COMPARISON OF LIGNIN YIELD FROM SUGARCANE BAGASSE PELLETS USING LIQUID HOT WATER AND IONIC LIQUID PRETREATMENT METHODS

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PREFACE

The work described in this thesis was performed by the author under the supervision of Professor. N. Deenadayalu at Durban University of Technology, Durban, South Africa and at Technische Universität Hamburg Harburg (TUHH), Institute of Thermal Separation Processes, Germany (collaboration) under the supervision of Lisa Schmidt from 2016 – 2018. The study presents original work by the author and has not been submitted in any form to another university. Where use is made of the work of others, it has been clearly stated in the text.

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Proverbs 3:6

"In all your ways acknowledge him and he will make your paths straight" I would like to send my sincere and warm gratitude to the:

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ABSTRACT

In this research work, lignin yield from sugarcane bagasse pellets (SBP) was investigated after treatment of sugar cane bagasse with liquid hot water (LHW) and enzymatic hydrolysis followed by ionic liquids (ILs) and only ionic liquids pretreatment methods.

In the LHW and ionic liquid methods, the SBP were first treated with LHW at 200 °C, for 30 minutes in a suitable reactor, for removal of hemicellulose. The complex cellulignin residue was treated separately with either of two ionic liquids namely: 1-ethyl-3- methylimidazolium acetate ([Emim][OAc]) or 1-butyl-3-methylimidazolium hydrogen sulphate ([Bmim][HSO_4]), using microwave digestion at varying time intervals.

The ionic liquid method involved the pretreatment of sugarcane bagasse pellets with either 1-ethyl-3-methylimidazolium acetate or 1-butyl-3-methylimidazolium hydrogen sulphate followed by microwave digestion at varying time intervals.

Ultraviolet (UV) spectroscopy at a wavelength of 280 nm was used as a tool for quantification of lignin. The different functional groups of the extracted lignin were confirmed using attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy. Thermogravimetric analysis (TGA) provided information on thermal characteristics of the extracted lignin. In addition to

material characterization, mixed factorial ANOVA was performed to compare the extracted lignin yield using the LHW and IL and the ionic liquid pretreatment methods. High performance liquid chromatography (HPLC) was used to identify the C_5 sugars in the hydrolysate after LHW pretreatment. X-ray diffraction (XRD) was used to identify cellulose peaks of cellulignin and SBP and ILs treated samples.

The results indicated that the lignin yield from sugarcane bagasse pellets after liquid hot water treatment and enzymatic hydrolysis was 37.8 % (m/v).

The highest percentage yield of lignin extracted from the complex cellulignin (LHW and IL) was found to be 68.00 % (m/v) and 32.04 % (m/v) for [Emim][OAc] and [Bmim][HSO_4], respectively for the optimized reaction time of 10 minutes. However, 67.25 % (m/v) and 48.94 % (m/v) of the extracted lignin were obtained for the pretreated SBP with [Emim][OAc] and [Bmim][HSO_4], respectively for a reaction time of 20 minutes. This comparative study revealed that, there is no significant difference between the yield of lignin extracted from the complex cellulignin (68.00%) and sugarcane bagasse pellets (67.25 %).The sugarcane bagasse pellets is the preferred method since it doesn't require high energy input.

Keywords:

Sugarcane bagasse pellets; Pretreatment; Liquid hot water; enzymatic hydrolysis; Ionic liquid.

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LIST OF SYMBOLS

Symbol	***Meaning***	***Units***
T	*Temperature*	*°C*
t	*Time*	*S*
N	*Number of samples*	-
% (m/v)	*Percentage mass per volume*	*g/L*
C	*Concentration*	*g/L*
$\sigma_{\bar{x}}$	*Standard deviation*	-

R^2	*Coefficient of determination*	-
λmax	*Maximum absorbance*	-

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Abbreviations	
LHW	Liquid hot water
SBP	Sugarcane bagasse pellets
[Emim][OAc]	1-Ethyl-3-methylimidazolium acetate
[Bmim][HSO_4]	1-Butyl-3-methylimidazolium hydrogen sulphate
EH	Enzymatic hydrolysis
LOI	Lateral order index
TCI	Total crystalline index
TGA	Thermogravimetric analysis
XRD	X-ray diffraction
HPLC	High performance liquid chromatography
HPAEC	High performance anion exchange chromatography

CHAPTER 1

INTRODUCTION

1.1 Background

In the 21st century renewable energy resources have become a top priority for many countries including South Africa. This is mainly due to the fluctuation of crude oil prices and rising concerns on environmental pollution. Biomass is considered to be a perfect resource to replace fossil fuels because it is sustainable and it is the most attractive renewable energy source for biodiesel and fine chemical production. Sugarcane bagasse is a potential renewable resource in South Africa since approximately 19.9 million tons of sugarcane produced each season from 14 sugar mills (Paterson-Jones 1989). Sugarcane bagasse (SB) is the most

abundant agro- industrial by-product used in various applications (Tita *et al.* 2002) in the following industries: pulp and paper making industry, production of levulinic acid and ethanol. The compositional analysis of sugarcane bagasse is: cellulose (44.8%), hemicellulose (28.5%), lignin (22.6%) and other components (2.9%) (Luz *et al*. 2007).

1.2 Lignin

Lignin is a phenolic polymer made up of three major three major C6-C_3 (phenylpropanoid) alcohol units, known as, syringyl alcohol, guaiacyl alcohol and *p*-coumaryl alcohol. Figure 1.1 illustrates the monomers of lignin.

Figure 1.1: Lignin monomers. Adapted from Shayesteh *et al*. (2016)

Lignin is the most abundant aromatic polymer on earth after cellulose. It is composed of up to 40% of dry weight of woody plants (Shayesteh Haghdan *et al*. 2016).

Lignin generates different functional groups depending on the extraction process and the chemical reagents. As a result, new aromatic structures may appear in the extracted lignin samples.

- **Lignin applications**

Lignin has several applications which are stated below:

- Dispersant in cement and gypsum blends (Yang *et al*. 2007)
- Chelating or emulsifier agent for removing heavy metals from industrial effluents (Sena – Martins *et al*. 2008).

- Antioxidant capacity mainly in cosmetics and pharmaceutical (Ugartondo *et al.* 2008).
- Composite materials such as biodegradable packing materials (Domenek *et al.* 2013).
- Lignin sulfonates provides anti-friction properties to grease ensuring longer life of lubricants.
- Lignin enhances performance of energy storage devices.

Major sectors of lignin applications are summarized in Figure 1.2.

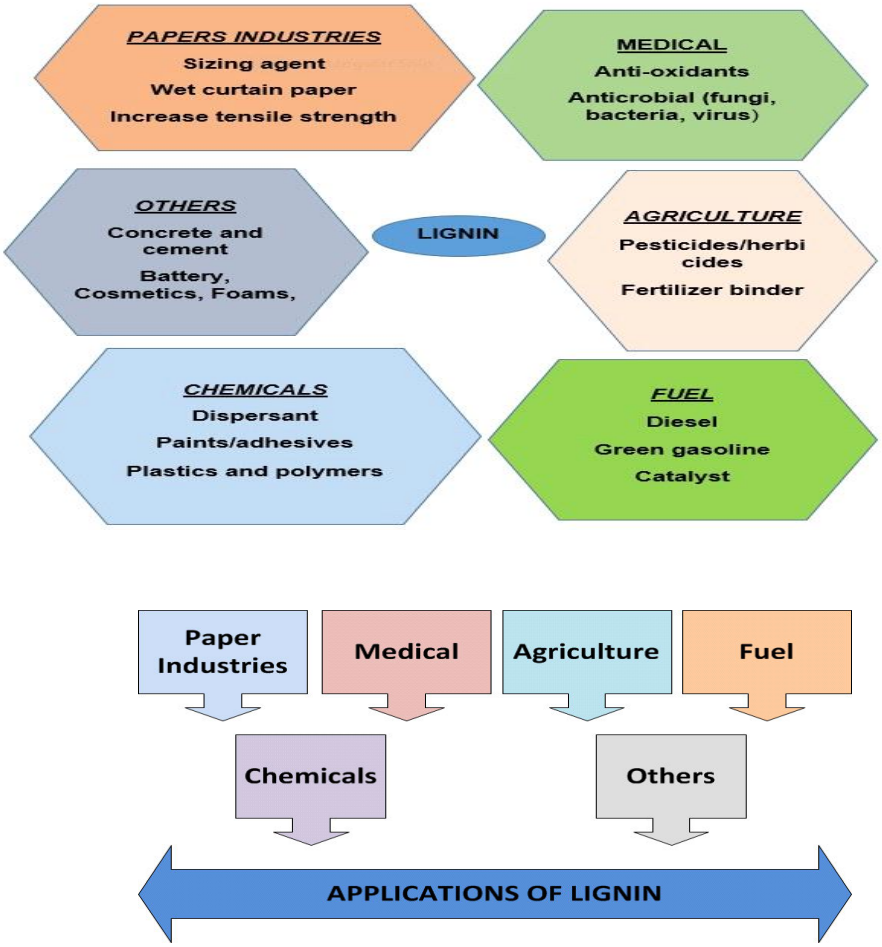


Figure 1.2: Applications of lignin. Abdel-Hamed *et al*. (2013)

1.3 Biomass pretreatment

The pretreatment process of biomass is a crucial step in the production of biofuels and chemicals from lignocellulosic biomass. Many pretreatment methods such as biological, mechanical, acid and ionic liquids have been proposed and found to change the physical and the chemical structure of lignocellulosic biomass components therefore making them more accessible to hydrolysis and /or enable it to be separated into individual components (Teymouri *et al*. 2014; Wang *et al*. 2012).

However, some of the challenges after the pretreatment process includes:

- Lower recovery of hemicellulose
- Insufficient extraction of lignin
- Formation of by-products that inhibit complete ethanol fermentation.

1.4 Summary of the study

In this research work two environmentally friendly pretreatment processes (LHW and IL) were used to pretreat the sugarcane bagasse pellets (SBP). The results were compared to evaluate the effectiveness of the pretreatment methods for the sugarcane bagasse pellets separation into its different components (hemicellulose, cellulose and lignin). The study was carried out in three parts.

- The first part of the study was the pretreatment of sugarcane bagasse pellets using liquid hot water to remove hemicellulose. The solid complex residue (cellulignin) was enzymatically hydrolyzed in order to separate the cellulose (as a sugar solution) from the complex cellulignin. The sugar solution was quantified by

the Thünen Institute of Wood Research in Hamburg, for galactose, glucose, mannose and lignin (in terms of Klason lignin) using high performance anion exchange chromatography (HPAEC) with photometric detection.

- The second part of the study is focused on the extraction of the lignin from the solid complex cellulignin using ionic liquids (ILs): 1-ethyl-3-methylimidazolium acetate ([Emim][OAc]) or 1-butyl-3-methylimidazolium hydrogen sulphate ([Bmim][HSO_4]) using microwave digestion with a fix temperature (180 ^oC) and electrical power (80 Watts) at varying time intervals (from 3 to 30 minutes).

