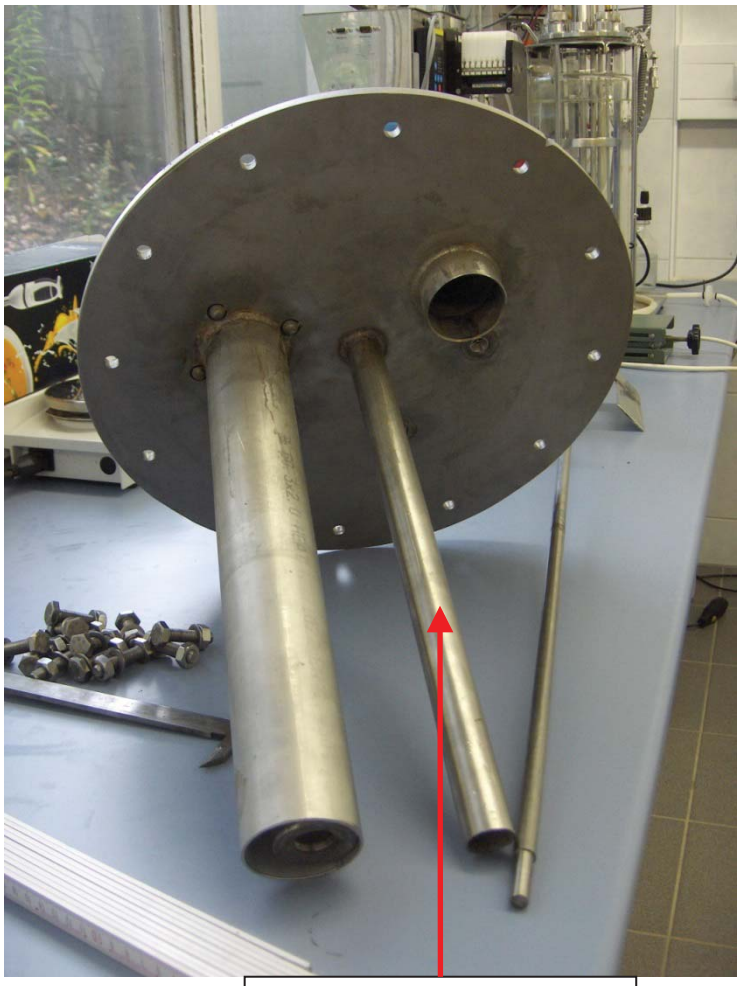


Anchor type impellers preferred for high solids slurries



Digester pictures





Dip pipe. Should be close to stirrer shaft, and be removable from the lid with a screw thread.

5.2.5 Function of Anaerobic Digestors

The purpose of the eight anaerobic digesters is to test various individual and mixtures of feed stocks for biogas production. This will be accomplished by maintaining the digesters under anaerobic conditions at 37 °C and continuous release of the gas produced. Total gas produced need to be monitored and gas release port should be suitable for collecting gas samples in Tedlar bags for gas quality analysis. The digesters should be operable under batch or semi-continuous feeding strategies using one or more feeding ports mounted on the head plate. Sample collection ports for intermittent sampling to be mounted at the bottom of the vessels for digestate analysis. Reactors should be able to run continuously for a minimum of 45 days with continuous mixing and without any interruption in process or data logging. Temperature should be controlled using jacketed reactor vessels and monitored via an internal probe with continuous logging. Once the run is complete, digester needs to be emptied from the bottom through the drain valve. Digesters should have two additional ports for future upgradation (pH and ORP measurements).

5.2.6 Required modifications to digestors bought from external supplier

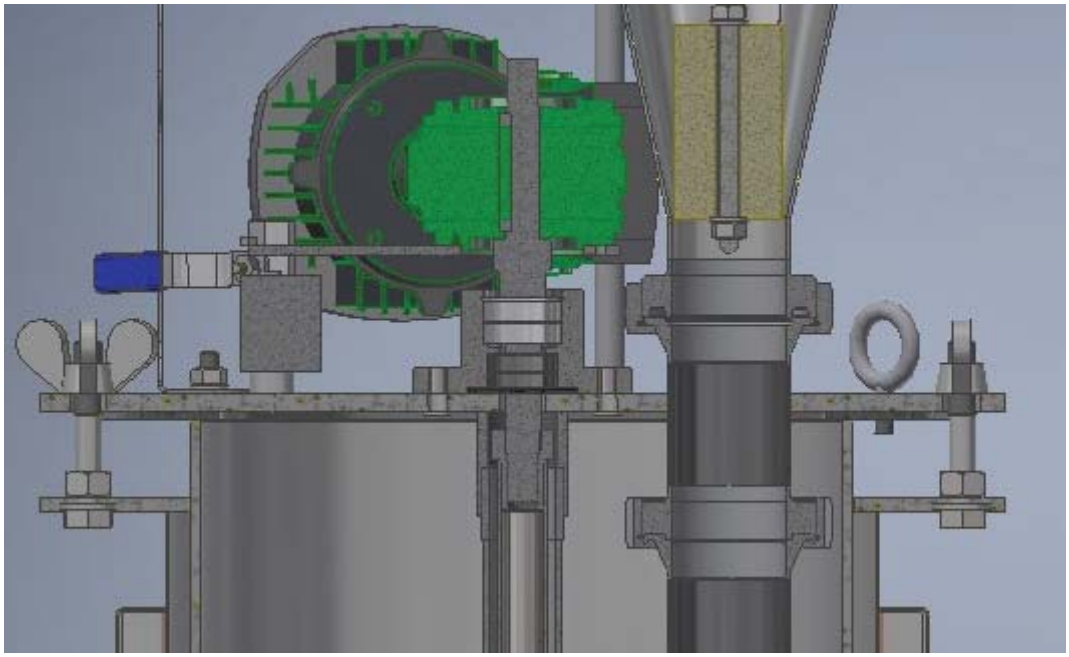
A set of unused, partially-commissioned 8 digestors became available during the course of the design exercise, which offered a more cost-effective solution to securing the required digestors. However, these digestors did not meet all of the design specifications required for the present project, requiring further modifications. In particular, the following modifications were required, resulting in digestors as represented in the drawings below:

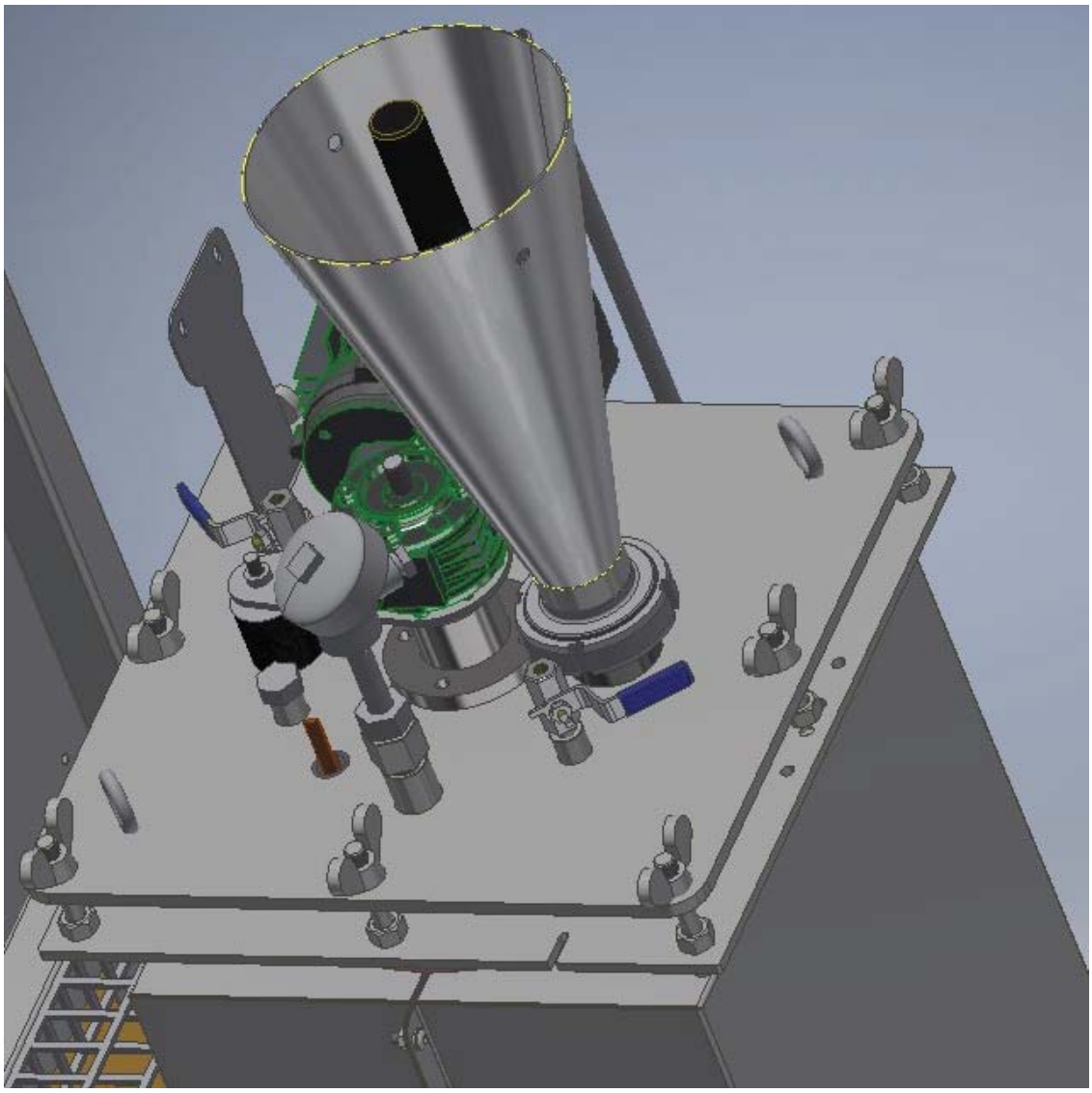
1. Overhead stirrer shaft drive with anchor type impellers. Shaft is to have a bed in the bottom of the reactor, to minimise vibration and movement. Improvements to shaft bearings required to ensure stability and resistance to reactor operation with solid wastes.
2. Existing agitation motor was too small, and therefore replaced with a more powerful motor of 140W and 200-400 N.cm torque. Agitation speed 50 to 500 RPM and frequency control with start and stop time options were included in the upgrade.
3. The digestor lids were replaced to include:
    - Screw-on, screw-off funnel for feeding; replaced with screw-on cap when funnel is not in use. Feed port on the head plate and port extension down into the reactor as short as possible, to avoid blockages with solid materials.
    - Plunger to push the solids from the feeding port to the digester.
    - Dip pipe as per photos above. Installed close to stirrer shaft, and removable from the lid with a screw thread. Dip pipe is 2-3cm into the liquid slurry inside the digestor.
    - One gas sample collection port and one gas release ports with ball valves. One gas release port is to release the gas for gas flow measurement and the second port to release the gas directly from the digester under pressurized conditions.
    - Additional space for two probes (pH and ORP) to future upgrade the digesters.

- A base addition port for controlled pH operation.
- A hole in the top plate closed with screw cap.
- Greased rubber seal between digester and lid. Sufficient number of bolts used to secure the lid in place.

4. The existing control system on the 8 digesters was re-programmed to ensure that data collection could be done continuously for 45 days, with no interruption in either reactor control or data logging.

The resulting digestors are depicted in the drawings below, and will be commissioned early in February 2017, and thus be ready in time for the experimental work scheduled for 2017.





CHAPTER 6: PRODUCTION OF ETHANOL AND BIOGAS FROM PAPER SLUDGE WASTE, TOGETHER WITH A PORTION OF WASTEWATER RECYCLING

6.1 CHARACTERIZATION OF PROCESSED WASTEWATER AND PAPER SLUDGE

6.1.1 Characterization of paper sludge

6.1.1.1 Composition of paper sludge

Compositional analysis was performed to determine the carbohydrate, lignin and ash content of three considered paper sludge (**Table 6-1**). The superior glucan fraction of VP-PS was similar to reported studies by (Boshoff, 2015). Corrugated recycle PS (CR-PS) had the highest xylan and lignin fractions. While tissue printed recycle PS (TPR-PS) had the highest ash fraction and the least glucan content. The high ash fraction of TPR-PS was as a result of repulping Newsprint, printing and recycled paper which contain high quantities of filler and ink (Robus, 2013; Boshoff, 2015).

Table 6-1: 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

6.1.1.2 Elemental analysis of paper sludge

Elemental components of the three-chosen paper sludge are displayed in **Table 6-2**. Elemental analysis was conducted to determine the carbon to nitrogen ratio of paper sludge which is of importance to optimal anaerobic digestion performance. Elemental analysis showed the nitrogen content of paper sludge was very low (<1% w/w) (**Table 6-2**). The highest carbon fraction was reported for VP-PS which also yielded the highest C:N ratio (**Table 6-2**). TPR-PS yielded a C:N ratio of 29:1 which was within optimum anaerobic digestion range (Kayhanian and Tchobanoglous, 1992).

Table 6-2: Elemental analysis of paper sludge

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

6.1.1.3 Water holding capacity (WHC) of paper sludge

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 higher the WHC, the greater the moisture content of paper sludge emanating from the belt

press of various mills (Boshoff, 2015). Consequently, the greater the amount of water that could be reclaimed instead of ending up in landfills. The high WHC of VP-PS and CR-PS were a direct consequence of the high volatile solid content and morphology of fibres in paper sludge (Boshoff, 2015; Cheng and Li, 2015).

6.1.2 Constituents of process water

The constituents of process water (PW) are displayed in **Table 6-3**. The low COD content of virgin pulp process water (VP-PW) is a testament of how effective the pulping process and effluent treatment system are in Mondi Richards Bay mill. The chemical pulping process significantly extract the lignin content of wood into black liquor leaving minor soluble degraded lignin compounds in effluent stream after pulp washing (Sixta, 2008). This reduces the COD load on effluent treatment system resulting in comparative cleaner process wastewater as compared to the other mills. The sodium content of VP-PW was very high, which is an indication of residual cooking liquor elements in effluent stream. CR-PW had the highest COD content due to the mechanical pulping process employed at Mpact Felixton mill. With mechanical pulping, there was no retrieval of lignin into black liquor and naturally, effluent stream contained significant amounts of soluble degraded lignin compounds. CR-PW had total suspended solids of 343 mg/L which was consistent with mechanical pulping wastewater (**Table 2-10**).

Table 6-3: Characteristic summary of paper sludge and dirty process water

	VP-PW	CR-PW	TPR-PW
COD (mg/l)	1194	4775	2618
pH	2.3	7.2	7.4
TSS (mg/l)	NA	343	NA
	µg/l	µg/l	µg/l
Boron	162	3810	3986
Vanadium	11	0.8	3.4
Chromium	1098	3.6	29
Cobalt	1.8	0.2	0.9
Nickel	27	1.8	4.7
Copper	43	1.0	4.5
Arsenic	3.1	1.7	2.7
Selenium	2.7	0.4	0.6
Strontium	964	815	297
Molybdenum	2.9	0.5	5.2
Cadmium	1.8	0.0	<0,02
Antimony	0.6	0.6	1.8
Barium	91	221	105
Mercury	<0.2	0.2	<0,2
Lead	17	0.2	0.2
Uranium			
	mg/l	mg/l	mg/l
Zinc	0.2	<0.2	<0.2

	VP-PW	CR-PW	TPR-PW
Aluminium	1.8	0.8	<0.2
Manganese	3.9	0.7	<0.2
Iron	5.3	1.6	<0.2
Calcium	280	476	188
Potassium	87	15	11
Magnesium	31	24	17
Sodium	1421	258	300
Phosphorous	8	<1	<1
Silicon	11	6.1	4.7

TSS – Total suspended solids; NA – Not Applicable

6.2 EFFECT OF CLARIFIED PROCESSED WASTEWATER ON YEAST, ENZYME AND ANAEROBIC BACTERIA

The potential effects of process water (PW) on fermentation and anaerobic digestion of paper sludge (PS) was investigated by conducting a series of fermentative screening and BMP tests as discussed in section 4.3. Consequentially, this resulted in an observable consensus that exclusive usage of PW without co-feeding with clean water (CW) in biochemical processing didn't have significant negative effect regarding biofuel production. Detailed discussions of experimental results are emphasized in the sections below.

6.2.1 Effect of dirty process wastewater on *S. cerevisiae* MH100 yeast strain (Fermentation in batch culture)

The effects of PW on yeast growth was investigated to determine whether employing just process water in fermentation is apposite and if not which co-feeding ratio of PW and CW will be suitable for ethanol production. As discussed in section 2.6, certain compounds identified in PW are potential toxicants that can affect fermentation performance. Thus, influence of PW on *S. cerevisiae* MH1000 was investigated pertaining to yeast growth and ethanol production. As described in section 4.3.1, all three PW samples were screened in fermentation batch culture for ethanol production at different PW/CW co-feeding ratios with the initial glucose concentration of 50 g/L over 6 days' incubation period.

6.2.1.1 Effect of process water on yeast growth

Process water had an adverse effect on yeast growth ($p < 0.05$). As seen from **Figure 6-1**, adverse effect were observed in process water media which yielded final yeast biomass concentrations 20-35% lesser than clean water fermentation media. This could be attributed to the synergistic repression obtained from a combination of the various inhibitory compounds found in process water (Vertes *et al.*, 2010).