- The third part of the research was based on the extraction of lignin from sugarcane bagasse pellet using ionic liquids: 1-ethyl-3-methylimidazolium acetate ([Emim][OAc]) or 1- butyl -3-methylimidazolium hydrogen sulphate ([Bmim][HSO_4]) using microwave digestion.

Analytical techniques used in this study include:

- **UV/VIS spectroscopy** to determine the concentration of the sugar and lignin at 280 nm.
- **High performance liquid chromatography (HPLC)** meant to identify the different sugars released from the Hydrolysate and the complex cellulignin after the enzymatic hydrolysis.
- **Attenuated total reflectance (ATR)** spectroscopy to identify the different functional groups of the complex cellulignin and SBP samples.

- **X-ray diffraction (XRD)** to determine the crystalline structure of the complex cellulignin and SBP samples.
- **Thermogravimetric analysis (TGA)** to understand the thermal decomposition of the complex cellulignin and SBP sample.

1.5 Aim

Land pollution is caused by the dumping of sugarcane bagasse residue, this research aims to valorize sugarcane bagasse residue by using two environmentally friendly methods for the extraction lignin.

1.6 Objectives of the study

- To determine the effects of LHW as a pretreatment method in the extraction of lignin.
- To identify the sugars released from the complex cellulignin using enzymatic hydrolysis.
- To determine the effect of ionic liquid as a pretreatment method in the extraction of lignin from cellulignin and SBP.
- To quantify the lignin extracted from the complex cellulignin and SBP.
- To characterize the complex cellulignin and the SBP samples using different analytical techniques.

CHAPTER 2

LITERATURE REVIEW

2.1 Biomass

The study conducted by Lee *et al.* (2008) evaluated the effect of an increasingly growing demand for energy, uncertain supply of petroleum and the rise of global warming by utilization of fossil fuels leading to researchers seeking alternate options by using renewable energy sources. The fossil fuel-based economy is faced with challenges like emissions of carbon dioxide, diminishing reserves and expanding costs. A potential answer for these issues could be the use of lignocellulosic biomass a second generation energy source and to create chemicals. Lignocellulosic biomass, for example, agricultural residues, waste paper and forestry waste are perceived as a potential source of sugars for biotransformation into biofuels and specialty bio based products (Himmel *et al.* 2007; Li *et al*. 2008).

Lignocellulosic biomass is the most abundant renewable carbon raw material on earth (Jorgensen *et al.* 2007), which is regarded as waste or left in agricultural fields as fertilizer. It can be used for the production of higher value compounds since it is approximately 75 % polysaccharides. Biomass sources include agricultral crops and residues, forestry crops, industrial residues, animal residues municipal solid waste and sewage are illustrated in Figure 2.1.

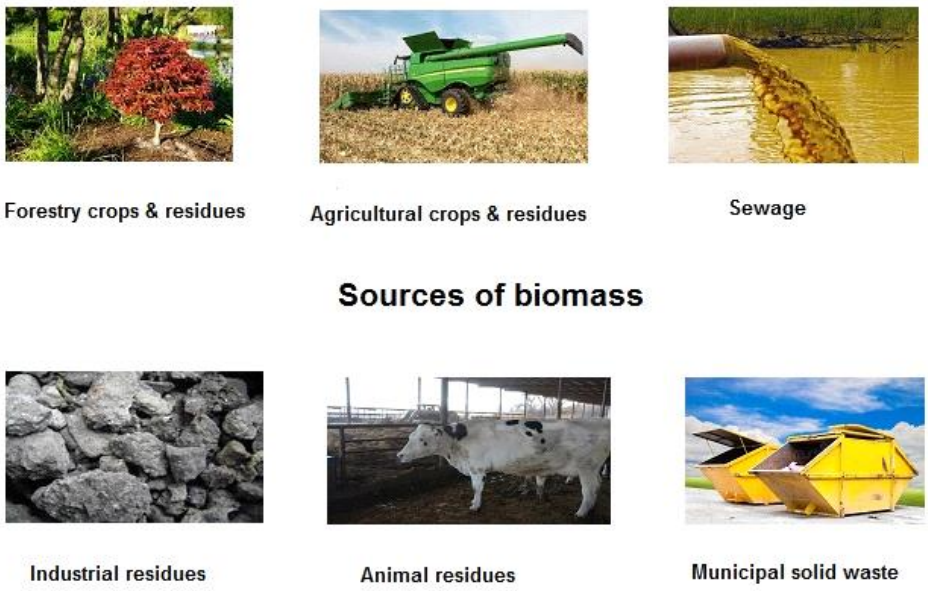


Figure 2.1: Biomass sources

2.2 Pretreatment methods

Pretreatment is an essential step in the biorefinery for production of biofuels and other beneficial by-products (such as chemicals) Xia *et al*. (2015).

It used for the separation of hemicellulose and lignin biomass component which allows cellulose to be accessible Alvira *et al*. (2010). Several techniques for pretreatment have been developed for example; biological, physical, including mechanical, chemical with the aid of acids, alkalis, ozone, organic solvents or ionic liquids and physic-chemical pretreatment involving steam explosion, ammonia fiber explosion (AFEX), CO_2 explosion, liquid hot water, wet oxidation, microwave and ultrasound (Alvira *et al.* 2010).

- **Biological pretreatment**

Biological pretreatment involves microorganisms, such as white, brown and soft rot-fungi are employed to degrade hemicellulose and lignin. The advantages of this pretreatment are: low energy required and mild operation conditions. Nevertheless, the rate of biological hydrolysis is usually very low, so this pretreatment requires long residence times (Cardona *et al.* 2007). The goal of the enzymatic hydrolysis is opening the biomass fibers to increase water access and enzyme accessibility in order to produce fermentable sugars.

2.2.2 Acid pretreatment

The use of strong acids for example H_2SO_4 and HCl have been generally utilized for treating lignocellulosic materials because they are powerful agents for cellulose hydrolysis (Sun *et al*. 2002), and no enzymes are needed subsequent to acid hydrolysis. Advantages of concentrated acid hydrolysis are the flexibility in terms of feedstock choice, high monomeric sugar yield as well as mild temperature conditions that are needed. Drawbacks of using concentrated acids are corrosive nature of the reaction and the need to recycle acids in order to lower cost. Several companies are in the process of commercializing strong acid hydrolysis of lignocellulosic biomass for microbial fermentation (Bluefireethanol. 2010).

2.2.3 Alkali pretreatment

The real impact of alkaline pretreatment is the removal of lignin from the biomass, thus improving the reactivity of the remaining polysaccharides. In addition, alkali pretreatments remove acetyl and the various uronic acid substitutions on hemicellulose that lower the accessibility of the enzyme to the hemicellulose and cellulose surface (Chang *et al*. 2000). It is reported that alkaline hydrolysis mechanism is based on saponification of intermolecular ester bonds crosslinking xylan hemicellulose and other components such as lignin (Sun *et al*. 2002).

2.2.4 Liquid hot water pretreatment

Recently liquid hot water has generated a widespread interest for conversion of bagasse to sugars in comparison to other pretreatment methods (Iryani *et al.* 2014). The drawbacks of organic solvents and ionic liquids pretreatment technologies include high chemical costs, high impact of residual chemical products on downstream processing, health and environmental impact, making them prohibitive towards the development of commercial processes for pretreating lignocellulosic biomass.

During LHW pretreatment, hydronium ions are generated in situ by auto-ionization of water at high temperature and pressure. The acetic acid is, therefore, generated by hydrolysis of hemicellulose which is retained in oligomeric and monomeric form in liquid fraction after LHW pretreatment Ruizet *et al.* (2013).

Ingram *et al.* (2009) during their study mentioned that the temperature for LHW process depends on the product of interest and confirmed the temperatures between 170 °C and 230 °C for hydrothermal pretreatment of rye straw

The research conducted by Zetzl *et al*. (2012) confirmed that to obtain high yield of glucose after enzymatic hydrolysis, removal of hemicellulose was needed, hence temperature above 200 °C was favorable.

The study conducted by Michele *et al*. (2016) concluded that LHW pretreatment resulted in hemicellulose solubilisation, and solids enriched in cellulose. LHW removed a large fraction of hemicellulose from raw feedstocks, confirmed by the observed decrease in its content in the solid fractions. The dissolved and/or degraded hemicellulose can also be confirmed through the hemicellulose content in the hydrolysates (Table 2.1).

Table 2.1: Chemical composition of hydrolysate of the feedstocks after LHW pretreatment. Borrega *et al*. (2011)

Components	**Composition**	
	Wheats straw	Corncob
Hydrolysates (g/L)		
Oligosaccharides		
Gluco-oligosaccharides	3.10	1.50
Xylo-oligosaccharides	15.50	6.00
Arabino-oligosaccharides	1.90	0.75
Acetyl groups-oligosaccharides	1.80	0.50
Monosaccharides		
Glucose	0.45	0.15
Xylose	0.63	0.76
Arabinose	1.50	1.65
Acetic acid	0.85	0.65
Degradation products		
HMF	0.08	0.07
Furfural	0.22	0.12
Hemicellulose extraction yield (%)	74.11	39.26

The fundamental objective of the LHW pretreatment at the Institute of Thermal Separation Processes at the Hamburg University of Technology (TUHH) is to isolate hemicellulose sugars from lignocellulosic biomass and to profit the enzymatic hydrolysis from cellulose to glucose, coproducing better quality of lignin.

2.3 Ionic liquids (ILs)

Ionic liquids are defined as a class of salts with melting points less than 100 °C, and are usually liquid at room temperature (Oghbaie *et al*. 2014). ILs are made of cations and anions. Their properties can be adjusted by choosing specific combinations of cations and anions (Nockemann *et al.* 2005). One of the main advantages of ionic liquids is that are easily recovered and reutilized therefore it reduces the quantity of waste generation on processes. Their designation as green solvents is related principally to their neglible vapour pressure (Dharaskar *et al.* 2015).

Graenacher in the 1930s first found that cellulose could be disintegrated in liquid N- ethylpyridinium chloride salt (Graenacher, 1934). Little consideration was paid to this for the most part in light of the high liquefying purpose of this liquid salt. In 2002, Swatloski and colleague's investigation on ILs demonstrated that some imidazolium- based ILs could break up cellulose productively at low temperatures (< 100 °C) without the arrangement of any catalysts (Swatloski *et al*. 2002). Nowadays, due to their unique properties, ionic liquids have attracted the attention of many researchers and increasingly have numerous applications in the industrial scale. Figure 2.2 shows the cations and anions used in the preparation of ionic liquids.