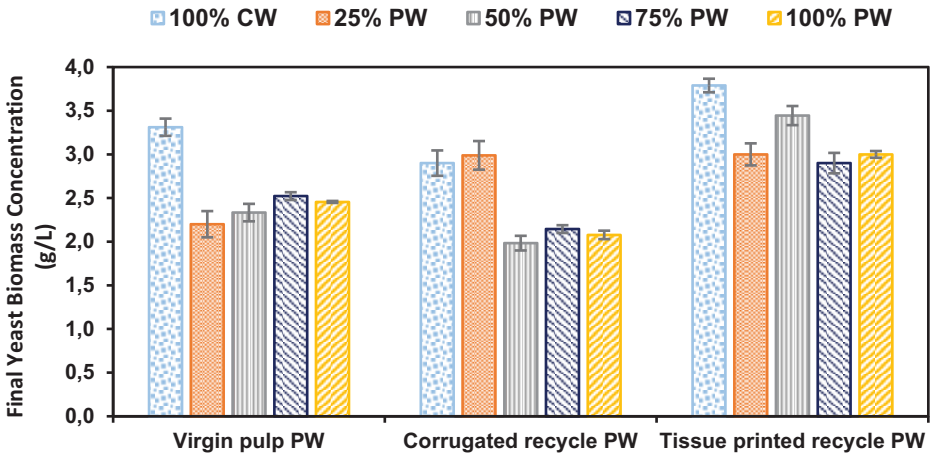


Figure 6-1: Final yeast biomass yield at different co-feeding of PW and CW ratios after fermentation

6.2.1.2 Effect of process water on ethanol production

Process water had no effect ethanol production ($p > 0.05$). Comparing to the maximum theoretical ethanol concentration of 25 g/L, **Figure 6-2** indicated successful ethanol production at different co-feeding ratios of PW and CW. These observations were consistent with previous literature studies on various engineered *S. cerevisiae* strains (Vertes *et al.*, 2010), which had demonstrated that successful production of ethanol could be attained albeit comparative reduction in cell growth due minimal concentrations of inhibitory species.

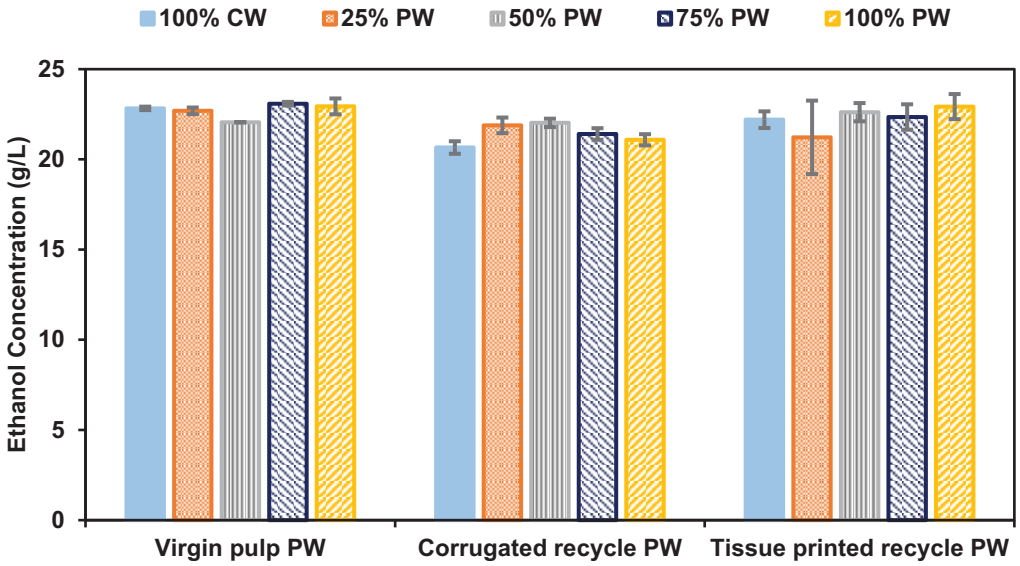


Figure 6-2: Ethanol production at different co-feeding ratios of processed wastewater and clean water

6.2.2 Effect of process water on cellulase performance

The effect of PW on cellulase performance during fermentation of PS was studied in SSF shake flask bottles at enzyme dosages of 5, 10, 15 and 20 FPU/gds. Paper sludge solids loading was limited to 6% (w/w) to avoid viscous slurries in shake flasks. Inferences established in section 6.2.1 indicated the exclusive usability of PW without impeding ethanol production. As such, experiments for the three types of PS were performed in 100% PW media at cellulase dosages stated earlier.

Except for cellulase dosage of 5 FPU/gds, significantly higher ethanol yields were obtained for VP-PS as compared to CR-PS and TPR-PS (**Figure 6-3**). This could be attributed to the superior glucan content of VP-PS as compared to CR-PS and TPR-PS (**Table 6-1**). Another important observation was the insignificant difference (t-test, p-value > 0.05) between control clean water and process water SSF at 15 FPU/gds. The similar ethanol production in control CW and PW SSF at 15 FPU/gds indicated no adverse effect of PW on cellulase performance.

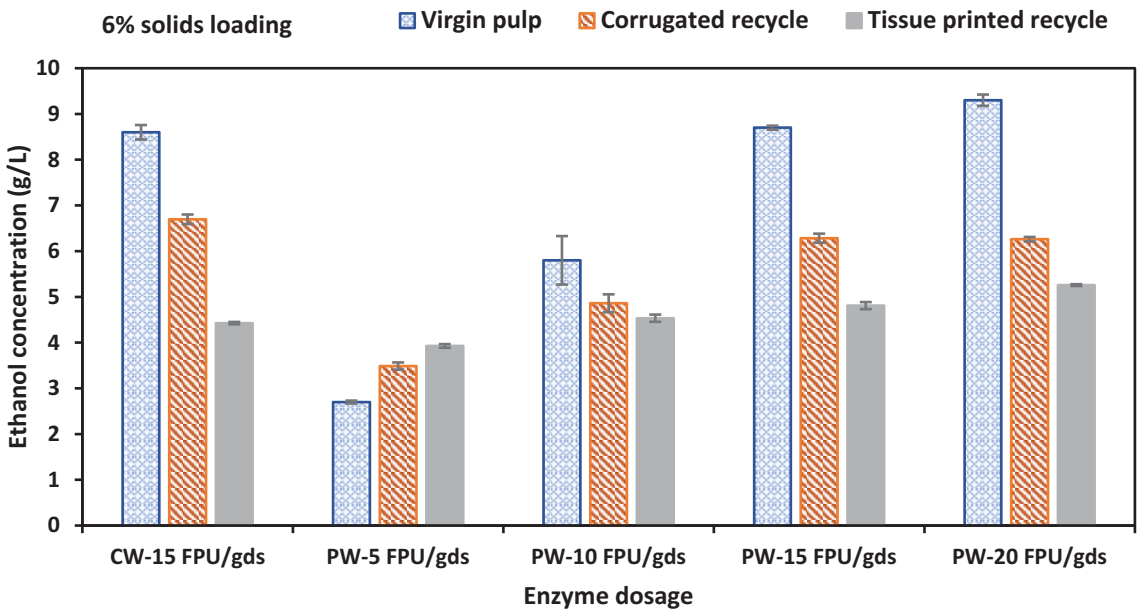


Figure 6-3: Ethanol yield at different cellulase dosages for fermentation of PS alongside PW

6.2.3 Effect of process water on anaerobic bacteria (Biomethane potential Screening)

The possible adverse effects of process water on bacteria community was investigated in anaerobic digestion of paper sludge. Consequently, as indicated in section 4.3.4, five different co-feeding ratios of PW and CW at 6% solids loading were tested over the period of 30-45 days to evaluate the methane potential for the chosen three types of PS. The PW/CW ratio was the significant parameter for this BMP assay test since the successful anaerobic digestion of PS with clean water to produce bio-methane is well established in literature (**Table 2-14)**. The cumulative biogas and methane production of the BMP assays were plotted over

the 30 to 45-day duration of the experiments for each substrate and its corresponding PW and CW mix. The data for the experimental runs are graphically presented in the following sub-sections.

6.2.3.1 BMP for virgin pulp paper sludge (VP-PS) at different co-feeding ratios of PW and CW

Significantly ($p < 0.05$) higher biogas and methane yields were obtained by substituting process water for clean water in virgin pulp PS BMP test (**Figure 6-4**). 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, Appendix**). Although, biogas yield in PW BMP assays were significantly higher as compared to clean water BMP, digestion performance decreased marginally as the PW concentration increased. Biogas and methane yields obtained from 75-100% PW BMP assays showed an extent of microbial inhibition at earlier periods of digestion. There was lower biogas production in 75-100% PW BMP as compared to 25-50% PW BMP assays within the first 22 days of incubation, **Figure**, **Appendix**. This observation could be attributed to ephemeral partial inhibition of microbial community as inoculum acclimatize to adverse conditions triggered by potential toxicants in VP-PW.

Experimental results indicated a co-feeding ratio of 25-50% VP-PW was best suited for VP-PS digestion but an acclimatized inoculum in 100% VP-PW would likely perform akin to the latter. The maximum usage of PW in anaerobic digestion without extensively decreasing biogas yield was a key objective of this study. Thus, a selection of 100% PW for virgin pulp PS digestion was acceptable due to its better biogas yield as compared to clean water digestion.

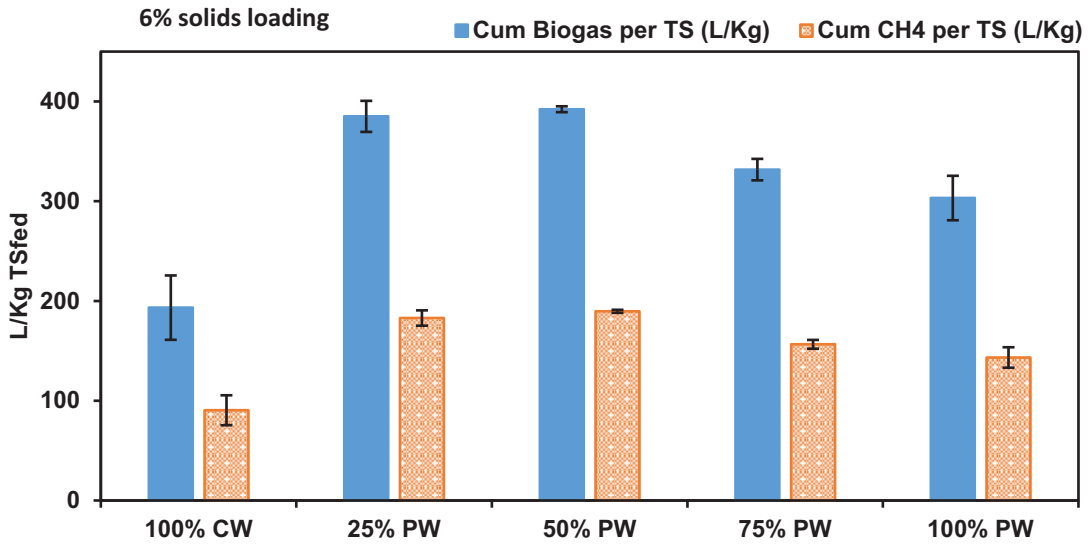


Figure 6-4: Cumulative biogas and biomethane production for VP-PS at different co-feeding ratios of PW and CW

6.2.3.2 BMP for Corrugated recycle paper sludge (CR-PS) at different co-feeding ratios of PW and CW

Process water had a significant effect on digestion of CR-PS ($p < 0.05$). Biogas and methane yields steadily decreased as the amount of crude CR-PW with suspended solids increased in BMP assays (**Figure 6-5).** The adverse effect on yield could be due to suspended solids found in CR-PW. The negative effects of suspended solids were established when biogas and methane yields increased steadily as the amount of supernatant CR-PW increased in a different set of BMP assays (**Figure 6-6**). 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.

Methane production was not harmfully impeded in crude CR-PW BMP. Biogas and methane yields in 100% crude CR-PW BMP were about 19% lesser than clean water anaerobic digestion of CR-PS. Thus, the exclusive use of crude CR-PW would be permissible for anaerobic digestion of CR-PS in an industrial setting considering the unfavorable step of a centrifuging out suspended solids.

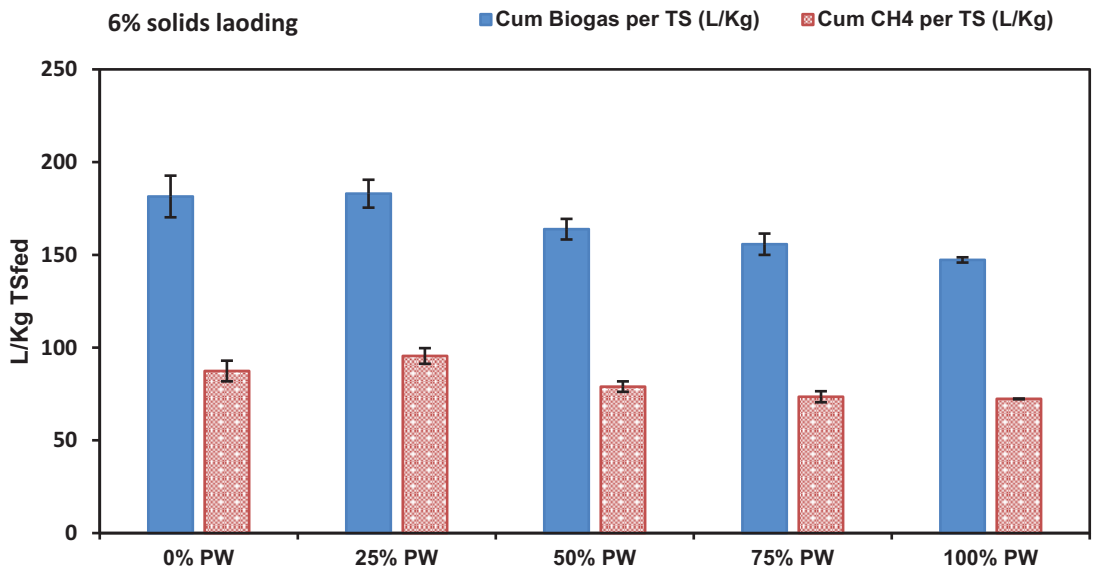


Figure 6-5: Cumulative biogas and biomethane production for crude CR-PW

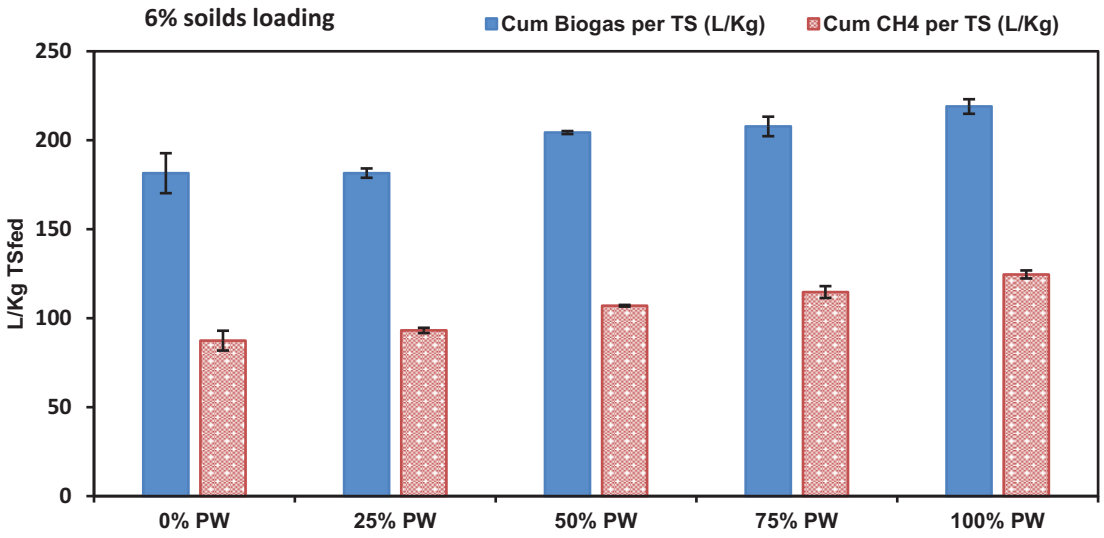


Figure 6-6: Cumulative biogas and biomethane production for supernatant CR-PW

6.2.3.3 BMP for Tissue printed recycle paper sludge (TPR-PS) at different co-feeding ratios of PW and CW

Anaerobic digestion of TPR-PS was unaffected by process water. As observed from **Figure 6-7**, similar biogas and methane yields were obtained independent of PW/CW co-feeding ratios ($p > 0.05$). The results indicated no inhibitory effect towards anaerobic microbes and thus it was feasible to exclusively utilize TPR-PW in anaerobic digestion of PS.

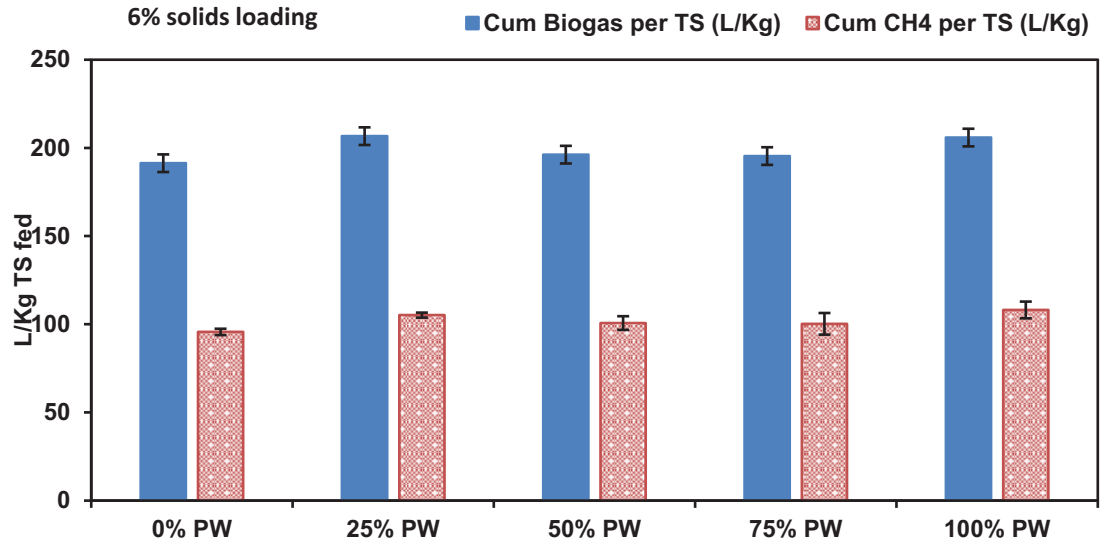


Figure 6-7: Cumulative biogas and biomethane production for TPR-PS at different co-feeding ratios of PW and CW

CHAPTER 7: SEQUENTIAL FERMENTATION AND ANAEROBIC DIGESTION OF PAPER SLUDGE

This chapter evaluates the added benefit of treating paper waste sludge using the fermentation and anaerobic digestion processes in sequence. The individual processes on their own contributes to:

1. Biofuel production. Paper sludge contain readily available carbon which through microbial activity can be converted to ethanol and methane.
2. Water reclamation. Raw paper sludge retains large amounts of water which 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. Use process instead of municipal water for bioprocessing.