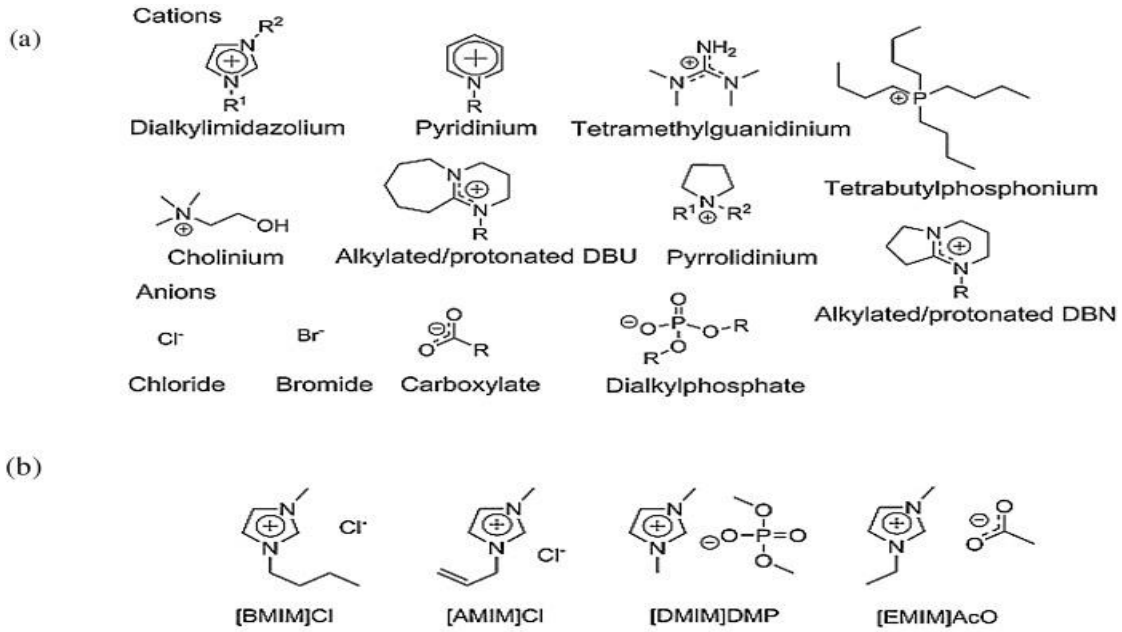


Figure 2.2: Cations and anions structures of ionic liquids (Amal *et al*. 2016)

the properties of ILs are summarized in Table 2.2 below.

Table 2.2: Properties of ionic liquids (Johnson 2007)

Property	Property value
Polarity	47- 49
Viscosity	< 100 Cp
Dielectic constant	≤ 30 ($F.m^{-1}$)
Electrochemical window	2 V – 4.5 V
Freezing point	< 373.15 K
Molar conductivity	< 10 $Scm^{-2}.mol^{-1}$
Liquidus range	298.15 – 473.15 K
Specific conductivity	< 10 $mS.cm^{-1}$
Vapour pressure	Usually negligible
Thermal stability	High
Solvent and /or catalyst	Excellent for many organic reactions

- **Ionic liquid pretreatment**

Qiu *et al*. (2012) evaluated the effect of 1-ethyl-3-methylimidazolium acetate ([Emim][OAc]) in pretreating energy cane bagasse (energy cane is a genetic modification of sugarcane having more fiber than sucrose in its composition) in terms of biomass composition. Energy cane bagasse was pre-treated with [Emim][OAc] (5

% (w/w)) at 120 °C for 30 min followed by hydrolysis with commercially available enzymes, Spezyme CP and Novozyme 188. IL treated energy cane bagasse resulted in significant lignin removal (32.0%) with slight glucan and xylan losses (8.8 % and 14.0 %, respectively).

The efficiency of ionic liquids as tested by Raquel *et al.* (2015) for lignin extraction. In this study, soda and organosolv lignins obtained from apple tree were purified using 1-butyl-3-methylimidazolium methylsulphate ([Bmim][$MeSO_4$]), and the lignin

extracted from raw material was tested comparing different conditions.

[Bmim][$MeSO_4$]

and 1-ethyl-3-methylimidazolium acetate ([Emim][OAc]) were the ionic liquids chosen

for the experimentation. The lignins obtained were characterized by FTIR, TGA, and HPLC, in order to determinate the influence of the different treatments on their structures. Lignin of 91.2 % purity was obtained directly from raw material using ionic liquids, whereas organosolv lignin purity ranges from 85.7 % to 90.9 %, and soda lignin from 12.9 % to 89.6 % after treatment with [Bmim][$MeSO_4$]. The experimental results confirmed efficient performance of 1-butyl-3-methylimidazolium methylsulphate up to the third cycle after which traces of ionic liquid appeared as contamination in the extracted lignin.

Sun *et al.* (2009) studied the complete dissolution and delignification of softwood (southern yellow pine) and hardwood (red oak) in 1-ethyl-3-methylimidazolium acetate

[Emim][OAc] after mild grinding, with red oak showing higher and faster dissolution an southern yellow pine. For pine, 59 % holocellulose (cellulose + hemicellulose) could be recovered in the reconstituted material, whereas 31 % and 38 % respectively of the original lignin was recovered. Thus, partial separation of wood components is possible with [Emim][OAc].

Li *et al*. (2011) noted that dissolution of bagasse and southern yellow pine has been achieved using the ionic liquid; 1-ethyl-3-methylimidazolium acetate ([C_2mim][OAc]) by using a dissolution temperature above the glass transition temperature of lignin (150˚C). Heating the solution at 185 ˚C for 10 min , the highest yields of lignin was obtained to be 31 % and 26 % for bagasse and southern yellow pine respectively.

Zang *et al.* (2011) used acidified aqueous ethylene glycol and ionic liquid in pre-treating sugarcane bagasse. The amount of lignins recovered from [Bmim]-Cl with HCl as the catalyst and [Bmim][CH_3SO_3] was 42 % and 35-36 % by ethylene glycol with HCl or H_2SO_4 as a catalyst respectively.

The research conducted by Tan *et al*. (2009) reported that an ionic liquid mixture containing the 1-ethyl-3-methylimidazolium cation and a mixture of alkyl benzene sulfonates with xylene sulfonate as the main anion was used to extract lignin from sugarcane plant waste at atmospheric pressure and elevated temperatures (170–190
°C). The lignin was recovered from the ionic liquid by precipitation, allowing the ionic liquid to be recycled. An extraction yield exceeding 93% was attained.
Haykir *et al*. (2013) examined the pretreatment of cotton stalk with a series of ionic liquids namely: 2-hydroxy ethyl ammonium formate, 1-allyl-3-methylimidazolium chloride, 1-buthyl-3- methylimidazolium chloride and 1-ethyl-3-methylimidazolium

acetate to enhance the enzymatic accessibility of the lignocellulosic feedstock. [Emim][OAc] was the best among each one of the ILs, for digestibility and essential changes in cotton stalk tests.

Biomass digestibility was 65 % for [Emim][OAc] pretreated cotton stalk after 72 h of

enzymatic hydrolysis.

Yoon *et al.* (2012) investigated the effect of temperature, time and solid stacking on reducing sugar (RS) yield from [Emim][OAc] pre-treated bagasse. Pretreatment at 145 ^{o}C, 15 min and 14 wt % solid stacking gave 69.7 % of RS. Although structural changes by [Emim][OAc] pretreated sugarcane bagasse (SCB) was not clear, the pretreated SCB appeared to have a more penetrable and less crystalline structure which is a desired structure for enzymatic hydrolysis step.

Karatzos *et al.* (2012) considered three ILs, [Bmim][Cl], [Emim][Cl] and [Emim][OAc] which were used to fractionate sugarcane bagasse at 130 ˚C after precipitation by adding an anti-solvent. Among the three ILs, [Emim][OAc] gave the best saccharification yield, material recovery and delignification as effects of [Emim][OAc] pre-treatment exhibited a resemblance to liquid acid neutralizer pre-prescriptions, while those of [Emim][Cl] and [Bmim][Cl] showed a resemblance to aqueous acid pretreatment. The usage of imidazolium IL solvents with shorter alkyl chains achieved dissolution and degradation. Lignin removals of 10 % mass of lignin in bagasse with [C$_4$mim]Cl, 50 % mass with [C$_2$mim]Cl and 60 % mass with [C$_2$mim]OAc, are achieved by limiting the amount of water added as antisolvent.

2. 3.2 Uses of ionic liquids

Imidazolium based ionic liquids have been increasingly used as the most environmentally friendly solvents to replace the volatile and relatively toxic organic solvents in homogeneous and heterogeneous catalysis, materials science, nano materials, lithium ion batteries and separation technology. Ionic liquids in lignocellulosic biomass, prove their ability to separate lignin, i.e delignification. In an analysis by Pu *et al*. (2007), a few ionic fluids were screened concerning their impact on solvency of Kraft lignin extracted from softwood. It was demonstrated that the anion nature influenced lignin dissolvability; [BMIM] based ionic fluids showed lignin solvency in the order: $MeSO_4$> Cl > Br > PF_6. Ionic liquids having large anions were poor in dissolving lignin. Pu *et al.* (2007) utilized the imidazoilum based ILs for disintegration of softwood lignin from a southern pine kraft pulp. ILs are seen as a progressive "green" solvent by the synthetic industry since it can break down cellulose (Swatloski *et al.* 2002 ; Pinkert *et al.* 2009). Owing to their properties and a variety of combinations, ILs have been used for forming cellulose into filaments, films, wipes, dabs and other cellulosic materials (Kosan *et al*. 2008).

2.3.2.1 Hydrogenation reaction

There are many ionic liquids that have been used in hydrogenation reactions. The ionic liquids used in hydrogenation reactions have two advantages. The rate of reaction rate by using ionic liquids is several times faster than using conventional solvents. Ionic liquids play two roles i.e. solvents and catalysts in the

hydrogenation reaction (Qiao *et al.* 2004), ionic liquids can be easily separated and sublimated when they are used in diesel fuel which mainly contain aromatics of the hydrogenation reaction.

2.3.2.2 Separation and purification technology

Ionic liquids are mostly used in the separation and purification technologies due to their unique physical and chemical properties. Many researchers have demonstrated that butanol can be extracted from the fermentation broth by using ionic liquids. Deng *et al*. (2006) have reported that ILs can be applied in the solid separation and 97 % recovery was achieved.

2.3.2.3 Pharmaceuticals

Stoimenovski *et al*. (2010) have confirmed that approximately 50 % of commercial pharmaceuticals products are organic salts. The combination of pharmaceutical active cations with pharmaceutically active anions conducts to a dual active ionic liquid in which the derivative of two drugs is combined. Table 2.3 is a summary of literature references of ILs used for lignin extraction.