The added benefit of employing both processes in sequence was highlighted by comparing it to the individual technologies. Fermentation and anaerobic digestion of paper waste, as the starting substrate, is covered in sections 7.1 and 7.2. The combined process is detailed in Section 0, where it is compared to the individual technologies.

7.1 FERMENTATION OF PAPER SLUDGE

Paper waste derived from Virgin Pulp, Corrugated Recycle Pulp and Tissue Printed Recycle Pulp was subjected to fed-batch fermentation. Operating conditions (**Table 7-1**) previously optimized for the individual substrate were used. For instance, a solid loading of 18% (w/w) was employed for Virgin Pulp which is less than the 33% (w/w) used for Tissue Printed Recycle Pulp. Enzyme dosage also depended on substrate type and ranges between 11 and 20 FPU/gds. Process water was used in all cases.

Table 7-1: Mass balance for the optimization runs for the SSF of PS with PW in 5 L 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
Ethanol concentration	g/L	49.6	41.1	44.6
Theoretical ethanol yield/ Y_{Et}	w/w	92.6%	79.4%	< 100%
Productivity	g/(L.hr)	0.459	0.343	0.362
Ethanol yield	g ethanol/g glucose consumed	0.489	0.495	NA
Ethanol yield	g ethanol/g glucose fed	0.426	0.366	NA
Overall ethanol yield	kg ethanol/ton dry PS	275.4	152.2	135.1

7.1.1 Bio-Fuel production through fermentation of paper sludge

Substrate VP-PS, CR-PS and TPR-PS produced an excess of 40 g/L ethanol within a 120-hr fermentation period (**Table 7-1**). The VP-PS fermentation yielded the highest ethanol concentration (i.e. 49.6 g/L) but also the fastest ethanol production rate (i.e. 0.459 g/L.hr). In comparison, VP-PS has the highest cellulose content, which favours ethanol production. Alternatively, VP-PS is also considered a cleaner substrate. Recycled products tend to accumulate ash which negatively affect enzymatic hydrolysis by forming irreversible bonds. Recycled Ink and filler contribute to the increased ash content. On the other hand, lactic acid, especially in the case of CR-PS, may also contributed to a lower ethanol performance compared to VP-PS (**Figure 7-1**). Lactic acid not only acts as a toxin to *S. cerevisiae* but also as a competing product. Lactic acid bacteria may find their way into fed-batch fermenter as it is difficult to maintain aseptic conditions.

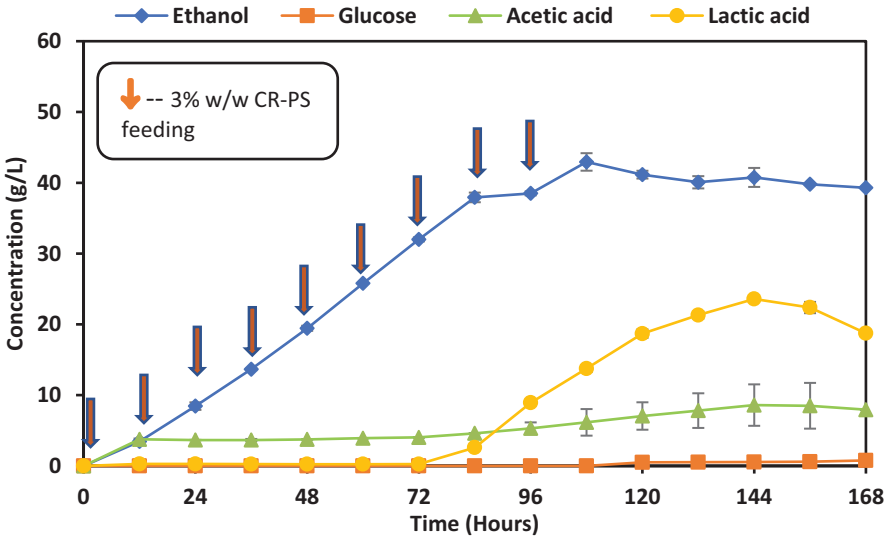


Figure 7-1: Time course for SSF of corrugated recycle PS with PW

7.1.2 Water reclamation through fermentation of paper sludge

Approximately 80% to 90% of previously entrapped water in paper sludge (PS) could be reclaimed through fermentation (**Table 7-2**). 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.

Table 7-2: 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

7.1.3 Water quality subsequent to fermentation

The Chemical Oxygen Demand (COD) of the supernatant water deteriorated as a result of fermentation. A more than ten-fold increase in COD was observed (**Table 7-3**). Fermentation releases organic matter that are otherwise recalcitrant to biological degradation. Fermentation can therefore not be viewed as a water purification technology if measured in terms of COD reduction.

Table 7-3: Chemical oxygen demand of process water and stillage after fermentation

Paper Sludge	Chemical Oxygen Demand (g/L)	
	Before	After
Virgin pulp	0.478	86.8
Tissue Printed Recycled	0.262	128.8
Corrugated Recycled	0.478	138.2

[1]Suspended solids are removed through centrifugation

7.1.4 Water reuse through fermentation of paper sludge

Process water was used in all fermentation processes. Hence, the use of municipal fresh water was avoided. However, the COD of the resulting liquid stream was severely affected by fermentation (**Table 7-3**). Thus, provided the existing pulp and paper plant wastewater treatment systems can handle the increase in COD, one may profit by using process water instead of municipal fresh water. Otherwise, benefits associated with using process water is nullified, if excessive amounts of municipal water are added in order to dilute the COD to within range.

7.2 ANAEROBIC DIGESTION OF PAPER SLUDGE

Paper waste derived from Virgin Pulp, Corrugated Recycle Pulp and Tissue Printed Recycle Pulp was subjected to anaerobic digestion. Biogas production took place in 30 L digesters batch reactors, set 37°C and a solid to liquid ratio of 10% (w/w). However, VP-PS was conducted at a slightly lower (i.e. 6% w/w) solids content to accommodate for the higher viscosity. Each process was initiated with an inoculum obtained from SAB. Biogas production was recoded on a continuous basis. The methane concentration of the biogas was determined on a weekly bases using an offline gas analyzer. Anaerobic digestion was completed within 30 days. All experiments were conducted with process water.

7.2.1 Bio-Fuel production through anaerobic digestion of paper sludge

Biogas production ranged between 130 and 180 L/kg total solids (**Table 7-4**). VP-PS yielded the highest biogas yield, followed by CR-PS and then TPR-PS. A similar observation was made for cumulative methane where VP-PS again yielded the highest methane production in excess of 95 L/kg total solids. An interesting phenomenon however, was the cumulative biogas produced per volatile solids basis by TPR-PS which was almost 50% higher than the other substrates involved. This may be due to a more accessible cellulose structure. However, the low cellulose content of TPR-PS prevents it from being the overall biogas producer based on total solids fed. Furthermore, the biogas production (on total solid basis) was 30% higher than the result obtained by Williams (2017) with a similar substrate. The enchantment is attributed to the use of a more advanced experimental design based on the impeller and leakage detection methodology.

Table 7-4: 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

7.2.2 Water reclamation through anaerobic digestion of paper sludge

Anaerobic digestion reduced the water holding capacity of all paper sludges by more than 40% (w/w) (**Table 7-5**). However, this reduction is 50% less than what was achieved through fermentation (section 7.1.2).

Table 7-5: 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

7.2.3 Water purification through anaerobic digestion of paper sludge

Anaerobic digestion was unable to rid the process water from its COD. However, at least anaerobic digestion did not contribute a higher COD as was the case with fermentation (section 7.1.3).

Table 7-6: Chemical oxygen demand of process water before and after anaerobic digestion

Paper Sludge	Chemical Oxygen Demand (mg/L)	
	Before	After
Virgin pulp	4780	3720
Tissue Printed Recycled	2620	1670
Corrugated Recycled	4780	3220

7.2.4 Water reuse through anaerobic conditions of paper sludge

Process instead of municipal water could be used for anaerobic digestion. Furthermore, since anaerobic digestion did not contribute to a higher COD the resulting liquid stream can be mixed with the process water of the plant without further modification.

7.3 SEQUENTIAL FERMENTATION AND ANAEROBIC DIGESTION

Sequential biochemical processing was achieved by fermenting paper sludge in 150 L bioreactor, 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 using conditions and commercial enzyme supplementation as established at bench scale (Section 7.1). Outcomes of the sequential fermentation and anaerobic digestion of paper sludge at such large scale reactor levels will thus provide sufficient proof of concept and data for transfer to a larger scale.

7.3.1 Scaled-up paper sludge fermentation in 150 L bioreactor

Fed-batch SSF was performed on virgin pulp PS (VP-PS), corrugated recycle PS (CR-PS) and tissue printed recycle PS (TPR-PS) in 150 L bioreactor with a working volume of 70 L using process water (PW). Operating conditions and cellulase dosages for the respective fermentation of the various paper sludges were based on 5 L bench scale fermentation runs (Section 7.1).

The ethanol production over a 168-hour period from the SSF of the two paper sludges are shown in **Figure 7-2, Figure 7-3** and **Figure 7-4**.

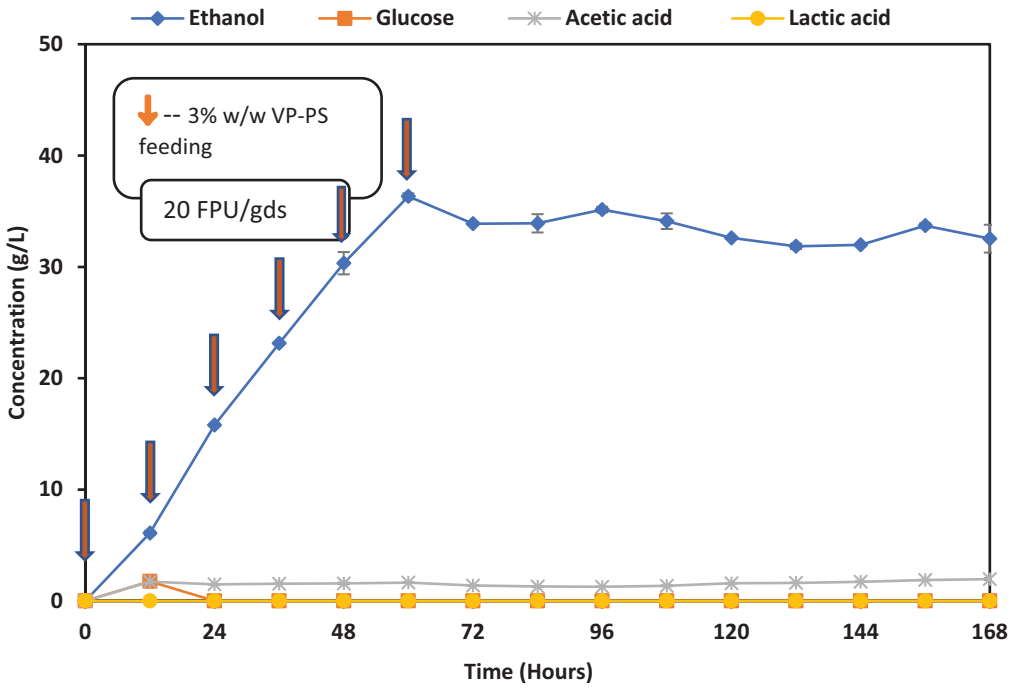


Figure 7-2: Ethanol Concentration profile for 150 L fermentation of virgin pulp PS with PW

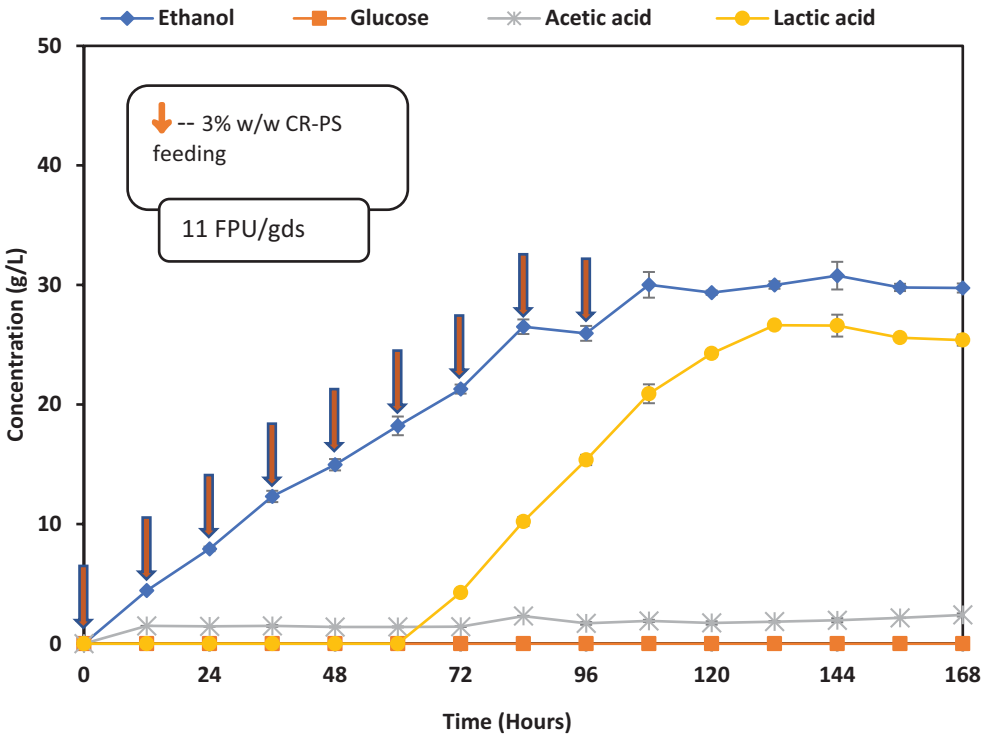


Figure 7-3: Ethanol Concentration profile for 150 L fermentation of corrugated recycle PS with PW

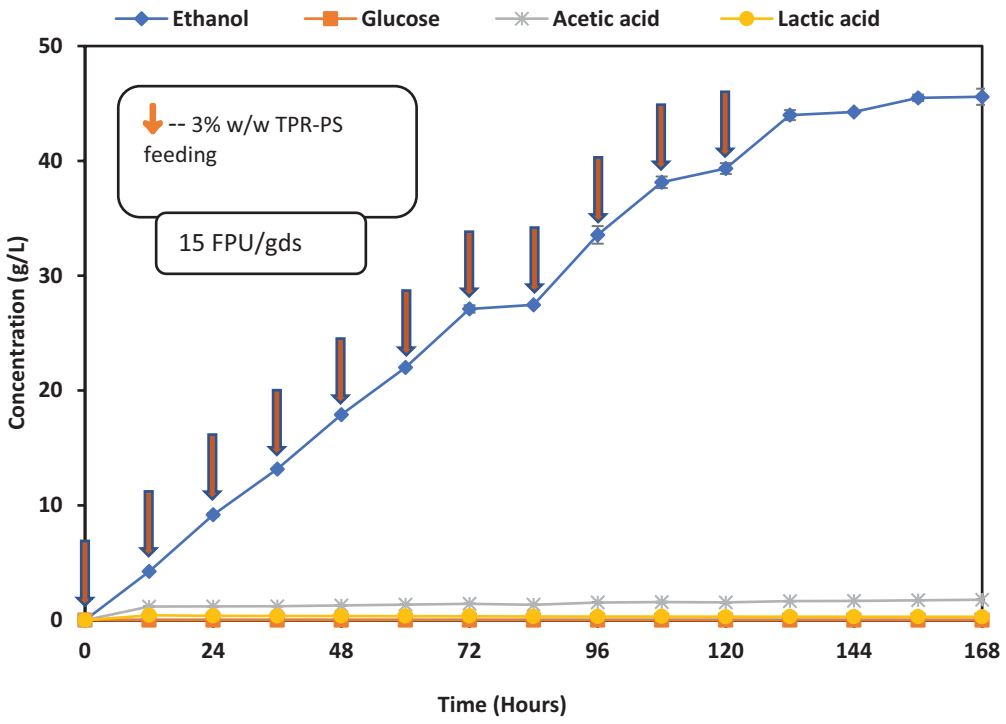


Figure 7-4: Ethanol Concentration profile for 150 L fermentation of tissue printed recycle PS with PW

In contrast to the bench scale fermentations, TPR-PS produced the highest ethanol concentration of 44.0 ± 0.6 g/L (**Figure 7-4**). 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 7-4**). VP-PS yielded the second best ethanol concentration of 36.3 ± 0.3 g/L at 60 hours (**Figure 7-2**). After the last feeding was added at 60 hours, there was no increase in ethanol concentration (**Figure 7-2**). 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 7-3**). Similar to bench scale fermentation of CR-PS (**Figure 7-1**), lactic acid production started after 72 hours of fermentation and increased to a high of 26 g/L (**Figure 7-3**). 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 7-1**). 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.

Table 7-7: Mass balance for the optimization runs for the SSF of PS in 150 L 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	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
Ethanol concentration	g/L	34.0	30.0	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)

In comparison to bench scale, ethanol production was only replicated in TPR-PS (**Figure 7-4**). 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 7-7**). 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 7-7**) as compared to bench scale fermentation (**Table 7-1**) and this resulted in more residual sugars in remaining solids (**Table 7-8**). Alternatively, the shorter time required for the VP-PS fermentation to reach its maximum ethanol concentration results in a higher productivity of 0.470 g/(L.hr) compared to 0.338 g/(L.hr) for TPR-PS (**Table 7-7**). Furthermore, even though the TPR-PS

produced a higher ethanol concentration, this difference was not enough to negate the higher amount of dry mass PS that would be needed to produce a higher overall yield compared to that of the VP-PS fermentation.

Ethanol production from CR-PS and VP-PS were significantly lower as compared to bench scale runs. However, fermentation of TPR-PS proved the feasibility of producing significantly higher ethanol concentrations at pilot scale level using process water as a make-up stream. The ethanol production from TPR-PS (44 g/L) was 10% higher than 40 g/L. Notably, 40 g/L has been set by industry as the target ethanol concentration to ensure economic distillation of ethanol (Kang *et al.*, 2011). Also, pilot scale fermentation of TPR-PS showed exceptional fermentation performance in comparison to Vehmaanpera *et al.* (2012) (the only pilot scale fermentation of paper sludge and waste fiber, Finnish study). TPR produced an ethanol concentration about 10% to 15% higher than that obtained by Vehmaanpera *et al.* (2012).

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. Thus additional pilot scale fermentations runs will be conducted in 2019. These further runs will be conducted with marine or axial impellers in 150 L to enhance ethanol production from CR-PS and VP-PS.

Table 7-8: Chemical composition of paper sludge and dried fermented solids

Constituents	Paper Sludge					
	Virgin Pulp		Corrugated recycle		Tissue printed recycle	
	Before	After	Before	After	Before	After
Cellulose (% w/w)	58.2	14.2	37.5	19.5	20.8	2.7
Xylan (% w/w)	12.2	3.1	13.1	6.1	4.9	0.9
Lignin (% w/w)	4.1	25.8	13.1	27.0	6.4	8.1
Extractives (% w/w)	5.4	17.1	10.4	18.1	5.1	7.6
Ash (% w/w)	20.8	39.8	25.9	30.9	62.9	80.7

7.3.2 Bio-Fuel production through anaerobic digestion of fermentation stillage

Biogas was produced from all stillage derived from fermentation of VP-PS, CR-PS and TPR-PS. Stillage produced 30% to 70% less biogas and methane compared to the paper sludge. However, a more desirable result was obtained with the stillage derived from CR-PS compared to that of the raw substrate (i.e. CR-PS before fermentation) (**Table 7-9**).

Table 7-9: 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

The chemical composition of the fermentation solids before and after anaerobic digestion remains approximately the same (**Table 7-10**). Thus, some other carbon source must therefore be responsible for methane production. Section 7.3.4 investigate the potential relationship between biogas production and COD reduction.

Table 7-10: Chemical composition of fermentation solids and solids following anaerobic digestion

3	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	32.0	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	31.0	30.9	30.2	80.7	84.6

7.3.2.1 Energy conversion efficiencies of individual and combined processes for fermentation and anaerobic digestion of paper sludge

The gross energy estimation gives a crude assessment of how efficiently a substrate was converted to bioenergy. The gross energy conversion was calculated for standalone and sequential biochemical processes (**Table 7-11**). The net heating values for ethanol and methane are 29.85 and 55.53 MJ/kg, respectively. While the higher heating values (measured using bomb calorimeter) for VP-PS, CR-PS and TPR-PS are 16.73, 14.54 and 11.10 MJ/kg respectively. Based on energy conversion efficiency, standalone fermentation process was better than anaerobic digestion of paper sludge with process water. However, energy conversion efficiency in sequential biochemical processing was about 6-35% and 20-45% higher than standalone fermentation and anaerobic digestion of PS with PW respectively.

Table 7-11: The heat values and energy conversion efficiencies for standalone and sequential biochemical processes

Process type	PS type	Product Yield (Kg/tonne PS)		Product Energy (MJ/tonne PS)	Energy Conversion Efficiency (%)
Fermentation only (**Table 7-1**)	VP	275.4		8 221	49.1
	CR	152.2		4 543	31.2
	TPR	135.1		4 033	33.4
Anaerobic digestion only (**Table 7-4**)	VP	67.7		3 759	22.5
	CR	52.9		2 454	20.2
	TPR	44.2		2 454	20.6
Sequential treatment (Current Section)	VP	275.4[1]	43.7[2]	10 650	63.7
	CR	152.2[1]	85.5[2]	9 288	63.9
	TPR	135.1[1]	15.1[2]	4 869	40.9

[1]Ethanol production through fermentation.
[2]Methane production through anaerobic digestion.

7.3.3 Water reclamation from paper sludge using sequential fermentation and anaerobic digestion

The amount of water reclaimed from the sequential bioprocessing was equivalent to that of the fermentation process (**Table 7-2**). Similar to the fermentation process only, more than 80% (w/w) of the water in paper sludge was recovered through the sequential bioprocessing of paper sludge. This was attributed to the no change in chemical composition of fermented solids before and after anaerobic digestion (**Table 7-10**).

7.3.4 Water purification using sequential fermentation and anaerobic digestion

The sequential fermentation and anaerobic digestion process does not serve as a means to purify water (**Table 7-12**). Fermentation of paper waste contribute to an effluent stream of which the COD is an average 32 time greater than that of the starting liquid, i.e. process water. Anaerobic digestion does, however, lower the COD of the fermentation stillage by 36%. However, this reduction still leaves a liquid that is 17 times dirtier than the starting liquid.

Table 7-12: 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	128 765	90 275
TRP	mg/L	2 618	138 217	95 394

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

32 Times Increase

36% Reduction

7.3.5 Water reuse through anaerobic digestion of fermentation stillage

The sequential fermentation and anaerobic digestion process do employ process instead of municipal water. However, 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 dilution with municipal water in order to reached required COD levels. The effluent stream as depicted in **Table 7-12** need to be dilute 24 times before it can be blend with process water.

CHAPTER 8: CHARACTERIZATION OF FERMENTED DIGESTED SOLID RESIDUE AND POTENTIAL INDUSTRIAL AND AGRICULTURAL USE

Solid residues from sequential bioprocessing of paper sludge with process water were characterized to determine potential industrial and agricultural benefits. Characteristics and produced quantities of fermented digested solid residues were summarized in **Table 8-1**.

Sequential bioprocessing resulted in a 59% volatile solids reduction in TPR-PS as compared to 6-8% decrease experienced in VP-PS and CR-PS. A high residual glucan fraction of 19.5% (w/w) and 14.2% (w/w) were observed in CR and VP residues respectively. Also, the volatile solids contents of CR and VP residues were dominated by 31.3% (w/w) and 32.0% (w/w) lignin respectively. TPR residues recorded the highest ash content of 85% (w/w), which was 64% higher than both CR and VP residues. The ash content of solid residues largely consisted of calcium. TPR residues recorded the highest calcium fraction of 91% (kg/kg ash), while CR and VP residues yielded 76 and 61% (kg/kg ash) respectively.

Table 8-1: Quantity and characterization of solid residues after sequential fermentation and anaerobic digestion of paper sludge with dirty process water

	Virgin pulp	Tissue printed recycle	Corrugated recycle
Solid residues (kg/kg dry TS_{fed})	0.275	0.732	0.613
Solid residues (kg/kg stillage)	0.035	0.167	0.133
Volatile solids (kg/kg dry fermented digested solids)	0.690	0.154	0.698
Ash content (kg/kg dry fermented digested solids)	0.310	0.846	0.302
Water holding capacity (kg water/kg fermented digested solids)	2.031	0.696	1.842
Higher heating value (MJ/kg fermented digested solids)	12.5	3.5	11.3
Boron (µg/kg)	23274	7890	15161
Vanadium (µg/kg)	10628	4986	13942
Chromium (µg/kg)	175686	14070	93175
Cobalt (µg/kg)	2270	1977	3848
Nickel (µg/kg)	29224	47813	11603
Copper (µg/kg)	84224	108472	37492
Arsenic (µg/kg)	5567	924	2827.0
Selenium (µg/kg)	556	157	299.0
Strontium (µg/kg)	36219	196206	1824
Molybdenum (µg/kg)	8723	2430	191.0
Cadmium (µg/kg)	588	151	532.0
Antimony (µg/kg)	504	38	57.0

	Virgin pulp	Tissue printed recycle	Corrugated recycle
Barium (µg/kg)	158981	50861	183943
Mercury (µg/kg)	459	2310	663.0
Lead (µg/kg)	34014	8797	21280.0
Uranium (µg/kg)	362	844	1204.0
Zinc (mg/kg)	2178	387	629.0
Aluminium (mg/kg)	6258	21374	18954
Manganese (mg/kg)	388	86	401
Iron (mg/kg)	3836	2816	6725
Calcium (mg/kg)	42513	342796	129033
Potassium (mg/kg)	995	983	1107
Magnesium (mg/kg)	1775	4606	3745
Sodium (mg/kg)	5495	1603	5254
Phosphorous (mg/kg)	4283	475	2583
Silicon (mg/kg)	1636	2634	1339

Fertilizer production from urine

A potential novel application of calcium rich TPR residue, is in water recovery and fertilizer production from urine collection (WISA conference, Randall, 2018). Fresh urine contains nutrient rich phosphorous and nitrogen compound which are important fertilizing agents. Using calcium carbonate, 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). In addition to already present macro and micronutrients, the high calcium content of TPR residue renders it an excellent capturing agent in this innovative process.

Nutrient supplement for poor soil environments

Solid residues after sequential biochemical processing of PS have the potential to moderately be used as nutrient supplements for plantation and natural forest soils (Demeyer *et al.*, 2001; Patterson, 2001; Goodwin and Burrow, 2006; Pitman, 2006). Solid residues had similar macro and micronutrient levels as wood ash (Scheepers, 2014). Solid residues in this study or wood ash lacks nitrogen and hence need to be balanced with adequate nitrogenous source (N-fertilizers) to achieve desired growth effects. However, some heavy metals concentrations in solid residues (chromium, copper, lead and zinc) exceeded the values set by the South African National Environmental Management: Waste Act, 2008 (Act no. 59 of 2008) which could lead to an accumulative effect in soil environment. The mechanics associated with leaching, bioavailability and soil toxicity are significantly affected by soil pH, metalloid concentrations, concentrations of organic and inorganic molecules, nutrients and microbial activity (Violante *et al.*, 2010). Consequently, the application of produced 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.

Clinker production

TPR solid residues because of its high ash content 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, Fe, Al and Si) essential for clinker production (Buruberri, Seabra and Labrincha, 2015).

CHAPTER 9: FRUIT POMACE. A POTENTIAL FEEDSTOCK FOR ETHANOL PRODUCTION IN THE WESTERN CAPE

9.1 ABSTRACT

The ethanol production potential of citrus, apple and grape pomace was investigated. The study focused on the fruit industries that are located within the boundaries of the Western Cape. The report was compiled by conducting both a literature review and an industrial survey. The literature survey presents an overview on the selected fruit industries, their pomace production potential and the ethanol yield that could theoretically be derived through their fermentation. The industrial survey, on the other hand, focused more on the technologies that are currently available and used by industry to either dispose or reuse pomace. Industry was also asked whether they consider the utilisation of their pomace for ethanol production as industrially and commercially relevant.

Literature Review: Citrus, apple and wine grape pomace are considered an untapped resource for bio-ethanol production in the Western Cape. Approximately 24.7 thousand tons of ethanol can theoretically be derived from these pomace on an annual basis. White grape pomace, in comparison to red grape, apple and citrus pomace, is considered the most promising feedstock for bioethanol production. This is attributed to its high production volume (with reference to the Western Cape) and high content of fermentable sugar which is present as a result of the wine making process. In contrast, the other also dominant fruits in the Western Cape are either produce at a much lower volume and/or their pomace contains less fermentable sugars compared to that of white grape pomace. White grape pomace are therefore the obvious choice when it comes to evaluating fruit pomace as a potential feedstock for bio-ethanol production.

Industrial Survey: The fruit Industry has only reach infancy when it comes to utilising pomace as a feedstock for value added product production. Pomace is generally landfill but has found some application in composting although somewhat reluctant due to it low nutritional value. Industry has not yet considered ethanol production from pomace.

9.2 STUDY OVERVIEW

The South African climate favours grapes, apples and citrus production (Khan et al., 2015). Most of these fruits are cultivated in the Overberg/Boland region which are centralised in the Western Cape Province. Based on production volume, most of these fruits are exported and sold on the international market. The balance are predominantly processed into value added products or to a lesser extent consumed locally (Department of Agriculture, Forestry and Fisheries, 2015, 2016a/b). Fruit processors generate large amounts of pomace which collectively includes the skin, pulp and the seeds of the fruits. Based on the weight of the individual fruit, citrus produces the largest amount of pomace. Half of the citrus fruit (based on weight) emerges as pomace compared to the 25% (w/w) obtained from apples and grapes (Negro, 2016, Shalini, 2010 and Dwyer, 2014). However, most of the pomace produced in the Western Cape derives from crushing grapes. This can be attributed to our flourishing wine industry, which as a by-product from the wine making process, produces up to 327 kt of grape pomace each year (Department of Agriculture, Forestry and Fisheries, 2016b).

Pomace is generally considered a low value product and carries a negative charge when landfilled. However, due to its sugar content (both free and structural), pomace can be used as a feedstock for ethanol production. Literature has demonstrated ethanol production from citrus, apple and grape pomace. Ethanol yields in excess of 0.43 g/g has been obtained by fermenting citrus pomace. However, citrus pomace contains limonene, an antimicrobial compound, the presence of which, would otherwise inhibits fermentation if not sufficiently removed from pomace using a suitable pre-treatment technology (Pourbafrani et al., 2010). Apple pomace, on the other hand, need not to be pre-treated, ethanol concentrations as high as 0.54 g/g has been obtained by only sterilising the feedstock before subjecting it to simultaneous saccharification and fermentation (Magyar M et al., 2016). The full potential of Grape pomace, despite being the obvious choice, due to it high fermentable sugar content, has not been fully recognised. This is a rather odd finding since grape pomace, especially white grape pomace, contains fermentable sugars that do not need to be pre-treated in order to be enzymatically digested.

A main research objective was therefore to derive the theoretically ethanol yield that could potentially be obtained from fermenting the pomace that is produce in the Western Cape. Secondly, to converse and acquire from industry their preferred method of disposing or reusing pomace and lastly, whether they have considered producing ethanol from pomace.

9.3 CITRUS POAMCE

Citrus fruits are frequently processed for their juice content. Peels, seeds and pulp are the by-products of this process. Approximately half of the fruit, based on weight basis, end up as being the waste. Fortunately not all citrus fruits are destined for juice making. In accordance with the Department of Agriculture, Forestry and Fisheries (2015) only about 26% of the produce in the Western Cape are processed for this purpose. The balance is predominantly exported (i.e. 57% based on total mass produced) and less than 10% (total mass produced) are consumed locally. The Western Cape produced on average approximately 51 kt citrus waste each year (Fig. 9-1). The waste contains between 70% and 80% moisture. The remaining solid is rich in carbohydrates as shown in Table 9-1.

Table 9-1: Chemical Composition of Citrus Waste

Constituents	Amount (g/100 g)
Cellulose	22.5-37.1%
Hemicellulose	5.6-11.0%
Protein	7.0-12.5%
Lignin	7.5-11.6%
Fat	0.5-4.0%
Pectin	8.5-23.0%
Free Sugars	8.1-10.1%

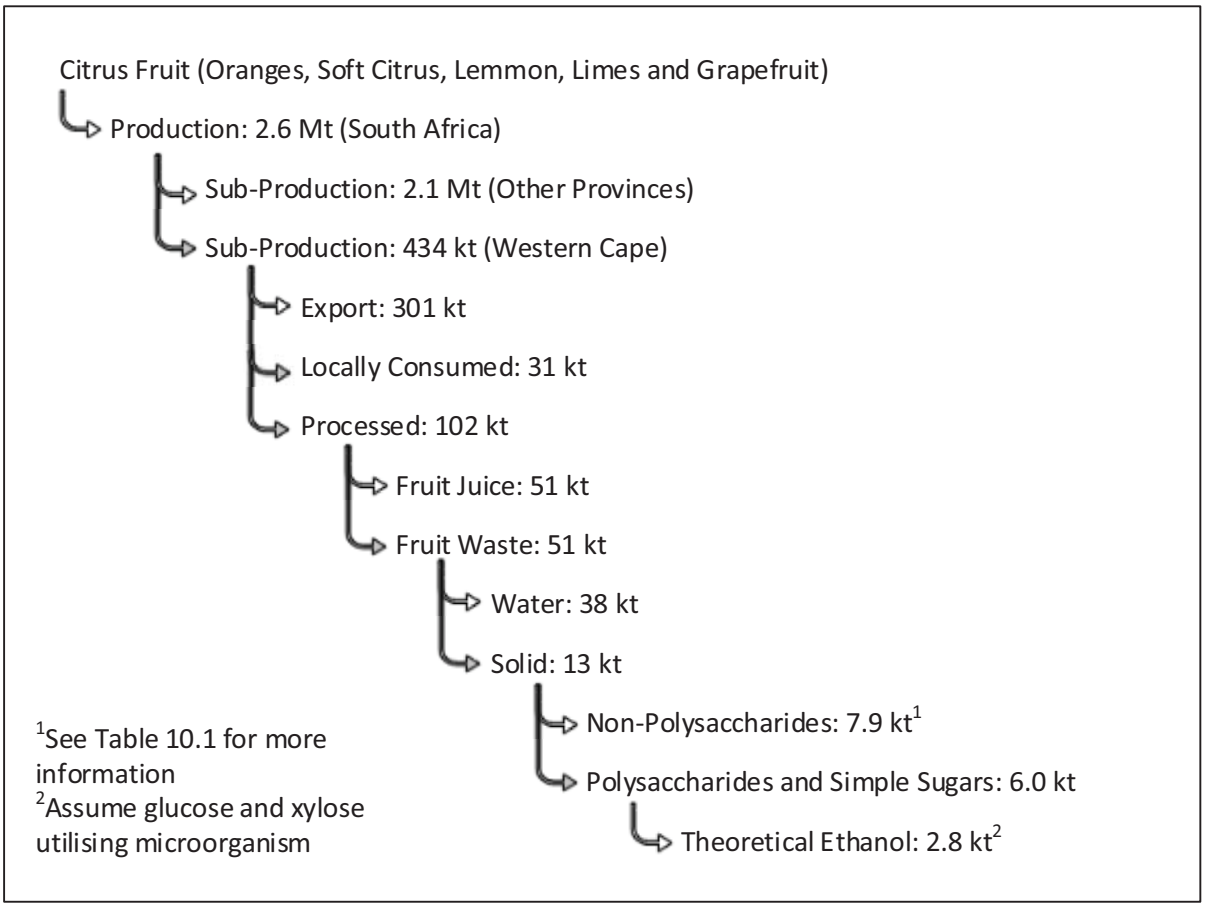


Figure 9-1: Theoretical ethanol yield from citrus pomace

A total of 2.8 kt of ethanol can theoretically be produced by fermenting the pomace annually derived from citrus in the Western Cape. This value assumes complete conversion of both free and structural sugars. Furthermore, a conversion factor of 0.51 g ethanol per g sugar was assumed. Ethanol production has been demonstrated from citrus waste but has been inhibited to some extent by a compound known as limonene. Limonene is founded in the peels of citrus and possess anti-microbial activity. Notwithstanding, ethanol yields in excess of 0.43 g/g has been obtained from citrus waste provided the limonene is flashed-off and the residual solid is subjected to pre-treatment in order to produce a more enzymatically accessible cellulose component (Pourbafrani et al., 2010).