Table 2.3: Summary of literature references of ILs used for lignin extraction

Ionic liquids	Subtract	Methods	Condition	Percent of lignin extracted	Characteristics	References
[Bmim][$MeSO_4$]	Apple Tree	Microwave Radiation	Maximum Power of 30 W for 3 min at 180 ^0C	**32.8%**	HPLC: sugars content. Pyrolysis : chemical structure of lignin was studied	Raquel *et al*. 2016
[Bmim][Cl	Palm oil biomass Sugarcane bagasse	IL dissolution and CO_2- AlK$(SO_4)2{\cdot}12H_2O$ precipitation processes	IL solution was purge with CO_2 gas for 30 min at 60 ^oC then 0.2 M AlK$(SO_4)2{\cdot}12H_2O$	**54%**		Chia *et al*. 2012
Bmim][Cl]	Corn Stover	Ultrasound-assisted	Lignin was successfully performed in	**89.9%**	Molecular weight and weight average were determine by GPC model, HPLC system.	Peng *et al*. 2015
[Emim][OAc]				**60%**		

[Emim][OAc]	Sugarcane bagasse Wheat straw	Fractional precipitation by stepwise addition of water Acid treated method	ultrasound-assisted ILs at a low reaction temperature of 70 ^{0}C for 3 h followed by alkaline extraction 150°C for 35 min with 25 min temperature ramp Precipitated lignin was separated, washed with acidified water and dried at 50 ^{0}C in an oven.	**67%**	NMR; HPLC and FTIR ATR-FTIR FTIR spectroscopy: characterization of fractionated sample. NMR: analysis of IL. HPLC :sugars analysis of the hydrolysate	Karatzos *et al*. 2012 Andre *et al.* 2013

Table 2.4: Summary of literature review for lignin recovered (%) and this experimental work

Subtract	**Method**	**Ionic liquids**	**Lignin recovered (%)**	**References**
Sugarcane bagasse	Fractional precipitation by stepwise addition of water	[Emim][OAc] [C_4mim][Cl] [C_2mim][Cl]	60 10 50	Karatzos *et al.* (2012)
Wheat straw	Acid pretreatment method	[Emim][OAc]	67 52.7	Andre *et al*. (2013) Fu *et al*. (2010 a)
Apple tree	Microwave radiation	[Bmim][$MeSO_4$]	32.8	Raquel et al. (2016)
Palm oil biomass	IL dissolution and CO_2-AlK $(SO_4)_2 \cdot 12H_2O$ precipitation processes	[Bmim][C]	54	Chia *et al.* (2012)
Sugarcane bagasse	Acid pretreatment method	[Emim][OAc] [$Hnet_3$][HSO_4]	28 17.2	Thandeka *et al*. (2016)
Sugarcane bagasse pellets Complex cellulignin	Liquid hot water and ionic liquid pretreatment methods	[Emim][OAc] [Bmim][HSO4]	67.20 68.00	This research work

Table 2.5 shows the advantages and disadvantages of lignocellulosic biomass pretreatment methods.

Table 2.5: Summary of the advantages and disadvantages of lignocellulosic biomass pretreatment methods (Amal *et al.* 2016)

Pretreatment method	**Category**	**Advantages**	**Disadvantages**
Milling	Physical	Cost effective	High energy input, inability to remove lignin which restricts the access of the enzymes to cellulose.
Steam explosion	Physico-chemical	Cost effective and chemical free	Excessive degradation of the physical and chemical properties of cellulose and release of inhibitors.
Liquid hot water	Physico-chemical	High recovery and lower formation of inhibitory components	Costly, does not significantly solubilize hemicellulose, ammonia must be recycled after the pretreatment to reduce the cost and the environment.
Ammonia fiber expansion	Physico-chemical	Reduces lignin fraction. Short retention time. No formation of inhibitory byproducts	Concentrated acid process is corrosive and dangerous. Formation of inhibitors at low pH.
Acid hydrolysis	Chemical	Remove lignin and part of hemicellulose. Decreases in polymerization degree	Low digestibility in softwoods. Large amount of water requires for washing
Microbial	Biological	Lignin degradation. Low energy requirement. Chemical free. Mild condition	Slow reaction time

The structures of the ILs used in this research are shown in Figure 2.3 and Figure 2.4 below:

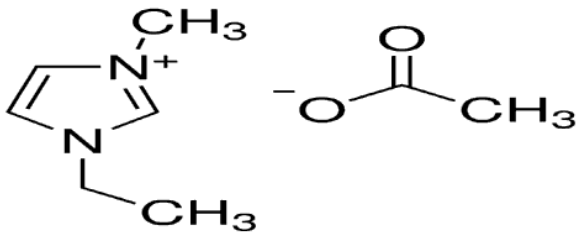


Figure 2.3: Chemical structure of 1-ethyl-3-methylimidazolium acetate: [Emim][OAc]

Figure 2.4: Chemical structure of 1-butyl-3-methylimidazolium hydrogen sulphate: [Bmim][HSO_4]

2.4 Statistical methods

In the study conducted by *Tazien et al*. (2018), the analysis of variance (ANOVA) was performed to identify the significant factors affecting the lignin extraction at p-value = 0.05. According to Guo *et al.* (2010), Sidiki *et al*. (2013) and Tan *et al*. (2011) when the p-value is < 0.05 the corresponding coefficient is more significant in the model. Based on the ANOVA analysis, the factors affecting the response (% lignin extraction) vary in the order as: extraction time > extraction temperature > biomass loading. The coefficient of determination (R^2) of the model was ≥0.9951 indicating that 99.51% of the experimental lignin extraction values matched the model predicted values

CHAPTER 3

INSTRUMENTATION

3.1 Liquid hot water process

Biomass pretreatment with liquid hot water (LHW) is a physicochemical process where pressurized water (typically between 160˚C and 230 °C) is used. The flow through reactor configuration in LHW treatment is considered as being superior

to batch methods for the solubilisation of lignin (Mosier. 2013). Structural characterization of pretreated solids from biomass pretreatment processes is important for understanding biomass decomposition processes, including the extraction of lignin. The preheater was fabricated from 1/8 inch stainless steel tubing and was heated using a mantel heater at temperatures of 100-150 °C. The 1/16 inch stainless-steel tube was used to introduce the hot water from the preheater to the reactor that was placed in an oven. The reactor was also made of stainless steel. After the biomass was introduced into the reactor and was installed in the system, water at room temperature was pumped through the reactor preheater for a few minutes to purge air. When the system reached the desired pressure (50 bar) and a steady state was achieved, an electric heater was applied to heat the water. During experiment, temperatures of the reactor water inlet (T_1) and outlet (T_2) were monitored around 200 °C. The outlet water was passed through the double-tube-type heat exchanger to quench the reaction. When the reaction is completed, the reactor is disconnected and preheater are switched off. The pressure is released while cooling the reactor and the pretreated biomass was removed from the reactor and weighed. The advantage of using LHW pretreatment is the avoidance of corrosive solvents and chemical additives. This reduces the plant complexity as well as the necessity for waste water treatment and recovery of catalyst chemicals (Andric *et al.* 2010). Figure 3.1 shows the schematic representation of LHW process.

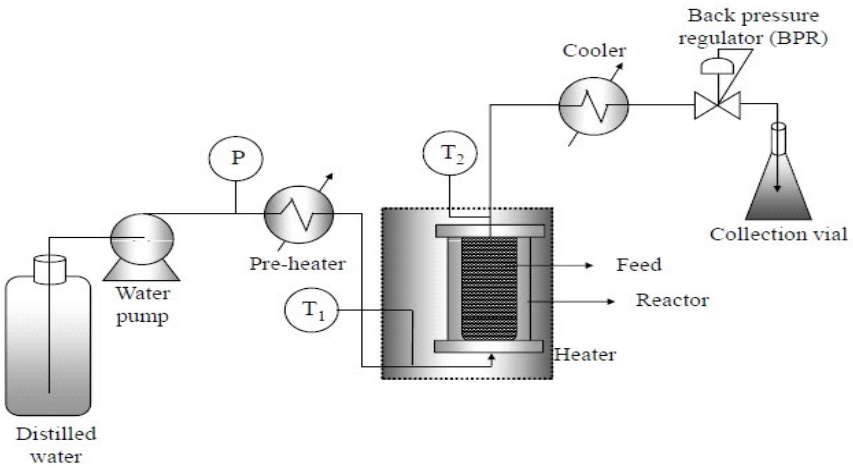


Figure 3.1 Schematic representation of LHW process (Machmudah *et al*. 2014)

3.2 UV- VIS spectrometer

UV-VIS spectrometry is one of the oldest instrumental techniques used for the determination of different analytes in a specific sample.

UV-VIS spectrum originates from the interaction of electromagnetic radiation in the UV-VIS region with molecules, ions or complexes. The major principles of UV-VIS spectroscopy are described below:

- Free atoms or gases generated in an atomizer can absorb radiation at a specific frequency
- Atoms absorb ultraviolet or visible light and make transitions to higher energy levels; therefore the concentration of the analyte is proportional to the resultant from the amount of absorption.

Figure 3.2 is a schematic of a modern of UV spectrometer.

Figure 3.2 Schematic modern of UV spectrometer. Adapted from (https://www.google.co.za/search?=schematic+modern+of+UV+spec trometer+picture). Date accessed 11th January 2018

The functions of a UV spectrometer's components are elaborated as follow:

- Monochromator

It isolates the specific wavelength of an element of interest from the other background wavelength and conducts it to the photomultiplier (detector).

- Sample cell

Composed of basic salts, such as sodium chloride or potassium bromide, and are frequently used because they will not absorb light.

- Detector

Measures the quantity of radiation that passes through the sample by converting it to an electrical signal.

- Amplifier

It provides an output which is greater than the input.

3.3 Microwave digestion

Compared to other heating mechanisms, control of microwave heating can be effective because of the ease of cessation of the application of energy, which instantly halts the heating. The direction of heat flow is reversed from conventional heating, as microwave energy is absorbed by the contents of the container, energy is also converted to heat, and the overall temperature of the contents rises.

Heat is generally transferred from the reagent and sample mixture to the container and dissipated through conduction to the surrounding atmosphere. In this technique, pressure and temperature feedback control mechanisms were quickly added to early commercial microwave digestion systems to fully utilize the instantaneous application of energy.

Microwave digestion is a pretreatment technique which consists of various rotors for a fast and completely closed vessel for the digestion of organic or inorganic samples under high pressure and temperatures. During the microwave digestion process, the solid materials in contact with organic molecules in a vessel are converted to liquid form prior to analysis by other instruments. Figure 3.3 describes the internal reactor of microwave digestion.

Figure 3.3 Schematic description of microwave digestion internal reactor (West *et al*. 2009)

Microwave digestion interacts directly with the reaction components, so the sample alone is heated with minimal need for energy to be expended in heating furnaces, containment materials, and the sample environment.

The advantages of using microwave digestion are elaborated below:

- Reduced time
- Better control over reaction
- Extraction efficiency
- Loss of volatile elements is prevented

- Reduced risk of contamination
- Provides an efficient and clean sample preparation for multi-element analytical techniques such as ICP-OES and ICP-MS.

Figure 3.4 below shows the image of Teflon sample vessel (55 mL) used in a microwave digestion.