9.4 APPLE WASTE

More than seventy percent of the apples produced in South Africa is cultivated in the Western Cape Province. In accordance with the Agriculture, Forestry & Fisheries report of 2016a, the Western Cape Province produce 568 kt apples between 2014 and 2015. Most of these apples were exported (46%), some were locally consumed (21%) and others (0.4%) were hydrated for a niche market. Approximately, 184 kt (32% of the produce) apples are locally processed into value added products such as juice. The juice only accounts for

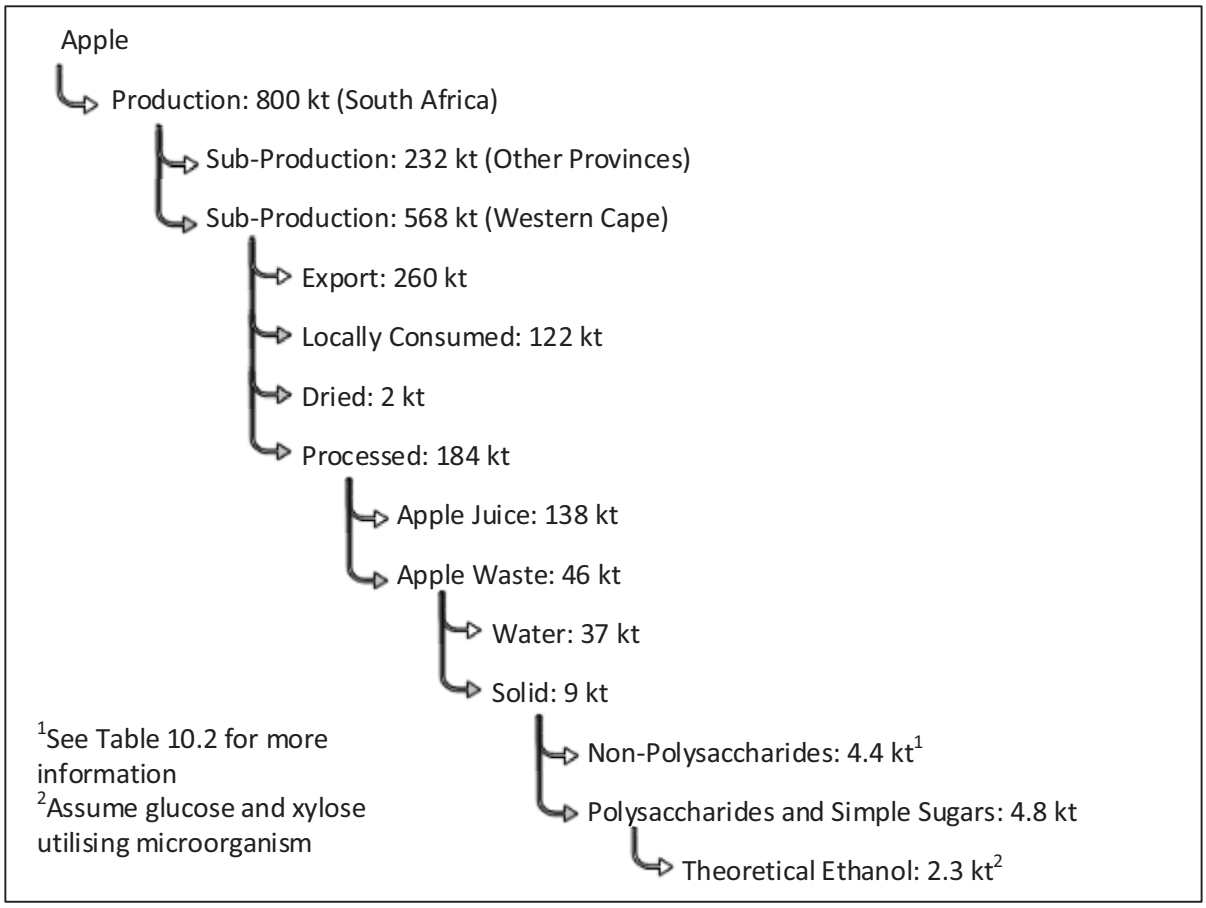


Figure 9-2: Theoretical ethanol yield from apple pomace

a portion of the apple. Twenty five percent of the apple remains as waste product known as pomace. The pomace is highly moist with a water content of 80% (w/w). However, it also contain polysaccharides and monosaccharides (Table 9-2) that can be converted into ethanol (Fig. 9-2).

Table 9-2: Chemical Composition of Apple Waste

Constituents	Amount (g/100 g)
Cellulose	21.0%
Hemicellulose	11.1%
Galactan	3.0%
Galacturonic acid	14.4%
Free Sugars	20.2%
Acid Insoluble Lignin	24.7%
Ash	2.2%

Apple pomace contains large amounts of fermentable sugar that can be converted into ethanol. An ethanol concentration of 53.8 g/L has been obtained by fermenting an apple pomace slurry with a 30% (w/w) solid content. In addition to containing fermentable sugars, apple pomace also comprise structural sugars which are enzymatically accessible and hence readily fermentable. Magyar M et al. (2016) found the enzymatic digestibility of treated and untreated apple pomace to be statistically the same. The study employed in their pre-treatment step both acid and base catalysis and concluded that, despite the high lignin content (24.7%),

which are generally associated with less enzymatically digestible solids, pre-treatment for apple pomace cannot be justify, since it does not influence the enzymatic digestibility of the resulting product.

9.5 GRAPE POMACE TO ETHANOL

Grape pomace is produced as a by-product in the wine making industry. South Africa crushes about 1.4 Mt of grapes annually. Most of the crushing takes place in the Western Cape where the grapes are also produced. About twenty to thirty percent of the grapes, as determined on a weight basis, will end up as pomace (Department of Agriculture, Forestry and Fisheries, 2016b). Grape pomace is best described as a wet solid with a moisture content of 60% to 70% (w/w) (Corbin KR et al., 2015). The chemical composition of the pomace depends on the type of grape cultivar used (Table 10-3). Pomace derived from white grapes generally contains more fermentable sugars compared to that of red grapes. The difference is not cultivar specific but rather technology orientated (Corbin KR et al., 2015). White grapes are crushed pressed before fermentation. Red grapes, alternatively, are crushed, fermented and then pressed. Red grape pomace spend more than with the grape juice in order to produce that authentic red colour of red wine. Two separate theoretical ethanol yields have therefore been determine for white and red grapes, respectively (Fig. 10-3).

Table 9-3: Chemical Composition of Grape Waste

Constituents	Amount (g/100 g)	
	White Grape	Red Grape
Cellulose	1.2	3.3
Hemicellulose	4.3	8.7
Free Sugars	37.6	4.6
Protein	7.2	10.8
Acid Insoluble Lignin	10.5	32.5
Ash	1.2	3.0

The amount of grape pomace that is produced in the Western Cape on an annual basis can theoretically be converted into 19.6 thousand tons of ethanol. Grape pomace and especially white grape pomace contains significant amounts of fermentable sugar which can be converted into bioethanol. Korkie et al. (2002) biologically converted these fermentable sugars into ethanol but failed to reach an ethanol concentration of 20 g/L. In an attempt to increase the ethanol yield, cellulase and pectinase were employed to release structural sugars but it had little effect on the overall ethanol yield. In another study by Corbin et al. (2015), grape pomace was subjected to pretreatment in order to make the resulting substrate more susceptible to enzymatic attack. Dilute acid pretreatment of red grape pomace produced a substrate that was enzymatically more digestible. Twenty five instead of sixteen percent cellulose could be liberated through enzymatic hydrolysis by subjecting red grape pomace to pre-treatment prior to enzymatic hydrolysis. Unfortunately, Corbin *et al*. (2015) did not preformed any fermentation experiments. Thus, no conclusion on the potential ethanol yield could be made.

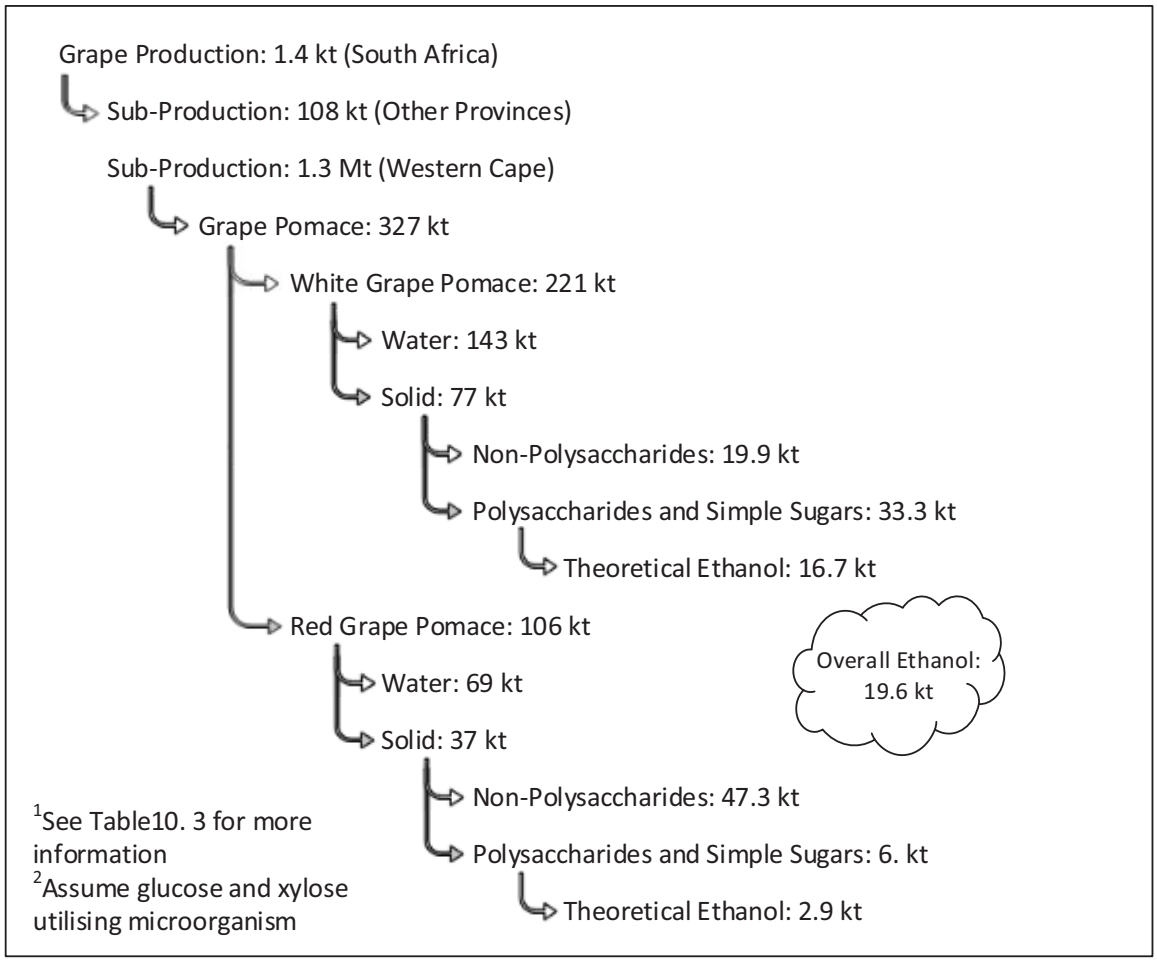


Figure 9-3: Theoretical ethanol produced form grape pomace

9.6 THEORETICAL ETHANOL POTENTIAL IN THE WESTERN CAPE

A total of 24.7 thousand tons of ethanol can theoretical be derived by fermenting citrus, apple and grape pomace produced in the Western Cape. Although this may sound like whole lot of ethanol, the value is dwarfed by the amount of petrol sold in the Western Cape alone. In actual fact, we would only be able to replace 1% (v/v) of the petrol. However, this should not discourage the fruit industry from utilising pomace as a feedstock for ethanol production. Ethanol has a rather high heating value which make it a valuable energy source to be used locally. The grape industry, and more specifically, the white grape industry may benefit most by converting their pomace into ethanol. In addition to producing large volume pomace, white grape pomace also contain free sugars that can be readily fermented into ethanol. One may also benefit by avoiding landfilling cost associated with discarding pomace.

9.7 MARKET SURVEY

Fruit industries, located within Western Cape Province, where consulted in terms of their preferred means for managing their fruit waste. Six companies including three from grape, two from citrus and one from the apple sector were contacted (Table 10.4). The companies were surveyed in terms of their waste production volume, pomace disposal or utilisation methodology, pomace sugar content and whether they have considered

ethanol production from pomace. All of the companies, except for Elgin Fruit Juice (i.e. apple sector), considered pomace as a low value product with limiting applications in composting and animal feed. Elgin Fruit Juice, on the other hand, are constantly trying new and innovating ideas to increase the value of their waste product. Methane is produced and consumed in-house by subjecting their apple pomace to anaerobic digestion. Pomace is also dehydrate in order to make it more suitable as an animal feed. As of late, Elgin Fruit Juice has set their sights on apple sauce production using apple pulp, the main ingredient in their pomace.

Table 9-4: Surveying fruit market regarding pomace production and application

Fruit	Company	Pomace (ton)	Current Application	Comment
Citrus	Mouton Citrus	2 500	Animal feed	Have not considered ethanol production but electricity generation
Citrus	ALG Estates	4 000-6 000	Animal feed	Have considered electricity production, Price: R 220/ton
Grape	Spier	1 000	Composting	Between 18-22 Brix in pomace
Grape	De Toren	50-70 m^3	Composting	Approximately 0.5 Brix in pomace
Grape	Blaauwklippen Vineyards	75	Landfilling	Between 22-23 Brix in pomace
Apple	Elgin Fruit Juice	10 600	Animal feed and anaerobic digestion	90 Rand/ton (wet), plans for apple source from pulp, between 2 - 4 Brix in pomace

The fruit industry acknowledge the presence of sugar in pomace but they displayed little interest in measuring or documenting the quantity or quality of these monosaccharides. Sugar and more specifically sucrose remains in pomace mainly due to the ineffectiveness of the juicing process. The fruit industry do not consider these sugars a loss, nor are they devising methods for their recovery. It should therefore come as no surprise that industry have not considered making ethanol from these sugars. Alternatively, industry is rather fixated on utilising pomace for electricity generation. However, due to economy of scale and the relative low cost of coal-based electricity, electricity generation is yet to become a reality. Producing ethanol from pomace may yet present an alternative way of producing energy and a means of utilising the “lost sugars” in a more effective manner. However, seeing that the industry that manufactures animal feed are willing to pay up to R200 per ton, pomace cannot be considered a valueless by-product and would need to be obtained at a cost when considered as feedstock for ethanol production.

9.8 CONCLUSION

Fruit pomace is predominantly considered a waste product and is landfill at a cost. Utilizing fruit pomace as a feedstock for bio-ethanol product may provide the fruit industry with a means to substitute their energy bill or an addition revenue stream. With the petrol price steadily increasing, ethanol may yet become a lucrative commodity with application in the food, chemical cosmetic and pharmaceutical industry.

CHAPTER 10: ECONOMIC ANALYSIS

The economic viability of three paper waste sludge technologies were evaluated. This included I) ethanol production through fermentation, II) methane production through anaerobic digestion and III) the sequential treatment of paper waste sludge using fermentation followed by anaerobic digestion in order to produce both ethanol and methane. Substrates VP-PS, CR-PS and TPR-PS served as feedstocks to these technologies. The processes described below were simulated in Aspen Plus® (Aspen Tech, 2015) to model the mass and energy balance and thereafter, economic analysis was carried out based on the mass and energy balance.

10.1 PROCESS DESCRIPTIONS

10.1.1 Ethanol from Paper Waste

The paper waste to ethanol process was designed as an add-on to an existing paper plant mill (Figure 10.1 A). Paper waste is charged into a sterilisation unit where direct steam is employed to sterilise the paper load. The sterilisation unit was modelled in ASPEN as a four-stage distillation column since they represent each other both in CAPEX and utility requirements. The feedstock following sterilisation is cooled using a heat exchanger. The feedstock enters the fermentation unit at room temperature. Please note that depending on the moisture content of the feedstock (i.e. sterilised paper waste), additional water may be added in order to maintain the solids to liquid ration in the fermenter at 10% (w/w). Other nutrients such as corn steep liquid and magnesium sulphate are filter sterilised before being added to the fermenter. The fermenter is modelled as a continuous stirred-tank reactor. Thus, the mass-flow rate of feedstock entering and product existing the fermenter remains the same. Two types of product namely a carbon dioxide/ethanol mixture (i.e. gaseous phase) and an ethanol-water slurry (i.e. liquid phase) exist the fermenter. However, small amounts of carbon dioxide also resides within the ethanol-water slurry as a dissolved compound. Through degasification, using a heated flash drum, carbon dioxide is removed from the ethanol-water slurry. However, due to the non-selectiveness of the degasification process, some ethanol is recovered together with carbon dioxide. A knocked-out drum and stripper column is applied to strip ethanol from carbon dioxide. All ethanol streams are merge before proceeding to a Beer Stripper column.

The ethanol-water slurry enters the Beer Stripper in the upper part of the column. Ethanol is being stripped off the slurry as it continuous down the column. The bottoms contain large amounts of water, unfermented residue such as unreacted cellulose, lignin and ash and trace amounts of ethanol. In contrast, most of the ethanol exist the top part of the Beer Stripper as an ethanol enriched product stream known as beer phlegm. Azeotrope quality ethanol is produced from the beer phlegm using a rectification column. In this step, ethanol is stripped off water to the point where the chemical compositional of liquid and vapour phase remains the same. A molecular sieve produces an almost pure ethanol stream by absorbing the remained of the water that resides within azeotrope ethanol-water mixture.