Figure 3.4 Teflon sample pressure vessel (55 mL)

3.4 Attenuated-total reflectance infrared (ATR-IR) spectrometer

It is a fast nondestructive instrumental technique which involves the twisting, bending, rotating and vibrational motions of many atoms in a molecule due to the electromagnetic radiation and confirms the presence of various functional groups due to the electromagnetic radiation. Attenuated Total Reflection (ATR) is an analytical method, combined with infrared spectroscopy which allows

samples to be examined directly in the solid or liquid state without any sample preparation. The main principle of ATR-IR is that the infrared light beam is transmitted through the ATR crystal which is overlapped on the top by the solid or the liquid sample such that the incident infrared light reflects internally after coming in contact with the crystal covered with the sample, forming an evanescent wave which extends into the sample, typically by a few microns. The incidence angle must be greater than the critical angle for total internal reflectance of the infrared light. When the beam exits the crystal, it is collected by a detector and analyzed and displayed in form of the ATR-IR spectra. Figure 3.5 describes the schematic diagram of ATR system.

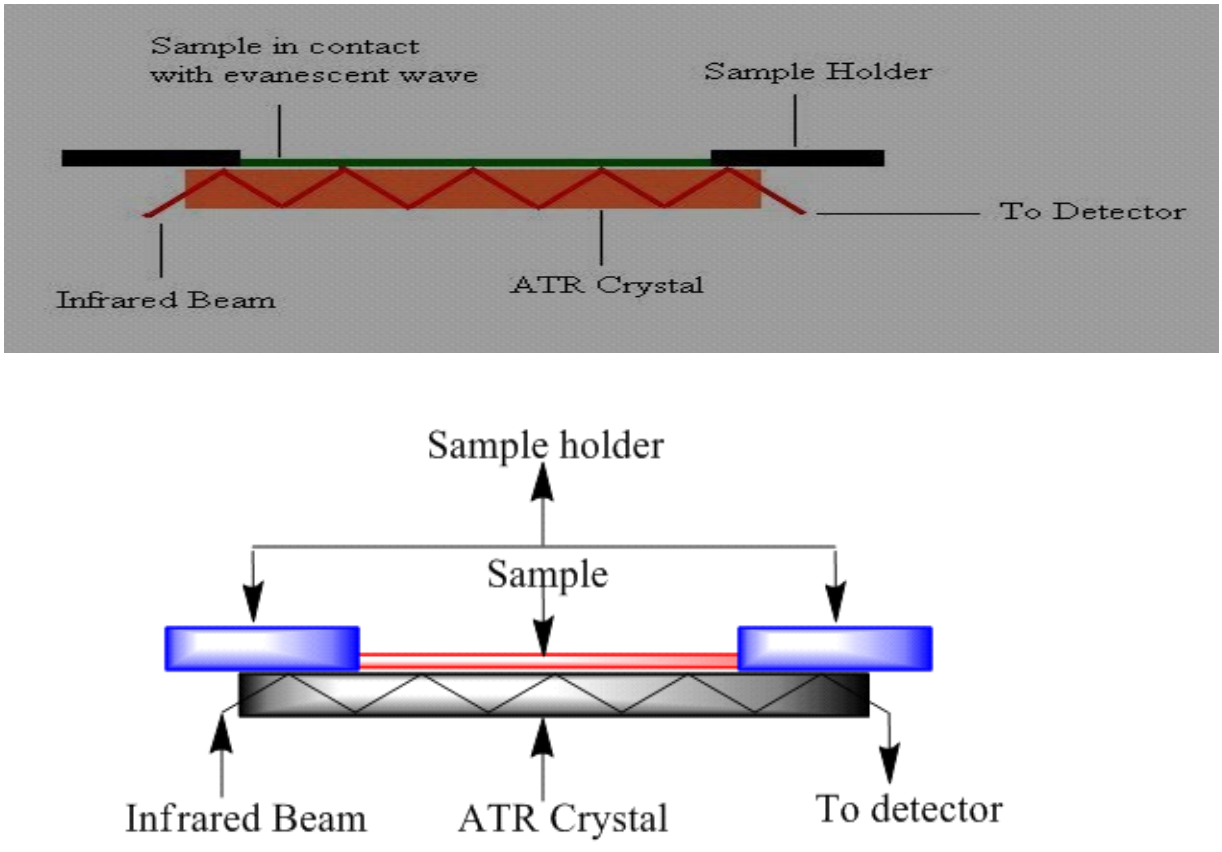


Figure 3.5 Schematic of a multiple reflection ATR system. (Adapted from (https://www.google .co.za/search?=schematic+ATR +system).

ThermoFisher Scientific, South Africa. Date accessed 03rd March 2018

3.5 Thermogravimetric (TG) analyzer

In the thermal analysis technique the physical property of a substance as a function of temperature is determined while the substance is subjected to a controlled temperature program. TG is the branch of thermal analysis used to understand the mass change of a sample as a function of temperature in the scanning mode or as a function of time in the isothermal mode (Willard *et al.* 1986). In the characterization of the decomposition and thermal stability of a liquid or solid sample, thermogravimetric analysis is mostly used to investigate the kinetics of the physicochemical processes. TG is used to provide an analysis of quantitative measurement of any mass change associated with a transition.

As the temperature is raised, the sample can undergo water loss because of water of crystallization and decomposition of the sample. A typical schematic of TGA components is shown in Figure 3.6 below.

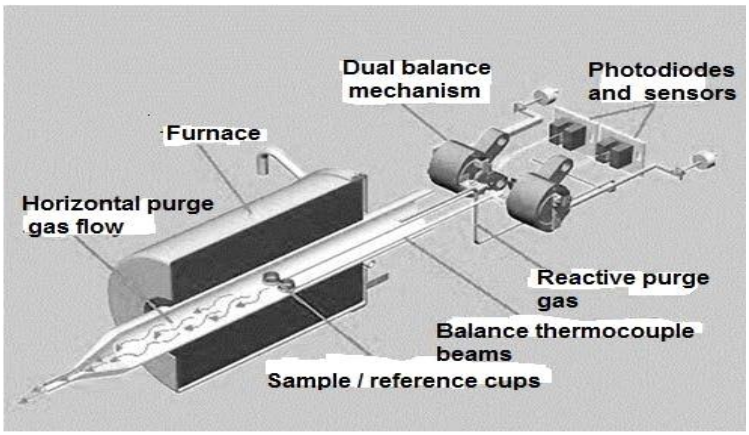


Figure 3.6 Schematic representations of TGA components adapted from (https//www.google.com/search?q=schematic+representation+of+TGA HYPERLINK "http://www.google.com/search?q=schematic%2Brepresentation%2Bof%2BTGA&t"& HYPERLINK "http://www.google.com/search?q=schematic%2Brepresentation%2Bof%2BTGA&t"t bm). Mettler Toledo, Australia. Date accessed 11th January 2018

3.6 High performance liquid chromatography (HPLC)

Chromatography is a technique used to separate mixtures into their components on the basis of their molecular structure and molecular composition. HPLC uses a stationary phase (a solid, or a liquid supported on a solid) and a mobile phase (a liquid or a gas). The mobile phase flows through the stationary phase and carries the components of the mixture with it (Giri *et al.* 2015).

High performance liquid chromatography is basically a highly improved form of column liquid chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster (Laurent *et al.* 2001). The HPLC technique involves the injection of a small volume of liquid sample into a tube

packed with porous particles. Then each component of the sample migrates along the column by a liquid moved by gravity (Laurent *et al.* 2001). Figure 3.7 below is a schematic representation of HPLC components.

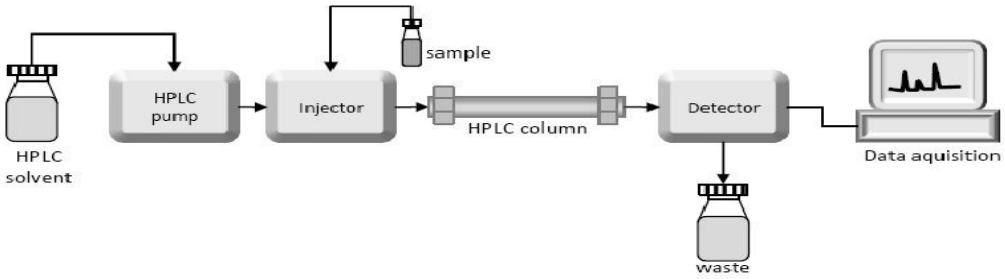


Figure 3.7 Schematic representation of HPLC. Adapted from (https://www.google .co.za/search?=schematic +representation+ HPLC+ PICTURE). PerkinElmer, USA. Date accessed 26th February 2018

CHAPTER 4

EXPERIMENTAL

4.1 Chemicals or reagents

All chemicals used in this study were commercially supplied. The chemicals used are summarized in Table 4.1 below.

Table 4.1: Chemicals, suppliers, purity and CAS number

Chemicals	Suppliers	Purity	CAS Number
1-Ethyl-3-methylimidazolium acetate	Sigma-Aldrich	≥ 95%	143314-17-4
1-Butyl-3-methylimidazolium hydrogen sulphate	Sigma-Aldrich	≥ 95%	262297-13-2
1-4-Dioxane	Sigma-Aldrich	≥ 99%	123-91-1
alkali lignin standard	Sigma-Aldrich	≥ 95%	8068-05-1
citric acid monohydrate	Carl Roth	≥99.5%)	77-92-9
3,5-Dinitrosalicylic acid (DNS)	Sigma-Aldrich	≥ 98.0 %)	609-99-4
Sodium hydroxide (NaOH)	Carl Roth	≥ 99.0 %	1310-73-2
Cellic CTec2	Novozyme A/S	≥ 90.0 %	_
Glucose	Capital lab	≥ 99.0 %	50-99-7
Mannose	Capital lab	≥ 95.0 %	3458-28-4
Galactose	Capital lab	≥ 99.0 %	59-23-4
Xylose	Capital lab	≥ 99.0 %	58-86-6

4.2 Experimental procedure

4.2.1 Sugarcane bagasse pelletization

Sugarcane bagasse pellets were manufactured following the different steps:

- Drying

The moisture of fresh sugarcane bagasse is about 48-50 %. First step is to dry the bagasse using a sawdust dryer model DLK 3000 with 14 m length and 2.5 m diameter.

- Grinding

Grinding the SCB with a pellets press type 14175 from Amandus Kahl (Reinbek, Germany) with the mesh size 3-5 mm.

- Pelletizing

The bagasse powder material is fed into the pellet mill (Hammer mill, Germany with motor of 60 HP and 3200 rpm) then compressed to form pellets by the movement of a die and a set of rollers inside the mill. Figure 4.1 bellow shows the biomass pellets production line from the plant

Figure 4.1: Photography of biomass pellets production line

- **Liquid hot water (LHW) pretreatment**

The experimental procedure for the pretreatment of SBP using LHW is presented in Figure 4.2 below.