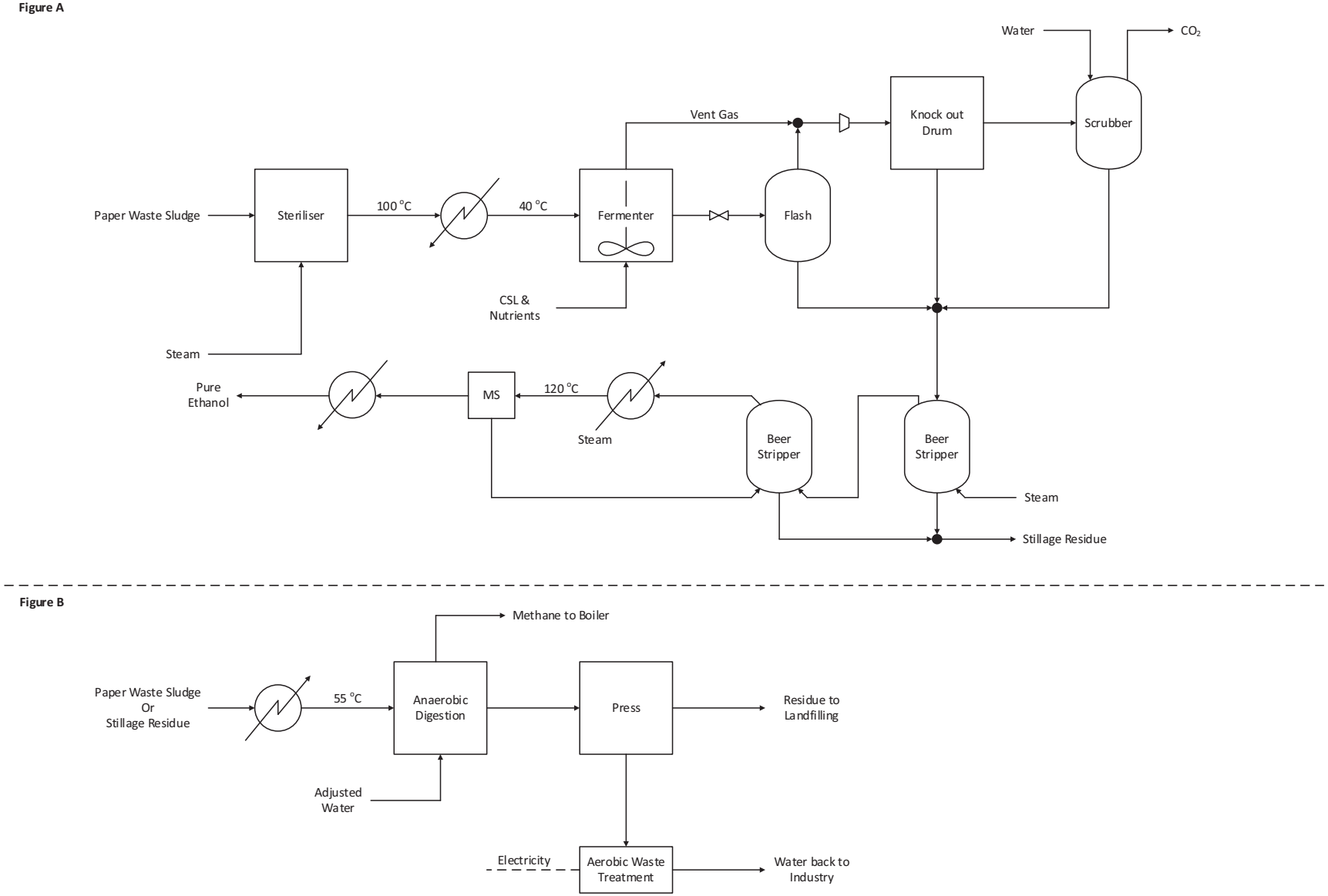


Figure 10-1: Mass and Energy Flow-Diagrams of Paper Waste Sludge Fermentation (A) and Anaerobic Digestion (B)

10.1.2 Methane from Paper Waste

The Anaerobic Digestion Plant operates within the battery limits of the Paper Plant (Figure 10-1 B). Paper waste is heat treated to a temperature of 55°C before entering the anaerobic digester. Water is added to the digester in order to maintain a solid to liquid ration of 5% (w/w). Microorganisms are cultured within the anaerobic digester that converts the volatile solid part of paper waste into biogas. Approximately 60% (v/v) of the biogas comprise methane with the balance carbon dioxide. The gas escapes from the Anaerobic Digester through a vent where it is recovered and used as a fuel source to provide heat such is the case in a boiler. The residue, following anaerobic digestion of paper waste, is dehydrated using a press. The solid component is landfilled and the liquid is send for treatment. In the case of anaerobic digestion, the COD of the resulting liquid is actually lower than the processed water used at the start of the process. Hence, no additional strain is added onto the Wastewater Treatment Plant. However, the scenario changes in the case of fermentation or the sequential process and are discussed in more detail in section 10.1.3.

10.1.3 Biofuels Production: Sequential Fermentation and Anaerobic Digestion

Stillage derived through fermentation of paper waste retains chemical potential that can be harvest through anaerobic digestion. The sequential fermentation of paper waste followed by anaerobic digestion of the stillage presents an improved alternative to the individual processes as additional energy can be harvest which would have otherwise been lost if only one or the other process were used. A similar processes description as defined in section '*Ethanol from Paper Waste*' was employed as the first step in our sequential fermentation and 'Anaerobic Digestion process' (Fig. 10-1 A & B). Also, the second step of the sequential process was based on section '*Methane from Paper Waste*'. However, instead of receiving paper waste as feedstock, the fermentation stillage is subjected to anaerobic digestion.

10.1.3.1 Wastewater Treatment

A major drawback of fermentation (with or without anaerobic digestion) is its negative contribution to COD. The resulting water mixture has such a high COD that it cannot simply be it recycled back into the paper plant but instead needs to be purified by sending it to the plant's existing aerobic digestion to be cleaned. In the bigger scheme of things, this may have little influence on the capacity of the plant to treat the resulting generated wastewater stream from the fermentation or sequential process. Compared to the quantity of clarifier process water generated, stillage from the fermentation or sequential process would constitute about 1% of the amount of clarifier process water generated. For example, about 50 to 150 kilolitres per day of stillage would be produced in an industrial scale simulation of this process as compared to about 3 000 to 15 000 kilolitres per day of process water generated by pulp mills (Personal communication, 2016). Mixing of both streams would produce a stream with COD ranging between 5 000 mg/L to 6 500 mg/L. The resulting mixed stream would have a COD about 5% to 35% higher than clarifier process water, this could be handled by wastewater treatment systems in the pulp and paper industry.

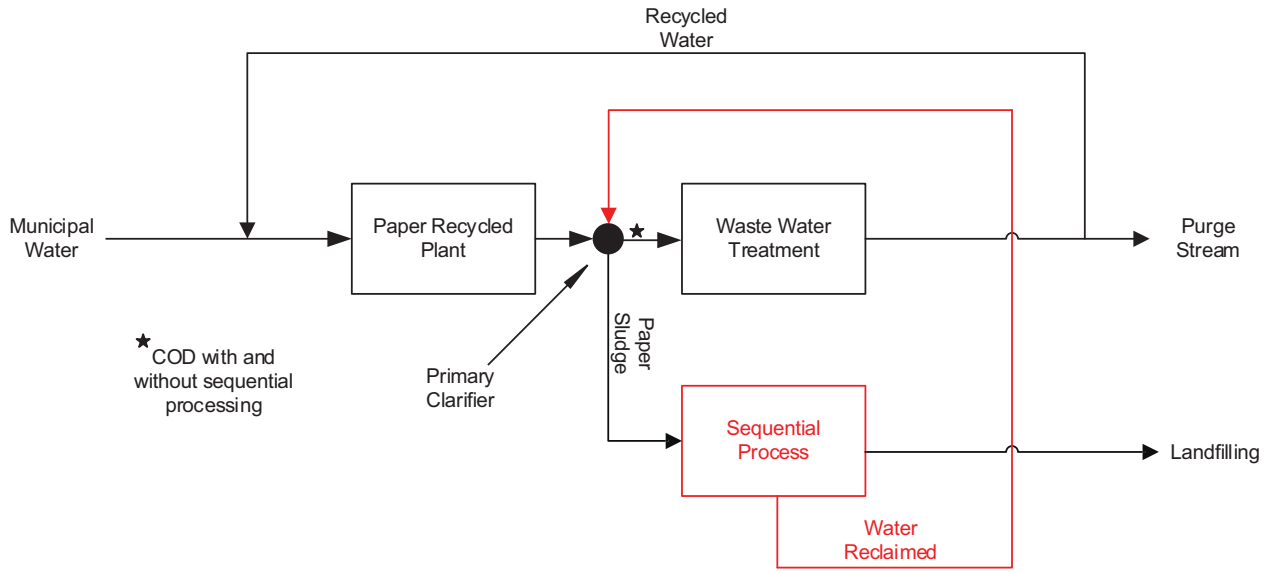


Figure 10-2: Wastewater treatment with and without treatment of paper sludge

Process economic analysis were carried out using simulated data from the mass and energy balance in Aspen Plus®. Data imported from the simulation was used to size processing units, and cost them using literature-based correlations that are dependent on a characteristic mass, energy flow or size characteristic (Petersen et al., 2017, Humbird et al., 2011). Further capital costs for installation, piping, controls, electrical, site development, contingencies, engineering and contracting fees were then accounted for as factors of equipment costs (Humbird et al., 2011). The simulated process inputs (water requirements, chemicals, enzymes, yeasts and energy utilities) as well as outputs (ethanol and biogas production, waste generation) were used to derive operating expenditures and incomes with applicable unit costs given by industry or found in literature. With capital (CAPEX) and operational expenses (OPEX), discount cash flow statements were constructed for determining the minimum ethanol price or minimum biogas selling price for the project to break even. The parameters on which the economic analysis was based are:

- Economic life of 30 years.
- Cost of Capital or Target IRR of 12%
- Company tax of 28%
- Straight line depreciation, with a salvage value of 0%.
- Real analysis

10.2 RESULTS AND DISCUSSION

10.2.1 Anaerobic Digestion

The results obtained for anaerobic digestion is presented in **Table 10-1**. The Virgin PS substrate had a high biogas production rate of 158 m^3/tonne(f), due to liquid COD reduction as well as a substantial digestion of carbohydrate in the solid species of 42%. Thus, the biogas selling price was 1.41 ZAR/m^3, which is about

30% lower than the natural gas cost on an energy equivalence. The reduction of cost due to feedstock diversion is quite significant, as it represents about 33% of the total income.

Table 10-1: Techno-economics of biodigestion of substrates. (VP – Virgin PS; CR – Corrugated Recycle PS; TPR – Tissue Printed Recycled PS)

Process Description	VP	CR	TPR
Biogas Yield (m^3/tonne(f))	110.69	78.61	76.13
Total Capital (million ZAR)	23.39	9.14	38.91
Feedstock Diversion (ZAR/m^3 – biogas)	0.69	0.98	0.40
Biogas Price (ZAR/m^3)	1.41	3.27	2.56

*(tonne(f) – values related to feed rate.

The CR-PS substrate had a low biogas yield of about 78 m^3/tonne(f), and is correspondingly obtained at biogas price of 3.27 ZAR/m^3. This is about 64% higher than the natural gas price on an energy equivalence. The high biogas price is primarily due to the small operating scale that is offered by the availability of this substrate, which is 27% in relation to the VP substrate.

The biogas price determined for the TPR-PS substrate is 2.56 ZAR/m^3 and is about 23% higher than the cost of natural gas on an equivalent energy basis. Even though the solids digestion was quite high at about 76%, the ash accounts for about 63% of the feed, leaving the carbohydrates at only 26%. Thus, the overall yield of biogas was only 76 m^3/tonne.

10.2.2 Fermentation and Sequential Digestion

The results for the fermentation scenarios, as well as those of fermentation with sequential digestion is presented in **Table 10-2** and **Figure 10.3** for all substrates. In **Figure 10-3**, overall capital estimate is given, in addition to the primary results of the mass and energy balance is presented, namely ethanol yields; energy demands and biogas yields. All are referenced to the dry feed rate of PS for each respective substrate. Following **Table 10-2** is **Figure 10-3**, where the minimum ethanol selling prices (MESPs) are presented, along with the breakdown in production costs.

Table 10-2: Primary results of mass and energy balance and capital estimate (VP –Virgin PS; CR – Corrugated Recycled PS; TPR – Tissue Printed Recycled PS; F – Fermentation only; F+D – Fermentation with sequential biodigestion)

Process Description	VP		CR		TPR	
	F[a]	F+D[b]	F	F+D	F	F+D
Ethanol Yield (m^3/tonne(f))	0.34	0.34	0.22	0.22	0.13	0.13
Electricity Demand(MWhr/tonne(f))	0.14	0.14	0.16	0.16	0.08	0.07
Steam demand (tonne/tonne(f))	2.46	2.46	2.51	2.51	1.11	1.11
Biogas yield (m^3/tonne(f))	N/A	0.10	N/A	0.11	N/A	0.18
Total Investment Capital (million ZAR)	**91**	**96**	**37**	**40**	**117**	**122**

[a] F – Fermentation Only, [b] F+D – Fermentation and Sequential Digestion,*(tonne(f)) – values related to feed rate.

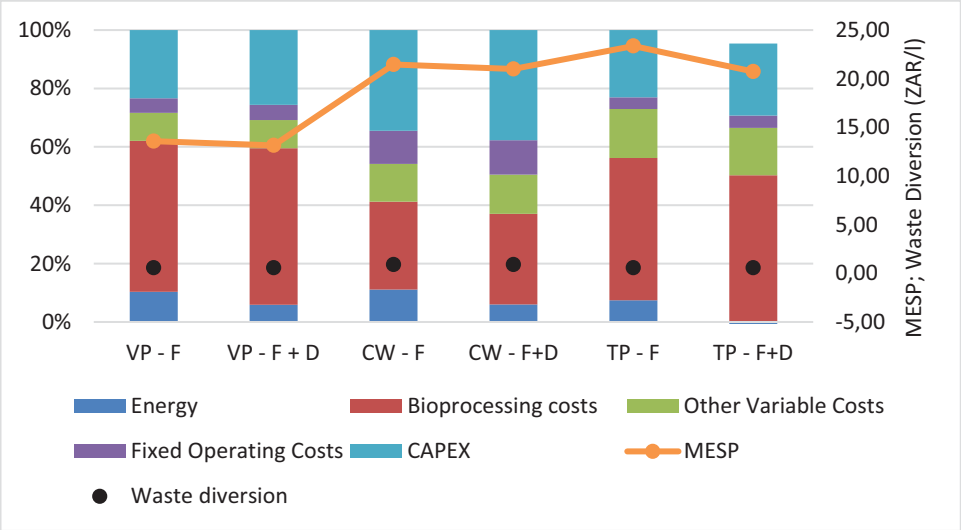


Figure 10-3: MESP and breakdown of production costs

10.2.3 Virgin PS

The ethanol yield was 0.34 m^3/tonne(f), and is obtained at a selling price of 13.6 ZAR/l, which is about 5% lower than the price 15 ZAR/l, at which ethanol is exported from South Africa (personal communication). The primary reason for the selling price is the high enzyme dosage of 20 FPU/g, which contributes a specific cost of 5.2 R per litre of ethanol. Thus, the bioprocessing costs, which encompasses enzymes, yeasts and nutrients, accounts for 52% of the production costs. If 50% of the required enzyme is provided by a CBP yeast, the MESP will be decreased to 11.52 ZAR/l.

The electricity demand of 0.14 MW/tonne(f) had resulted primarily from cooling duties of reflux requirements in distillation and substrate cooling after sterilisation; while the steam demand of 2.41 tonne/tonne(f) resulted primarily from direct steam use in distillation and substrate sterilisation. The overall contribution of the energy utilities to the overall production cost is 10%. Regarding the CAPEX of 91 million rand, it accounted for 23% of the total production cost.

When anaerobic digestion follows fermentation, the MESP reduces by 3%, since the biogas is sold as a value added energy product. The electricity demand remained similar since the digestion only reduced the COD of the effluent after digestion by 17%, and thus the electricity required for aeration in the aerobic water treatment remained similar. Thus, the increased capital cost of 4% for sequential biodigestion was recovered, since the contribution of total energy utilities, which is credited by the biogas production, is reduced to 4%.

10.2.4 Corrugated Wood PS

The MESPs was calculated at 21.5 ZAR/l, since the yield of ethanol was only 0.17 m^3/tonne(f). The MESP was about 1.43 times the export price of ethanol from South Africa. Though enzyme dosages for this substrates was 11 FPU/g which resulted in a contribution of only 32% from bioprocessing costs, the low ethanol yield increases the specific costs. So even if 50% of the required enzyme is provided by a CBP yeast, the MESP will only be decreased to 19.7 ZAR/l, which is about 1.3 times the export price of ethanol from South Africa. If the scale of the ethanol plant is tripled, by including a similar PS streams generated by nearby paper mills, then the selling price reduces to 17.7 ZAR/l, which is then only 20% higher than the export price of 15 ZAR/l.

The energy contribution to production costs was 12%, which resulted from an electricity demand of 0.16 MWhr/tonne(f) and a steam demand of 2.51 tonne/tonne(f). The total CAPEX of 37 million accounted for 36% of the total production cost. With anaerobic digestion after fermentation, the MESP decreased by 2.5%, given that the biogas is sold as a value added energy product due to a reduction of the COD of the effluent by 31%. Thus, the increase in capital cost of 4% for sequential biodigestion was recovered due to the biogas production.

10.2.5 Tissue Paper PS

The yield of ethanol is only at 0.11 m^3/tonne(f), due to the high ash contents of 63% (Table 6-1), and thus, it is expected that the capital payback per litre of ethanol will be high. Hence, the MESP for this substrate was calculated at 23.4 ZAR/l, which is about 1.6 times the basic fuel price, on an energy equivalence. The energy contribution to production costs for this substrate however, was only 7% since fermentation took place at a high substrate loading of 33%. Thus, the electricity demand was only 0.07 MWhr/tonne(f) while the steam demand was only 1.11 tonne/tonne(f) due to low processing volumes. The total CAPEX of 117 million ZAR accounted for 23% of the total production cost, since most of the production costs were caused by the bioprocessing costs (i.e. 49%, since the enzyme dosage was 15 FPU/g).

With anaerobic digestion after fermentation, the MESP had a drastic decreased of 11% since its biogas yield was 0.18 m^3/tonne(f). This is explained by the high percentage solids of the fermentation needing an excess of process water to satisfy the solids loading of 10% of the biodigestion reactor, and subsequently, the COD loading from the process water provides for more biogas production. Thus, the increase in capital cost of 4% for sequential biodigestion was recovered due to the additional biogas production.

10.3 CONCLUSIONS FROM ECONOMIC ASSESSMENT

The Virgin PS produced biogas at a selling price was 1.41 ZAR/m^3, which was about 30% lower than the natural gas cost on an energy equivalence, due to the biogas production rate of 158 m^3/tonne(f), The CR-PS substrate produced biogas at a selling price that was 64% higher than the natural gas cost on an energy equivalence due to a small processing scale. The selling price of biogas from the TP substrate was 23% higher than the natural gas price on an energy equivalence, due to the high ash content of the feed. In general, the avoided disposal costs of the feedstock had significantly reduced the selling price of biogas, by a range of 13-33%.

Ethanol production from Virgin PS is economically viable at the given enzyme price and experimental performance, due to the large scale and yield (0.34 m^3/tonne(f)). The MESP of 13.6 R/l is 5% lower than the export ethanol price of 15 ZAR/l and could even reduce to as low as 11 ZAR/l, if half the enzyme dosage is provided by CBP yeasts. Ethanol production from the CW is not economically viable, due to the low production scale. If the scale increases by 3 fold, economic viability is approached as the MESP of ethanol would then be about 17.7 ZAR/l, which is only 20% greater than the ethanol export price. If TP is used as an ethanol substrate, ethanol production is unviable, due to the high ash content, which reduces the ethanol yields, and consequently causes a MESP of 23.4 ZAR/l.

When fermentation is sequentially followed by digestion, the MESP had decreased for the Virgin PS by 3% and the MESP for the CR-PS was reduced by 2.5%. Thus, the additional capital costs required for digestion was recovered in either case. For the TPR-PS substrate, the MESP was reduced by 11%, as the high solids loading in fermentation required more dilution with plant process water for digestion, supplementing the dissolved digestible substrates.

CHAPTER 11: CONCLUSIONS & RECOMMENDATIONS

11.1 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, together with bio-energy production. This was achieved by performing sequential bioprocessing and standalone fermentation and anaerobic digestion on paper sludge obtained from three different South Africa pulp mills.

11.1.1 Influence of recycled process water on fermentation of paper sludge

Recycled process water 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. However, the ethanol production in recycled process water fermentative batch cultures was similar to clean water control cultures at identical cellulase dosage. This allowed for the exclusive usage of process water in fermentation of paper sludge at various reactor levels.

11.1.2 Influence of recycled process water on anaerobic digestion of paper sludge

Anaerobic digestion of paper sludge was either adversely or favourably affected by the type of recycled process water utilized. 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. 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. 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.

11.1.3 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. The better water reclamation experienced in fermentation could be attributed to the action of cellulase on the lignocellulose structure. In contrast to anaerobic digestion, cellulase employed in the fermentation process appeared to be able to 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.

11.1.4 Quality of process water after sequential bioprocessing and individual fermentation and anaerobic digestion of paper sludge.

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. In contrast to fermentation, anaerobic digestion decreased the COD of subsequent process water by about 20% to 40%. 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.

11.1.5 Biofuel and bioenergy from sequential bioprocessing and standalone fermentation and anaerobic digestion of paper sludge with recycled process water.

11.1.5.1 Ethanol production from paper sludge with recycled process water in bench and pilot scale fermenters

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. Virgin pulp produced the highest ethanol concentration of 49.6 g/L in 5 L bioreactors as a result of its superior glucan content. 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. 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. Also, bacterial contamination and lactic acid production were observed with fermentation of CR-PS, both in 5 L and 150 L fermenters. This possibly led to inhibition of *S. cerevisiae* and contributed to the lower ethanol yield attained for CR-PS. Pilot scale fermentation of TPR PS produced an ethanol concentration 10% to 15% higher than bench scale. Thus, proof of concept was illustrated with TRP-PS as similar ethanol concentration were obtained using both the 5 L and 150 L fermenter. We are of the opinion that VP-PS and CR-PS can also be successfully up scaled provided some modification are made to the fermenter such as replacing the Rushton impeller with a marine impeller.

11.1.5.2 Biogas and methane production from paper sludge and fermentation stillage in 30 L digesters

Corrugated recycle stillage produced the highest biogas yield of 184.4 $\pm$ 2.3 L/kg TSf_{ed}, this was about 40% to 60% higher than biogas yields obtained from virgin pulp and tissue printed recycle stillages. 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.

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 CH4/kg $TSf_{ed.}$). 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 favourable effect of virgin pulp process water also contributed to the high methane yield obtain from VP-PS.

11.1.5.3 Bioenergy production from sequential bioprocess and standalone fermentation and anaerobic digestion of paper sludge

Fermentation of paper sludge gave 35% to 55% more bioenergy as compared to standalone anaerobic digestion of paper sludge. Additionally, sequential bioprocessing of paper sludge also produced about 20% to 60% more energy than individual fermentation of paper sludge. 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. 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.

11.1.6 Potential industrial and/or agricultural use of solid residues after sequential bioprocessing of paper sludge

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, aluminium, 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.

11.1.7 Investigating the ethanol production potential of fruit pomace in the Western Cape

Fruit pomace has the potential to serve as feedstock for the production of ethanol. Large amount of sugar and structure sugar are contained in pomace can be fermented with or without the use of enzymatic hydrolysis. To date industry have focused on using pomace specifically for animal feed or composting. However, lower nutritional value has motivated industry to seek other alternatives for pomace. Electrical generation has been proposed as an alternative option. However, economy of scale and the low ethanol selling price has hampered

progress in this area. In this study white grape pomace was identified as the most promising feedstock for ethanol production. Due to its large wine industry, the Western Cape Province produces up to 1105 thousand tons white grapes which are ultimately reduced to 221 thousand tons of pomace. These pomace contains large amount of fermentable sugars which can be readily converted in to 16.7 thousand tons of ethanol per annum. Take in consideration the pomace also derived from citrus and apple, a total 24.7 thousand tons per annum of ethanol can theoretically be produced in the Western Cape.

11.1.8 The economic analysis of producing bio-fuels from paper waste sludge using fermentation, anaerobic digestion and the combination of these two process

Only a plant based on the Virgin PS stream was economically feasible since the scale of production and yield were high enough. Ethanol could be sold at a price of 13.6 ZAR/l for the project to break even, which is below the competitive value of 15 R/l. Similarly for biogas, only the Virgin PS stream produced biogas at a feasible price of 1.41 ZAR, which was 30% lower than the price of natural gas price on an energy equivalent basis. Where fermentation is sequentially followed by biogas production, the overall profitability improved, despite increased capital costs. Biogas and ethanol plants based on the CR-PS and TPR-PS streams, would not be feasible since the flow rate of the CR-PS stream is too low, while on the other hand, the ash contents of the TPR-PS stream is too high.

11.2 RECOMMENDATIONS

11.2.1 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). Thus, axial flow impellers such as marine impellers are recommended for future experiments.

11.2.2 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 fibre.

11.2.3 Consolidated bioprocessing (CBP) of paper sludge with recycled process water

Consolidated bioprocessing of paper sludge with recycled process water would merge important biological processes such as cellulase production, polysaccharide hydrolysis and hexose and pentose sugars fermentation into a single reactor process (Vertes et al., 2010). The combination of the biological steps could be achieved by a single microorganism or a consortium of microorganisms that would be capable of fermenting paper sludge without the addition of enzymes. Such a process would eliminate the need to separately produce enzymes and could also significantly improve process economics since the cost enzymes was determined to be a major factor affecting economics associated with industrial scale application of paper sludge fermentation (Robus et al., 2016; Williams, 2017).

REFERENCES

ADEN A and FOUST T (2009) Technoeconomic analysis of the dilute sulfuric acid and enzymatic hydrolysis process for the conversion of corn stover to ethanol. Cellulose **16 (4)** 535-545.

AHRING BK, ALATRISTE-MONDRAGON F, WESTERMANN P and MAH R (1991) Effects of cations on Methanosarcina thermophila TM-1 growing on moderate concentrations of acetate: production of single cells. Applied Microbiology and Biotechnology **35 (5)** 686-689.

AHRING BK (2003) Perspectives for Anaerobic Digestion. Advanced Biochemical Engineering Biotechnology **81** 1-30.

ALI M and SREEKRISHNAN TR (2001) Aquatic toxicity from pulp and paper mill effluents: A review. Advances in Environmental Research **5 (2)** 175-196.

ANDO S, ARAI I, KIYOTO K and HANAI S (1986) Identification of aromatic monomers in steam-exploded poplar and their influences on ethanol fermentation by Saccharomyces cerevisiae. Journal of Fermentation Technology **64 (6)** 567-570.

ANGENENT LT, KARIM K, AL-DAHHAN MH, WRENN BA and DOMÍGUEZ-ESPINOSA R (2004) Production of bioenergy and biochemicals from industrial and agricultural wastewater. Trends in Biotechnology **22 (9)** 477-485.

ANNE-MARIE B (2015) *Optimisation and Scale-up of Biogas Production from Paper Sludge*. Department of Process Engineering, Stellenbosch University.

DE BAERE LA, DEVOCHT M, VAN ASSCHE P and VERSTRAETE W (1984) Influence of high NaCl and NH4Cl salt levels on methanogenic associations. Water Research **18 (5)** 543-548.

BAJPAI P (2015) Management of pulp and paper mill waste. Management of Pulp and Paper Mill Waste 1-197.

BALDWIN GN (1951) Basic Effects of Sulfur Dioxide on Yeast Growth. American Journal of Enology and Viticulture **2 (1)** 43-53.

BALLESTEROS M, OLIVA JM, MANZANARES P, NEGRO MJ and BALLESTEROS I (2002) Ethanol production from paper material using a simultaneous saccharification and fermentation system in a fed-batch basis. World Journal of Microbiology & Biotechnology **18 (6)** 559-561.

BAYR S and RINTALA J (2012) Thermophilic anaerobic digestion of pulp and paper mill primary sludge and co-digestion of primary and secondary sludge. Water Research **46 (15)** 4713-4720.

BENJAMIN MM, WOODS SL and FERGUSON JF (1984) Anaerobic toxicity and biodegradability of pulp mill waste constituents. Water Research **18 (5)** 601-607.

BLACKWELL BR, MACKAY WB, MURRAY FE and OLDHAM WK (1979) Review of kraft foul condensates. Sources, quantities, chemical composition and environmental effects. TAPPI Journal 33-7.

BLUM DJW and SPEECE RE (1991) A database environmental interspecies of chemical bacteria to toxicity in and its use and correlations comparisons. Water Environment Federation **63 (3)** 198-207.

BOSHOFF S (2015) *Characterization and fermentation of waste paper sludge for bioethanol production*. Department of Process Engineering, Stellenbosch University.

BOSHOFF S, GOTTUMUKKALA LD, VAN RENSBURG E and GÖRGENS J (2016) Paper sludge (PS) to bioethanol: Evaluation of virgin and recycle mill sludge for low enzyme, high-solids fermentation. Bioresource Technology **203** 103-111.

CABIROL N, BARRAGÁN EJ, DURÁN A and NOYOLA A (2003) Effect of aluminum and sulphate on anaerobic digestion of sludge from wastewater enhanced primary treatment. Water Sci Technol **48 (February 2003)** 235-240.

CSRSC (2004) *Pulp and Paper Sector Summit Resource Book*, CEPPWAWU, South Africa.

CHEN Y, CHENG JJ and CREAMER KS (2008) Inhibition of anaerobic digestion process: A review. Bioresource Technology **99 (10)** 4044-4064.

CLARK TA and MACKIE KL (1984) Fermentation inhibitors in wood hydrolysates derived from the softwood Pinus radiata. Journal of Chemical Technology and Biotechnology **34 (2)**101-110.

DALWAI I (2012) *A comparison of technical and environmental merits of producing bioethanol and biomethane from waste paper sludge*. Department of Chemical Engineering, University of Cape Town.

DEMIREL B and SCHERER P (2008) The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: A review. Reviews in Environmental Science and Biotechnology **7 (2)** 173-190.

ENVIRONMENT CANADA & HEALTH CANADA (1991) *Effluents from Pulp Mills Using Bleaching Effluents from Pulp Mills Using Bleaching.* Priority Substances List Assessment Report No. 2, Environment Canada, Ottawa (Ontario), Canada.

FAN Z, SOUTH C, LYFORD K, MUNSIE J, WALSUM PV and LYND LR (2003) Conversion of paper sludge to ethanol in a semicontinuous solids-fed reactor. Bioprocess and Biosystems Engineering **26 (2)** 93-101.

FAN Z and LYND LR (2007) Conversion of paper sludge to ethanol. I: Impact of feeding frequency and mixing energy characterization. Bioprocess and Biosystems Engineering **30 (1)** 27-34.

FERGUSON JF (1994) Anaerobic and Aerobic Treatment for AOX Removal. Water Science and Technology **29 (5-6)**149-162.

FIELD JA and LETTINGA G (1987) The methanogenic toxicity and anaerobic degradability of a hydrolyzable tannin. Water Research **21 (3)** 367-374.

FIBRE PROCESSING AND MANUFACTURING SECTOR EDUCATION AND TRAINING AUTHORITY (2014) *A profile of the paper and pulp sub-sector*, South Africa.

GÍRIO FM, FONSECA C, CARVALHEIRO F, DUARTE LC, MARQUES S and BOGEL-ŁUKASIK R (2010) Hemicelluloses for fuel ethanol: A review. Bioresource Technology **101 (13)** 4775-4800.

GOTTUMUKKALA LD, HAIGH K, COLLARD FX, VAN RENSBURG E and GÖRGENS J (2016) Opportunities and prospects of biorefinery-based valorisation of pulp and paper sludge. Bioresource Technology **215** 37-49

HABETS LHA and DE VEGT AL (1991) Anaerobic Treatment of Bleached TMP and CTMP Effluent in the Biopaq UASB System. Water Science and Technology **24 (3-4)** 331-345.

HAGELQVIST A (2013a) Batchwise mesophilic anaerobic co-digestion of secondary sludge from pulp and paper industry and municipal sewage sludge. Waste Management **33 (4)** 820-824.

HAGELQVIST A (2013b) *Sludge from pulp and paper mills for biogas production: Strategies to improve energy performance in wastewater*. Faculty of Health, Science and Technology Environmental, Karlstad University Studies.

HOFFMANN RA, GARCIA ML, VESKIVAR M, KARIM K, AL-DAHHAN MH and ANGENENT LT (2008) Effect of shear on performance and microbial ecology of continuously stirred anaerobic digesters treating animal manure. Biotechnology and Bioengineering **100(1)** 38-48.

HUBBE MA (2007) Water and papermaking 2. White water components. Paper Technology **48 (2)** 31.

HUILIÑIR C, QUINTRIQUEO A, ANTILEO C and MONTALVO S (2014) Methane production from secondary paper and pulp sludge: Effect of natural zeolite and modeling. Chemical Engineering Journal **257** 131-137.

JÖNSSON LJ, PALMQVIST E, NILVEBRANT NO and HAHN-HÄGERDAL B (1998) Detoxification of wood hydrolysates with laccase and peroxidase from the white-rot fungus Trametes versicolor. Applied Microbiology and Biotechnology **49 (6)** 691-697.

KAMALI M, GAMEIRO T, COSTA MEV and CAPELA I (2016) Anaerobic digestion of pulp and paper mill wastes – An overview of the developments and improvement opportunities. Chemical Engineering Journal **298** 162-182.

KANG L, WANG W, PALLAPOLU VR and LEE YY (2011) Enhanced ethanol production from de-ashed paper sludge by simultaneous saccharification and fermentation and simultaneous saccharification and Co-Fermentation. BioResources **6 (4)** 3791-3808.

KANG L, WANG W and LEE YY (2010) Bioconversion of kraft paper mill sludges to ethanol by SSF and SSCF. Applied Biochemistry and Biotechnology **161 (1-8)** 53-66.

KARIM K, KLASSON KT, HOFFMANN R, DRESCHER SR, DEPAOLI DW and AL-DAHHAN MH (2005) Anaerobic digestion of animal waste: Effect of mixing. Bioresource Technology **96 (14)** 1607-1612.

KAYHANIAN M and TCHOBANOGLOUS G (1992) Computation of C/N Ratios for Various Organic Fractions. BioCycle **(May)** 58-60.

KELLEHER BP, LEAHY JJ, HENIHAN AM, O'DWYER TF, SUTTON D and LEAHY MJ (2002) Advances in poultry litter disposal technology--a review. Bioresource Technology, **83 (1)** 27-36.

KIM M AHN YH and SPEECE RE (2002). Comparative process stability and efficiency of anaerobic digestion; mesophilic vs. thermophilic. Water Research **36 (17)** 4369-4385.

KIM SH, HAN SK and SHIN HS (2004) Kinetics of LCFA Inhibition on Acetoclastic Methanogenesis, Propionate Degradation and β-Oxidation. Journal of Environmental Science and Health, Part A **39 (4)** 1025-1037.

KLEIN-MARCUSCHAMER D, OLESKOWICZ-POPIEL P, SIMMONS BA and BLANCH HW (2012) The Challenge of Enzyme Cost in the Production of Lignocellulosic Biofuels **109 (4)** 1083-1087.

KOPLAN S, OKUN DT, BRAGG LM, MILLER ME AND HILLMAN JA (2002*) Industry and trade summary: Wood Pulp and Waste Paper*. USITC Publication, Washington, DC, USA.

KOSTER IW and CRAMER A (1987) Inhibition of Methanogenesis from Acetate in Granular Sludge by Long-Chain Fatty Acids Inhibition of Methanogenesis from Acetate in Granular Sludge by Long-Chain Fatty Acids. Applied and Environmental Microbiology **53 (2)** 403-409.