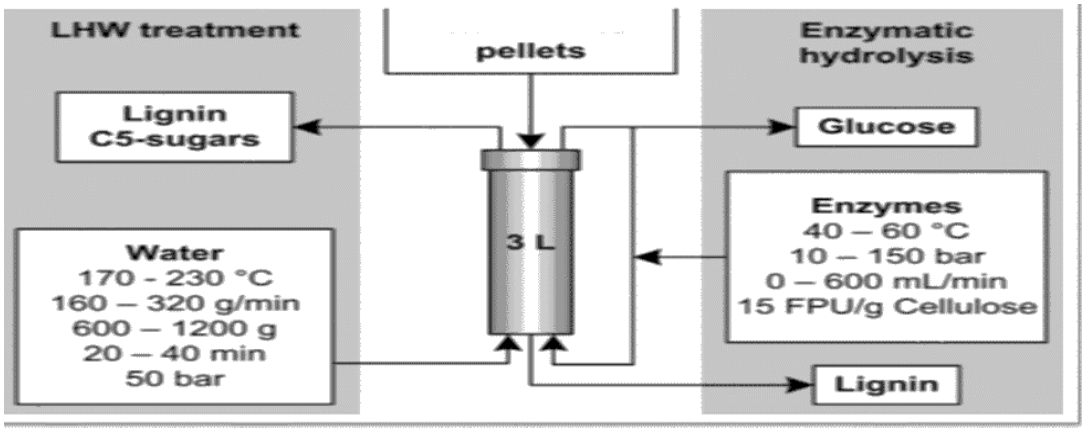


Figure 4.2: Liquid hot water process followed by enzymatic hydrolysis. Wienke *et al*. (2015)

A 1.0 kg of pelletized bagasse was used for the LHW hydrolysis at a temperature of 200 ˚C in a 3 L fixed bed reactor at high pressure for 30 minutes with a volume flow of 250 ml/min of water. Hemicellulose was dissolved in the water that was flowing throughout the reaction and a solid residue (the complex cellulignin)

was collected from the reactor. The experimental setup and procedure for the liquid hot water hydrolysis in a 3 L laboratory unit were previously described Reynolds *et al.* (2015).

4.2.3 Enzymatic hydrolysis of the complex cellulignin

The solid complex cellulignin from the LHW process was enzymatically treated in triplicate using the magnetic hot plates (stirrer 250 rpm) at a temperature of 50 °C for 72 hours as shown in Figure 4.3 below.

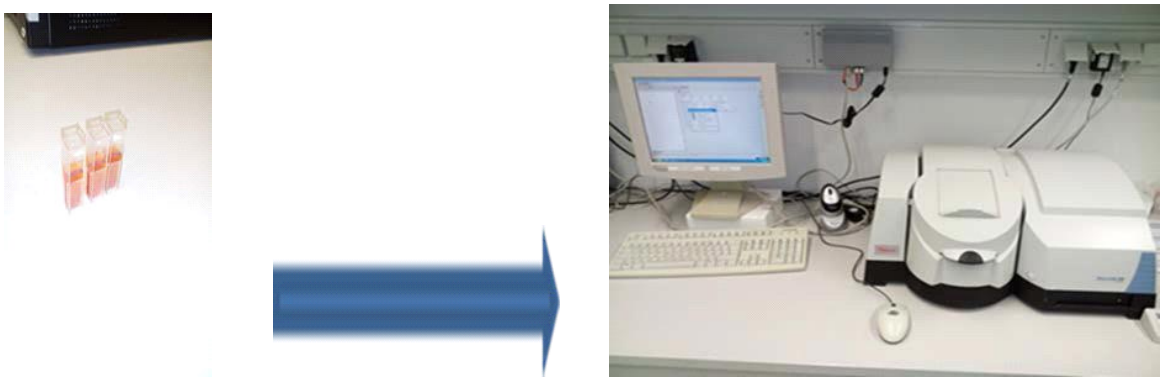
Figure 4.3: Photograph of enzymatic hydrolysis of cellulignin using hot plates

Approximately 60 g of biomass (complex cellulignin) and 600 g of deionized water was used for enzymatic hydrolysis. During the first reaction, 300 µl of Cellic CTec2 enzyme was added to the mixture and the pH was adjusted to 4.80 using 2 M NaOH solution. In the second reaction a ratio 75:25 of Metaplus/Rapidas enzyme was added to the mixture (biomass + water). The sample was thereafter collected from 0 to 72 hours, centrifuged at 2500 rpm for 10 minutes for separation. The liquid solution (glucose solution) was kept in the refrigerator for analysis and the solid

residue (lignin) was dried in an oven overnight at 50 °C and it was ready for compositional analysis (see results in Table 5.6 chapter 5).

- **Analysis of sugars from cellulignin**

The supernatant from the hydrolysate sample (liquid fraction obtained after LHW process) was diluted in order to match the valid absorbance range of the UV photometer. Thereafter 0.167 ml of the diluted sample with 0.333 ml of 0.05 M citrate buffer and 1 ml DNS acid reagent were transferred into 2 ml centrifuge tubes and thoroughly mixed. The mixture was boiled in water for a duration time of 5 minutes and cooled in iced water immediately in order to stop the reaction. The color of the samples should have changed from bright yellow to orange or red. Approximately 0.1 ml of the boiled sample was diluted with 1.25 ml of deionized water in a polystyrene cuvette and UV absorbance was measured at 540 nm. A calibration curve for glucose in a range of 0 to 10 g/L has been conducted prior to the sample analyses. Figure 4.5 describes the photograph of sugar, sample ready for photometry analysis and the UV photometer used in this study.



a b

Figure 4.4: Photograph of sugar sample ready for photometry analysis (a) and the UV photometer used in this study (b)

- **Compositional analysis**

4.2.5.1 Compositional analysis of SBP

- **Moisture content**

The moisture content of SBP was determined gravimetrically as follows: approximately 10 g of SBP sample was weighed in triplicate using aluminum pan and dried at a temperature 105 °C for 4 hours. The samples were cooled in desiccator at room temperature. All masses of samples (mass before drying and mass after drying) were recorded. The moisture content of SBP was determined using the equation 4.1 below.

Moisture content = {(mass before drying – mass after drying) ÷ mass before drying} × 100 **(4.1)**

- **Compositional analysis of hemicellulose, cellulose, lignin and residue**

The composition analysis of hemicellulose, cellulose, lignin and residue from SBP was done using the National Renewable Energy Laboratory (NREL) TP-510-42619 method (Sluiter *et.al* 2008) by Thunen institute of wood research in Hamburg Germany.

4.2.5.2 Compositional analysis of the complex cellulignin from LHW

The complex cellulignin from LHW was analyzed by the Thünen institute of Wood research in Hamburg, Germany. Lignin and sugar contents were determined using soxhlet extraction after two-step sulfuric acid hydrolysis (72 % and 4 % respectively). The remaining solid was dried and weighed. The total lignin was the sum of the klason lignin and the acid soluble lignin. The acidic solution was analyzed by high performance anion exchange chromatography (HPAEC) with photometric detection.

4.2.5.3 Compositional analysis of the hydrolysate from LHW

The hydrolysate composition analysis was performed by the central laboratory of TUUH for sugar monomer and oligomer content. The analysis was performed on a Agilent high performance liquid chromatography (HPLC) with refraction index detection. The column used was VA 300/7.8 Nucleogel sugar Na (Machery-Nagel).

The LHW hydrolysate was treated with 4 % sulfuric acid hydrolysis and neutralization with calcium carbonate.

4.2.5.4 Compositional analysis of the complex cellulignin after enzymatic hydrolysis

The procedure of the compositional analysis the complex cellulignin after enzymatic hydrolysis is similar to section 4.2.5.2 described on page 35.

4.2.5.5 Lignin extraction procedure using microwave digestion

A ratio 1:10 biomass (complex cellulignin or SBP: 0.5 g) and ionic liquid ([Emim][OAc] or [Bmim][HSO_4]: 5 g) was weighed in duplicate and transferred into 65 mL Teflon vessels which have been numbered before weighing the samples Raquel *et al*. (2013). Each vessel was placed into the rotor of the microwave digestion. The parameters such as electrical power (80 Watt); ramp time (10 minutes); temperature control of 180 °C and different hold times of 3; 10; 15; 20; 25; and 30 minutes were set for each run.

After each run the mixture was transferred into a 100 mL beaker from the Teflon vessel and thoroughly rinsed with 10 mL solution containing a mixture of 1-4-dioxane/water 95:5 (v/v) to make sure that all sample was removed. Thereafter the sample was filtered using a vacuum filtration. The filtrate (lignin) was then transferred

quantitatively into a 50 mL volumetric flask and diluted with a mixture of 1-4-dioxane/water 50:50

(v/v) up to the calibration mark ready for UV analysis of lignin at 280 nm from the complex cellulignin and SBP respectively.

Approximately 0.1 g of the solid remained (lignin) after vacuum filtration was dissolved in a mixture of 1-4-dioxane/water 50:50 (v/v) into 10 mL volumetric flask. Lignin was thereafter quantified using UV 2401 PC Shimadzu Japan (spectrometer) at 280 nm.

- **Characterization**

4.2.6.1 UV-VIS spectroscopy

Based on the results from the compositional analysis of the complex cellulignin and the sugarcane bagasse pellets, different concentrations of alkali lignin standard were prepared (from 0.01 % m/v to 0.30 % m/v) in order to plot a linear calibration curve Raquel *et al*. (2012).

Example of stock solution (0.05%) preparation procedure: approximately 5 mg of alkali lignin standard was weighed on analytical balance then transferred into 50 mL volumetric flask. The sample was dissolved with a mixture 1-4-dioxane/water 95:50 (v/v) into 10 mL volumetric flask up to the calibration mark. Thereafter 1 mL of the aliquot was transferred into 10 mL volumetric flask and diluted with a mixture 1-4-dioxane/water 50:50 (v/v). Solution ready for UV analysis was performed in the range of 800-190 nm using UV-2401 PC, Shimadzu Japan. The extracted lignin absorbance peak was quantified at 280 nm.

4.2.6.2 Attenuated total reflectance infra-red (ATR-IR) spectroscopy

All the samples (cellulignin and SBP) were characterized by attenuated-total reflectance infrared (ATR-IR) spectroscopy by direct transmittance in a single reflection ATR System (ATR top plate fixed to an optical beam condensing unit with

ZnSe lens) with a FTIR spectrometer instrument (Perkin Elmer Spectrum 100) Lilia *et al*. (2013). The measurement for each spectrum was performed within 45 seconds in the region 500 – 4000 cm^{-1}.

4.2.6.3 Thermogravimetric (TGA) analysis

The thermogravimetric analysis was also carried out to characterize thermal stability of cellulignin and SBP samples. The thermal stability of the samples was carried under Nitrogen atmosphere using TGA/DSC 1 STAR system Mettler Toledo instrument Melieh *et al.* (2014). The heating rate was set at 5 °C/min from 25 to 550 °C. All analyses were performed in aluminum oxide crucible.

4.2.6.4 X-ray diffraction (XRD) analysis

X-ray diffraction analysis of cellulignin and SBP samples was performed using a Pan Analytical X'Pert PRO X-ray Diffractometer instrument fitted with a Cu Kα radiation source Saksit *et al*. (2014). The Wide-angle-X-ray intensities were recorded from 5° to 80° in 2θ, at a speed of 3°/min operating V&I = 45 kV, 40 mA.