KUMAR D and MURTHY GS (2011) Impact of pretreatment and downstream processing technologies on economics and energy in cellulosic ethanol production. Biotechnology for biofuels **4** 27.

LALMAN JA and BAGLEY DM (2000) Anaerobic degradation and inhibitory effects. Water Res **34 (17)** 4220-4228.

LALMAN J and BAGLEY DM (2002) Effects of C18 long chain fatty acids on glucose, butyrate and hydrogen degradation. Water Research **36 (13)** 3307-3313.

LARK N, XIA Y, QIN CG, GONG CS and TSAO GT (1997) Production of ethanol from recycled paper sludge using cellulase and yeast, Kluveromyces marxianus. Biomass and Bioenergy **12 (2)** 135-143.

LARSSON S, REIMANN A, NILVEBRANT NO and JÖNSSON LJ (1999) Comparison of Different Methods for the Detoxification of Lignocellulose Hydrolyzates of Spruce. Applied Biochemistry and Biotechnology **77 (1-3)** 91-104.

LEE DH, BEHERA SK, KIM JW and PARK HS (2009) Methane production potential of leachate generated from Korean food waste recycling facilities: A lab-scale study. Waste Management **29 (2)** 876-882.

LEONARD RH and HAJNY GJ (1945) Fermentation of wood sugars to ethyl alcohol. Industrial and Engineering Chemistry **37 (4)** 390-395.

LINDMARK J, ERIKSSON P and THORIN E (2014) The effects of different mixing intensities during anaerobic digestion of the organic fraction of municipal solid waste. Waste Management **34 (8)** 1391-1397.

LIU Y, XU J, ZHANG Y, YUAN Z, HE M, LIANG C, ZHUANG X and XIE J (2015) Sequential bioethanol and biogas production from sugarcane bagasse based on high solids fed-batch SSF. Energy **90** 1199-1205.

LIVER SF and HALL ER (1996) Interactions of resin acids with aerobic and anaerobic biomass—I. Degradation by non-acclimated inocula. Water Research **30 (3)** 663-671.

MA J, ZHAO QB, LAURENS LLM, JARVIS EE, NAGLE NJ, CHEN S and FREAR CS (2015) Mechanism, kinetics and microbiology of inhibition caused by long-chain fatty acids in anaerobic digestion of algal biomass. Biotechnology for biofuels **8** 141.

MAGHANAKI MM, GHOBADIAN B, NAJAFI G and GALOGAH RJ (2013) Potential of biogas production in Iran. Renewable and Sustainable Energy Reviews **28** 702-714.

MAHMOOD T and ELLIOTT A (2006) A review of secondary sludge reduction technologies for the pulp and paper industry. Water Research **40 (11)** 2093-2112.

MALIK RK, SINGH R and TAURO P (1987) Effect of inorganic nitrogen supplementation on biogas production. Biological Wastes **21 (2)** 139-142.

MAO C, FENG Y, WANG X and REN G (2015) Review on research achievements of biogas from anaerobic digestion. Renewable and Sustainable Energy Reviews **45** 540-555.

MARQUES S, ALVES L, ROSEIRO J C and GIRIO FM (2008) Conversion of recycled paper sludge to ethanol by SHF and SSF using Pichia stipitis. Biomass and Bioenergy **32 (5)** 400-406.

MCCARTHY PJ, KENNEDY KJ and DROSTE RL (1990) Role of resin acids in the anaerobic toxicity of chemithermomechanical pulp wastewater. Water Research **24 (11)** 1401-1405.

McCARTY PL (1964) Anaerobic Waste Treatment Fundamentals. Chemistry and microbiology **95 (9)** 107-112.

MCKENDRY P (2002) Energy production from biomass (part 1): overview of biomass. Bioresource Technol **83 (1)** 37-46.

MEYER T and EDWARDS EA (2014) Anaerobic digestion of pulp and paper mill wastewater and sludge. Water Research **65** 321-349.

MONTE MC, FUENTE E, BLANCO A and NEGRO C (2009) Waste management from pulp and paper production in the European Union. Waste Management **29 (1)** 293-308..

MORGAN-KISS RM, PRISCU JC, POCOCK T, GUDYNAITE-SAVITCH L and HUNER, NPA (2006) Adaptation and Acclimation of Photosynthetic Microorganisms to Permanently Cold Environments. Microbiology and Molecular Biology Reviews **70 (1)** 222-252.

MUSSATTO SI and ROBERTO IC (2004) Alternatives for detoxification of diluted-acid lignocellulosic hydrolyzates for use in fermentative processes: A review*. Bioresource Technology* **93(1)** 1-10.

NILSSON B and STRAND O (1994) Evaporator Condensate and Caustic Extraction Liquor from a Pulp Factory Treated with an Anaerobic Process. Water Science and Technology **29 (5-6)** 399-407.

OLOFSSON K, BERTILSSON M and LIDÉN G (2008) A short review on SSF – an interesting process option for ethanol production from lignocellulosic feedstocks. Biotechnology for biofuels **1 (1)** 7.

OWENS JM and CHYNOWETH DP (1993) Biochemical methane potential of municipal solid waste (MSW) components. Water Science and Technology 1-14.

PALMQVIST E and HAHN-HÄGERDAL B (2000) Fermentation of lignocellulosic hydrolysates. II: Inhibitors and mechanisms of inhibition. Bioresource Technology **74 (1)** 25-33.

PAMSA (2012) South African pulp and paper industry. South Africa.

PARKIN GF, LYNCH NA, KUO W, KEUREN ELV, BHATTACHARYA SK, PARKIN F and LYNCH A (1990) Interaction between Sulfate Reducers and Methanogens Fed Acetate and Propionate. Research Journal of the Water Pollution Control Federation **62 (6)** 780-788.

PARKIN GF, SPEECE RE, YANG CHJ and KOCHER WM (1983) Response of Methane Fermentation Systems to Industrial Toxicants. Journal (Water Pollution Control Federation) **55 (1)** 44-53.

PATEL GB, AGNEW BJ and DICAIRE CJ (1991) Inhibition of pure cultures of methanogens by benzene ring compounds. Applied and Environmental Microbiology **57 (10)** 2969-2974.

PENG L and CHEN Y (2011) Conversion of paper sludge to ethanol by separate hydrolysis and fermentation (SHF) using Saccharomyces cerevisiae. Biomass and Bioenergy **35 (4)**1600-1606.

PIRINGER G and BHATTACHARYA SK (1999) Toxicity and fate of pentachlorophenol in anaerobic acidogenic systems. Water Research **33 (11)** 2674-2682.

POKHREL D and VIRARAGHAVAN T (2004) Treatment of pulp and paper mill wastewater – A review. Science of the Total Environment **333 (1-3)** 37-58.

PRASETYO J, NARUSE K, KATO T, BOONCHIRD C, HARASHIMA S and PARK EY (2011) Bioconversion of paper sludge to biofuel by simultaneous saccharification and fermentation using a cellulase of paper sludge origin and thermotolerant Saccharomyces cerevisiaeTJ14. Biotechnology for Biofuels **4 (1)** 1-13..

PRASETYO J and PARK EY (2013) Waste paper sludge as a potential biomass for bio-ethanol production. Korean Journel of Chemical Engineering **30 (2)** 253-261.

PUYOL D, SANZ JL, RODRIGUEZ JJ and MOHEDANO AF (2012) Inhibition of methanogenesis by chlorophenols: A kinetic approach. New Biotechnology **30 (1)** 51-61. Available at: http://dx.doi.org/10.1016/j.nbt.2012.07.011.

REXFELT J and SAMUELSON O (1970) The composition of condensates from the evaporation of sulfite spent liquor. Svensk Papperstidning **73** 689-95.

RANDALL D (2018) A household urine collection device to save water and produce fertilizer. Water Institute of Southern Africa Conference 2018 session 36.

RINTALA JA and PUHAKKA JA (1994) Anaerobic treatment in pulp and paper mill waste management: A review. Bioresource Technology **47 (1)** 1-18.

ROBERTSON JA and EASTWOOD MA (1981) An examination of factors which may affect the water holding capacity of dietary fibre. British Journal of Nutrition **45 (1)** 83-88.

ROBERTSON S (1990) *Water and waste-water management in the paper and pulp industry*. Research report no.145/49/90, Water Research Commission, Pretoria, South Africa.

ROBUS CLL (2013) *Production of bioethanol from paper sludge using simultaneous saccharification and fermentation*. Department of Process Engineering, Stellenbosch University.

SANCHEZ J, VALLE L, RODRIGUEZ F, MORIÑIGO M and BORREGO J (1996) Inhibition of methanogenesis by several heavy metals using pure cultures. Letters in Applied Microbiology **23 (6)** 439-444.

SIERRA-ALVAREZ R, FIELD JA, KORTEKAAS S and LETTINGA G (1994) Overview of the anaerobic toxicity caused by forest industry wastewater pollutants. Water Science Technology **29 (5-6)** 353-363.

SIERRA-ALVAREZ R and LETTINGA G (1991) The methanogenic toxicity of wastewater lignins and lignin related compounds. Journal of Chemical Technology & Biotechnology **50(4)** 443-455.

SIERRA-ALVAREZ R and LETTINGA G (1990) The methanogenic toxicity of wood resin constituents. Biological Wastes **33 (3)** 211-226.

SINGH L, MAURYA M S, SAI RAM M and ALAM SI (1993) Short Communication: Biogas Production from Night Soil – Effects of Loading and Temperature. Bioresource Technology **20** 59-61.

SINGHAL A & THAKUR IS (2009) Decolourization and detoxification of pulp and paper mill effluent by Emericella nidulans var. nidulans. Journal of Hazardous Materials **171(1-3)** 619-625.

SIXTA H (2008) *Handbook of Pulp*, WILEY-VCH Verlag GmbH and Co. KGaA, Weinheim, Germany.

SOTO M, MÉNDEZ R and LEMA JM (1993) Methanogenic and non-methanogenic activity tests. Theoretical basis and experimental set up. Water Research **27 (8)** 1361-1376.

SPEECE RE, BOONYAKITSOMBUT S, KIM M, AZBAR N and URSILLO P (2006). Overview of Anaerobic Treatment: Thermophilic and Propionate Implications. In: *Keynote Address—Association of Environmental Engineering and Science Professors—78th Annual Water Environment Federation Technical Exposition and Conference*, Washington D.C., U.S.A October 29th-November 2nd, 2005, **78(5)** 460-473. Water Environment Research.

STENSTROM MK, NG SA, BHUNIA, PRASANTA K and ABRAMSON SD (1983) Anaerobic Digestion of Municipal Solid Waste. Journal of Environmental Engineering **109 (5)** 1148-1158.

SUBRAMANIAN B and PAGILLA KR (2014) Anaerobic digester foaming in full-scale cylindrical digesters – Effects of organic loading rate, feed characteristics, and mixing. Bioresource Technology **159** 182-192. Available at: http://dx.doi.org/10.1016/j.biortech.2014.02.089.

SUHR M, KLEIN G, KOURTI I, GONZALO MR, SANTONJA GG, ROUDIER S and SANCHO LD (2015) *Best Available Techniques (BAT) Reference Document for the Production of Pulp , Paper and Board*. Report EUR 27235 EN, Publications Office of the European Union, Luxembourg.

SUNDRARAJAN R, JAYANTHI A and ELANGO R (1997) Anaerobic digestion of organic fractions of municipal solid waste and domestic sewage of Coimbatore. Indian J.Environ. Health **39 (3)** 193-196.

SUNTIO LR, SHIU WY and MACKAY D 1988. A review of the nature and properties of chemicals present in pulp mill effluents. Chemosphere **17 (7)** 1249-1290.

TAKIZAWA N, UMETSU K, TAKAHATA H and HOSHIBA H (1994) Temperature effects on continuously expanding anaerobic digester with dairy manure slurry. Research Bulletin of Obihiro University Natural Science **19 (1)** 31-36.

TIAN L, ZOU D, YUAN H, WANG L, ZHANG X and LI X (2015) Identifying proper agitation interval to prevent floating layers formation of corn stover and improve biogas production in anaerobic digestion. Bioresource Technology **186** 1-7. Available at: http://dx.doi.org/10.1016/j.biortech.2015.03.018.

VERTES AA, NASIB Q, HANS PB, HIDEAKI Y (2010) *Biomass to Biofuels: Strategies for Global Industries*, John Wiley & Sons Ltd, West Sussex, United Kingdom.

WATSON NE, PRIOR BA, LATEGAN PM and LUSSI M (1984) Factors in acid treated bagasse inhibiting ethanol production from d-xylose by Pachysolen tannophilus. Enzyme and Microbial Technology **6 (10)** 451-456.

WILLIAMS A (2017) *The production of bioethanol and biogas from paper sludge*. Department of Process Engineering, Stellenbosch University.

WU WM, BHATNAGAR L and ZEIKUS JG (1993) Performance of anaerobic granules for degradation of pentachlorophenol. Applied and Environmental Microbiology **59 (2)** 389-397.

XIAO Z, ZHANG X, GREGG DJ and SADDLER JN (2004) Effects of sugar inhibition on cellulases and beta-glucosidase during enzymatic hydrolysis of softwood substrates. Applied biochemistry and biotechnology **113-116** 1115-1126.

YADVIKA, SANTOSH, SREEKRISHNAN TR, KOHLI S and RANA V (2004) Enhancement of biogas production from solid substrates using different techniques – A review. Bioresource Technology **95 (1)** 1-10.

ZENNAKI-BENSOUDA Z, ZAID A, LAMINI H, AUBINEAU M and BOULIF M (1996). Methane fermentation from cattle wastes: study over time of the hydraulic retention, temperature and concentration of the substrate. Tropicultura **14** 134-140.

ZHANG J & LYND LR (2010) Ethanol production from paper sludge by simultaneous saccharification and co-fermentation using recombinant xylose-fermenting microorganisms. Biotechnology and Bioengineering **107 (2)** 235-244.

ZHANG T, LIU L, SONG Z, REN G, FENG Y, HAN X and YANG G (2013) Biogas Production by Co-Digestion of Goat Manure with Three Crop Residues. PLOS ONE **8 (6)** 1-7.

ZHENG X et al. (2015) Effects of titanium dioxide and zinc oxide nanoparticles on methane production from anaerobic co-digestion of primary and excess sludge. Journal of environmental science and health **50 (9)** 913-21. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26061204.

ZHU M, XU W and LI X (2012) Bioconversion of Different Paper Sludge to Ethanol by Yeast Using Separate Hydrolysis and Fermentation. **3** 141-145.

APPENDIX

ADDITIONAL EXPERIMENTAL RESULTS

Table A-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)	Final Yeast Biomass concentration (g/L)
Virgin pulp	0	22.827	0.457	89.343	0.951	3.311
	25	22.690	0.454	88.807	0.945	2.200
	50	22.056	0.441	86.326	1.838	2.333
	75	23.085	0.462	90.354	1.924	2.522
	100	22.937	0.459	89.773	1.911	2.456
Corrugated recycle	0□	20.657	0.413	80.849	0.861	2.900
	25□	21.887	0.438	85.663	0.912	2.989
	50□	22.023	0.440	86.196	0.918	1.983
	75□	21.407	0.428	83.785	0.892	2.144
	100□	21.083	0.422	82.517	0.878	2.078
	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	3.789
	25	21.222	0.424	83.060	0.884	3.000
	50	22.615	0.452	88.514	0.942	3.444
	75	22.349	0.447	87.472	0.931	2.900
	100	22.925	0.458	89.725	0.955	3.000

(*) – Crude Corrugated recycle PW ; **(□)** – Centrifuged Corrugated recycle PW; **(N/A)** – Not applicable

Table A-2: Summary of yields for BMP test of paper sludge with dirty 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	
	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		

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				
Tissue printed recycle		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

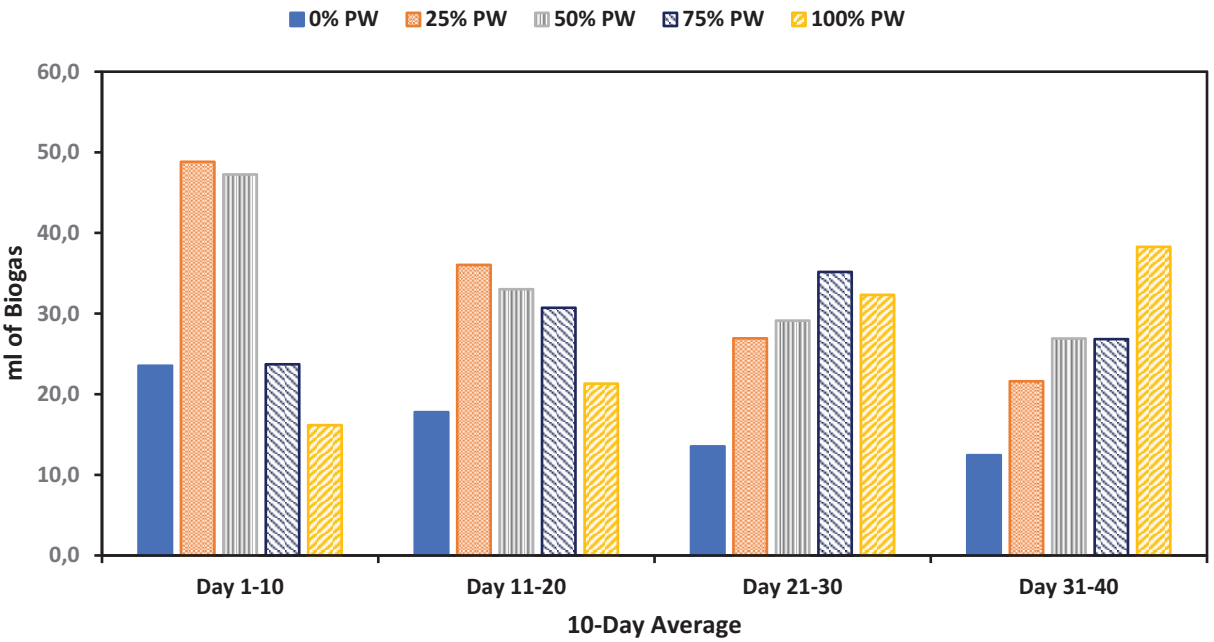


Figure A-1: 10-day average biogas production during incubation period stillage