4.2.6.5 High performance liquid chromatography (HPLC)

The HPLC analysis done is this research was performed by the Thünen institute of wood research in Hamburg, Germany Wienke *et al.* (2014). The Agilent high performance liquid chromatography with a refractive index detector, VA300/7.8 Nucleogel sugar Na (Machery-Nagel) column was used with a detection limit of 100 mg/L.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 Compositional analysis

5.1.1 Compositional analysis of the untreated sugarcane bagasse pellets (SBP)

5.1.1.1 Moisture content

The average moisture content of the untreated SBP was found to be 5.21 % and was calculated using equation (4.1).

The moisture content of SBP was compared in Table 5.1 with the moisture content from different biomass feedstocks.

Table 5.1 Moisture content from different biomass feedstocks

Biomass feedstock	Moisture content %	References
Corn stover	70	Womac *et al*. 2005
Corn stover pellets	28 -38	Nicoleta *et al*. 2018
Sugarcane mill bagasse	52	Manickavasagam *et al.* 2018
Wheat straw	10.4 – 20.3	Guo *et al.* 2017
Barley straw pellets	19 – 23	Serrano *et al.* 2011
Starch pellets (cellulosic material)	8 – 12	Liu *et al.* (2005)
Dry wood	5 – 12	Li *et al.* (2003)
Sugarcane bagasse pellets	5.21	Current research work

Moisture content of biomass feedstocks is the main factor that affects the properties of pellets, such as the bulk density or mechanical durability during storage and transportation as observed by Nicoleta *et al*. (2018). Low moisture content of SBP is probably due to high temperature steam used in the pelletizing operations to activate natural binders and lubricants in biomass. During pelletizing, high fiber feeds cannot absorb moisture in the conditioning

chamber therefore water stays on particle surface as observed by Nicoleta et al. (2018). Low moisture content of SBP is due to the compressibility of water during the pelletizing process and water evaporation. The study conducted by Nicoleta *et al.* (2018) confirmed that pelletizing relies upon the moisture content of biomass density, the size of particles, fiber quality, biomass lubricating attributes and the contribution of natural binders. In other research guided by Liu *et al.* (2005), it has been discovered that the ideal moisture content for pelletization of cellulosic material is 8 -12 % while for materials containing starch and protein can be up to 20 %. Li *et al.* (2003) during their study, reported moisture content of 5 -12 % was able to produce a good quality pellets (in terms of optimal density and pellets durability). The value for the moisture content for SBP for this work (5.21 %) is within the range for SBP samples.

5.1.1.2 Hemicellulose, lignin, cellulose and residue

As shown in Table 5.2, the compositional analysis of the untreated SBP constituted 40 % cellulose, 25 % hemicellulose, 25 % lignin and 10 % inorganic matters. This finding is consistent with that reported by Konstantin-Gabov *et al.* (2017) that sugarcane bagasse consists of 40 – 45 % of cellulose, 20 – 25 % of lignin, 25 – 30 % of hemicellulose and inorganic material, and other extracts (1 - 5 %).

Table 5.2 Compositional analysis of SBP

Compounds	**Mass % (m/m)**	**% RSD (total)**
Cellulose	40	± 5.5
Hemicellulose	25	
Lignin	25	
Residue	10	

5.1.2 Liquid hot water pretreatment of SBP

- **Composition of the hydrolysate from LHW**

The composition analysis (liquid fraction) is given in Table 5.3

Table 5.3 Liquid fraction or hydrolysate compositional analysis from LHW. Mthembu (2015)

Compounds	Monomers (mg/L)	Oligomers (mg\L)	Total (g/L)	Mass of compound in 4L	% hydrosylate purity inSBP
Cellubiose	<50	460	510×10^{-3}	2.04	0.2
Glucose	115	1200	1315×10^{-3}	5.26	0.53
Xylose	1400	21300	22700×10^{-3}	90.8	9.08
Arabinose	1100	2300	3400×10^{-3}	13.60	1.36
Formic acid	<50	<100	150×10^{-3}	0.6	0.06
Acetic acid	1100	3700	4800×10^{-3}	19.20	1.92
Levulinic acid	<50	<100	150×10^{-3}	0.6	0.06
HMF	<50	<100	150×10^{-3}	0.6	0.06
Furfural	370	570	940×10^{-3}	3.76	0.38

The hydrolysate from LHW contains sugars, acids, HMF and furfural. The majority of the hemicellulose is retained in oligomeric and monomeric forms in the liquid fraction (Mthembu. 2015). The superheated liquid water auto ionizes into hydronium ions which act as a promoter to break the ester bonds of acetyl side chains of hemicellulose involving formation of acetic acid and played the major role in converting the hemicellulose chains into the liquid form Teo *et al*. (2010).

The high xylose content of 21300 mg/L indicates that hemicellulose was removed.

In order to determine the total amount of hemicellulose removed in the hydrolysate, it was assumed that all the hydrolysate were obtained from hemicellulose.

- SBP contains 25 % of hemicellulose as mentioned in Table 5.3
- Total components in the hydrolysate (monomers + oligomers) = 34115 mg/L = 34.115 g/L.
- After LHW process 5 L of hydrolysate was retained
- Total components in 5 L hydrolysate = (34.115 g/L × 5) = 170.575 g/L.

- Since 1 kg of SBP was used in LHW process, therefore
- The percentage of hemicellulose removed from 1 kg SBP = (170.575 ÷ 1000) × 100 = 17.07 %.
- Percentage of hemicellulose remained in the complex cellulignin after LHW process should be = 25 – 17.07 = 7.94 % (theoretically value) which is lower compared to 11 % of hemicellulose from Table 5.5.

This is probably due to the removal of cellulose as well during LHW processing of SBP and sugars removed from cellulose is present in the hydrolysate.

5.1.2.1 Composition analysis of cellulignin

The complex cellulignin in its solid form remained inside the reactor after the liquid hot water process on sugarcane bagasse pellets. The compositional analysis of cellulignin after LHW process is reported in Table 5.4

Table 5.4 Compositional analysis of cellulignin. Mthembu (2015)

Compounds	**Mass % (m/m)**
Cellulose	56
Total lignin	29
Hemicellulose	11
Residue	4

It was observed that when comparing the composition of the untreated SBP (Table 5.3) and cellulignin, the percentage of hemicellulose decreased from 25 % to 11 %. Therefore this indicates that LHW was successful in removing hemicellulose in SBP during the LHW process. Lignin content increased from 25 % to 29 %.

Cellulose content increased from 40 % to 56 % while hemicellulose was reduced from 25 % to 11 %.

5.1.2.2 Compositional analysis of the hydrolysate after enzymatic hydrolysis of cellulignin

Table 5.6 below shows the results of the liquid fraction after the enzymatic hydrolysis of the complex cellulignin.

Table 5.5 Compositional analysis of the liquid fraction obtained after enzymatic hydrolysis. Mthembu (2015)

Compounds	Monomers (mg/L)	Oligomers (mg\L)
Cellubiose	1500	730
Glucose	42800	44000
Xylose	9600	11700
Arabinose	500	600
Formic acid	500	0.0
Acetic acid	2600	0.0
Levulinic acid	<50	0.0
HMF	60	160
Furfural	400	550

High sugar content (111430 mg/L) was observed in the liquid fraction after the enzymatic hydrolysis of the complex cellulignin (Table 5.5). This observation is probably due to the strong modification in the cellulignin structure brought about by the hemicellulose and lignin extraction from the sample. As an outcome, cellulose fibers were isolated being progressively susceptible to enzymatic hydrolysis. It is, therefore, reasoned that the lower the hemicellulose and lignin quantities in the complex cellulignin, the higher the effectiveness of cellulose hydrolysis compared to the untreated sample (Solange *et al.* 2008). These results were also supported in the study conducted by Jin *et al.* (2016) who reported that the highest increase in glucose yield was assigned to higher removal of

hemicellulose, which provides more efficiency of enzyme accessibility, therefore, release more sugars in the liquid fraction.

5.1.2.3 Compositional analysis of lignin (solid residue) after liquid hot water process

The composition analysis of the lignin (solid residue) recovered from LHW process followed by enzymatic hydrolysis was tabulated in Table 5.6 below.

Table 5.6 Compositional analysis of the solid residue (lignin recovered)

Components	**Mass % (m/v)**
Klason lignin	36.1
Acid soluble lignin	1.7
Total lignin	37.8
Total sugars	57.7
Xylose	8.8
Glucose	47.9
Mannose	0.2
Galactose	0.2
Arabinose	0.5
Rhamnose	0.1
Residue	4.5

The results from Table 5.7 shows that the percentage yield of the total lignin extracted from SBP using the LHW pretreatment method followed by enzymatic hydrolysis has found to be 37.8 %. In addition, the sugars decreased from 67 % to 57.7 % with total l i g n i n content which increased from 29 % to 37.8 % in solid residue. This indicates that cellulose in the hydrolysate was from the liquid hot water process.

5.2 Percentage lignin recovered from cellulignin and SBP using ionic liquid and microwave digestion

Table 5.7 shows lignin recovered (%) (m/v) from the complex cellulignin and SBP treated with two different ionic liquids ([Emim][OAc] or [Bmim][HSO_4]) using microwave digestion at different time intervals.

Table 5.7 Lignin recovered (%) from cellulignin and sugarcane bagasse pellets using

[Emim][OAc] or [Bmim][HSO4]

Time / min						
Experimental number	3	10	15	20	25	30
	Lignin concentration (%) (m/v) from the complex cellulignin treated with [Emim][OAc]					
Run 1	48.04	67.46	39.80	36.19	Was not done because 10 min was the optimum time.	
Run 2	51.00	68.75	39.56	37.59		
Average	49.52	**68.00**	39.68	36.89		
Experimental Number	Lignin concentration (%) (m/v) from the complex cellulignin treated with [Bmim][HSO_4]					
Run 1	16.04	30.98	16.65	17.97	Was not done because 10 min was the optimum time.	
Run 2	13.15	33.11	21.23	16.35		
Average	14.59	**32.04**	18.94	17.16		
Experimental Number	Lignin concentration (%) (m/v) from SBP treated with [Emim][OAc]					
Run 1	31.49	30.64	59.50	65.84	59.67	56.58
Run 2	30.25	68.23	57.31	68.65	60.28	58.92
Average	30.87	29.64	58.15	**67.20**	59.98	57.75
Experimental Number	Lignin concentration (%) (m/v) from SBP treated with [Bmim][HSO_4]					
Run 1	24.92	36.00	45.08	47.84	35.23	35.96
Run 2	22.61	36.21	46.37	50.04	37.87	35.07
Average	23.77	36.11	45.73	**48.94**	36.55	35.51

The lignin recovered (%) was calculated as follows:

- Total mass of lignin from the compositional analysis of cellulignin and sugarcane bagasse pellets (total mass expected).
- Ionic liquid fraction.
- Total mass of the extracted lignin (liquid and solid fractions) using microwave digestion.

The following equation was used to calculate lignin recovered percentage

Figure 5.1 is a graphical representation of the results tabulated in Table 5.8 which showed the mean lignin recovered (%) from the complex cellulignin and sugarcane bagasse pellets for the two ionic liquids.

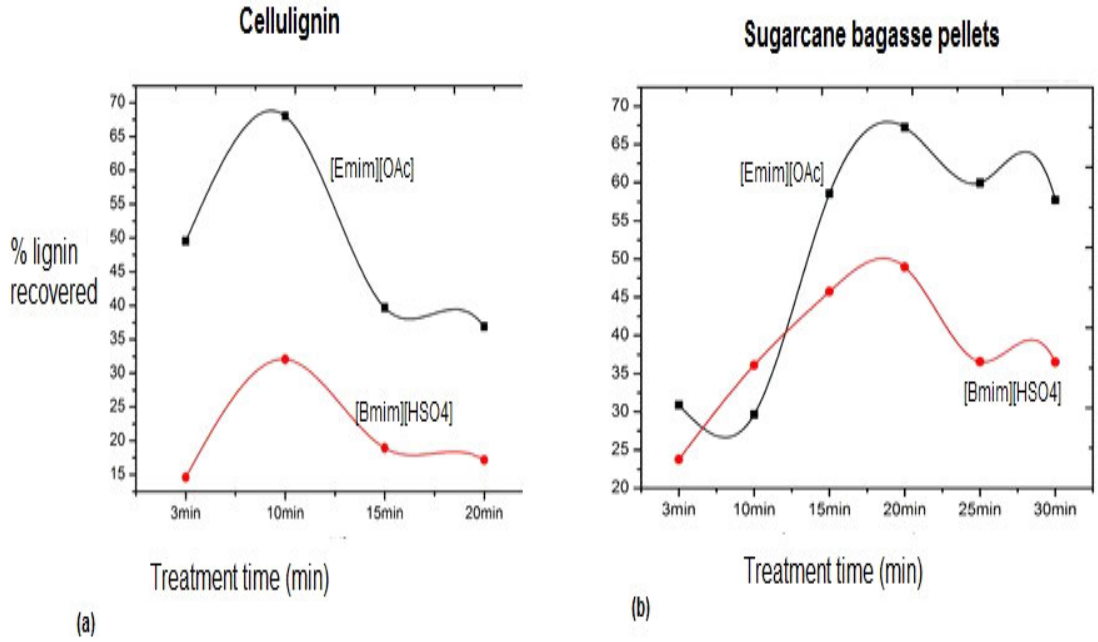


Figure 5.1 Graphs of lignin recovered (%) from (a) cellulignin and from (b) sugarcane bagasse pellets

From Table 5.7 and Figure 5.1 it can be observed that at 10 min and 20 min treatment time the maximum lignin recovered (%) was obtained for ionic liquids (1-ethyl-3- methylimidazolium acetate or 1-butyl-3-methylimidazolium hydrogen sulphate) for the complex cellulignin and SBP, respectively. But at 10 min treatment time 68 % of lignin was recovered from the complex cellulignin treated with [Emim][OAc] ionic liquid. At 20 min treatment time 67.20 % of lignin was obtained from sugarcane bagasse pellets treated with [Emim][OAc]. At 10 min treatment time 32.04 % of lignin was recovered from the complex cellulignin treated with [Bmim][HSO_4] ionic liquid. But at 20 min treatment time, the maximum % lignin obtained from sugarcane bagasse pellets treated with [Bmim][HSO_4] was found to be 48.94 %. Therefore, 10 min and 20 min were considered as the optimum treatment time for lignin

extraction in this research work. The ionic liquid [Emim][OAc] gave better yields of the lignin extracted from the complex cellulignin and sugarcane bagasse pellets compare to [Bmim][HSO_4] ionic liquid.

This can be explained due to the greater affinity between the aromatic ring of [Emim][OAc] ionic liquid and the aromatic ring of lignin complex compared to [Bmim][HSO_4] ionic liquid. According to George *et al.* (2011), when using imidazolium based ionic liquid, the α-aryl ether linkages cleavage of lignin is due to dehydration reaction, catalyzed by imidazolium anion basicity and its affinity towards water. Furthermore, rather than acting as nucleophiles or a catalyst to cleave β-O-4 linkages of lignin, acetate anion bonded to 1-ethyl-3-methyl imidazolium cation acts as a weak nucleophile to remove OH groups from guaiacyl units of lignin components. The strong affinity of the imidazolium cation to lignin could be attributed to attractive forces between the cation component of the ionic liquid and the OH^- groups of the lignin, leading to strong bonding (Idi *et al.* 2011).

[Emim][OAc] ionic liquid has a shorter alkyl chain therefore provides more affinity with the polar compound (lignin) compared to [Bmim][HSO_4] ionic liquid. The hydrogen in the OH groups of lignin have high affinity with [Emim][OAc] ionic liquid anion (acetyl group) because both interact through hydrogen bonding. The explanation agrees with the observations by Espinoza *et al*. (2014) and confirmed that the main force involved in the dissolution process of lignocellulosic biomass components is hydrogen bonding. The study conducted by Haykir *et al*. (2013) found that the pretreatment of cotton stalk with ionic liquids, namely, 2-hydroxy ethyl ammonium format, 1-allyl-3- methylimidazolium chloride, 1-butyl-3-methylimidazolium chloride and 1-ethyl-3- methylimidazolium acetate before

enzymatic hydrolysis of the lignocellulosic feedstock showed that [Emim][OAc] was the best ionic liquid. In addition,

Karatzos *et al*. (2012) successfully used three ILs, 1-butyl-3-methylimidazolium chloride (Bmim][Cl]),1-ethyl-3-methylimidazolium chloride ([Emim][Cl]), 1-ethyl-3-methylimidazolium acetate ([Emim][OAc]) to pretreat and fractionate sugarcane bagasse. In these ILs based pretreatment the biomass was completely or partially dissolved in ILs at temperatures greater than 130°C and then precipitated by the addition of an anti-solvent to the IL biomass mixture. Sugarcane bagasse pretreated with [Emim][Cl] and [Emim][OAc] recovered 50 and 60 % of lignin respectively.

Recent studies have indicated that the ionic liquid [Emim][OAc] is effective for removing lignin Samayam *et al*. (2010).

Six ionic liquids evaluated for removing lignin in flax, triticale and wheat straw (Fu *et al*. 2010 a) showed optimal results of 52.7% acid insoluble lignin in triticale straw using [Emim][OAc] ionic liquid and was proved to be the more efficient ionic liquid than the others. Lynam *et al.* (2012) determined the effect of [Emim][OAc],[Amim][Cl] and [Hmim][Cl] on the fractionation of the main constituents of rice hulls. [Emim][OAc] (110 ^{o}C, 4 h) removed 46 % of lignin. The additional time allow [Emim][OAc] to penetrate further into rice hull. This indicate that lignin extraction yield improve with long incubation time and high temperature, therefore, the extraction condition affects lignin removal and yield. The advantage of releasing more lignin yield from the biomass using [Emim][OAc] was also confirmed in the study conducted by Espinoza *et al.* (2014); the structure of the ionic liquid contains an imidazolium based salt and two alkyl groups; because of the cationic nitrogen in the imidazolium, the ionic liquid maybe physically and

chemically associated with lignin such as β-O-4, α-O-4 and β-β linkage. Furthermore Lee *et al*. (2009) confirmed that lignin removal using [Emim][OAc] depend on the anion interaction of the ionic liquid and the biomass.

5.3 Statistical analysis

Lignin recovered (%) from the complex cellulignin and sugarcane bagasse pellets treated with both ionic liquids: [Emim][OAc] and [Bmim][HSO_4] is shown in the Table 5.8

Table 5.8 Lignin recovered (%) from complex cellulignin and sugarcane bagasse pellets treated with [Emim][OAc] and [Bmim][HSO_4]

Complex cellulignin			
Time	Ionic liquid	Lignin recovered (%)	Std. Deviation
3 min	[Emim][OAc]	49.52	2.09
	[Bmim][HSO_4]	14.59	2.04
10 min	[Emim][OAc]	68.00	1.05
	[Bmim][HSO_4]	32.04	1.51
15 min	[Emim][OAc]	39.68	0.17
	[Bmim][HSO_4]	18.94	3.24
20 min	[Emim][OAc]	36.89	0.99
	[Bmim][HSO_4]	17.16	1.15
Sugarcane bagasse pellets (SBP)			
3 minutes	[Emim][OAc]	30.87	0.88
	[Bmim][HSO_4]	23.77	1.63
10 minutes	[Emim][OAc]	29.64	1.42
	[Bmim][HSO_4]	36.11	0.15
15 minutes	[Emim][OAc]	58.15	0.60
	[Bmim][HSO_4]	45.73	0.91
20 minutes	[Emim][OAc]	67.20	1.99
	[Bmim][HSO_4]	48.94	1.56
25 minutes	[Emim][OAc]	59.98	0.43
	[Bmim][HSO_4]	36.55	1.87
30 minutes	[Emim][OAc]	57.75	1.66
	[Bmim][HSO_4]	35.51	0.78

From Table 5.8, a subtle difference of lignin recovered (%) was observed for the treated cellulignin and the SBP with both ionic liquids with respect to the treatment time.

The highest % lignin recovered (68.00 %) was obtained for the cellulignin treated with [Emim][OAc] ionic liquid at 10 minutes reaction time while the lowest lignin recovered (%) was found to be 36.89 % at 20 minutes. On the contrary the cellulignin treated with [Bmim][HSO_4] ionic liquid, had the highest lignin recovered (%) at 10 minutes (32.04 %) and the lowest was obtained at 3 minutes (14.59 %).

At 3 and 10 minutes, the percentage of lignin achieved from SBP treated with [Emim][OAc], were reduced from 30.87 % to 29.64 %, respectively. The lignin

recovered (%) increased from 58.15 % at 15 minutes to 67.20 % at 20 minutes reaction time. However, a decrease in % lignin recovered after 20 minutes of SBP treated with [Emim][OAc] was noted. On the other hand, SBP treated with [Bmim][HSO_4] showed an increase in lignin recovered (%) as the treatment time increased from 3 minutes to 20 minutes, but decrease in lignin recovered (%) after 20 minutes of treatment was observed, hence suggesting that 20 minutes as the optimal time of treatment. Lignin recovered (%) obtained from the samples (cellulignin and the SBP) treated with [Emim][OAc] were consistently higher than those treated with [Bmim][HSO_4] with respect to the time of treatment.

The results showed that 10 minutes and 20 minutes are the optimum times for the two ionic liquid pretreatment methods for cellulignin and SBP, respectively.

During this research work, the experiment was done in duplicate per each treatment time. Therefore the standard deviation values for example 1.05 for the cellulignin treated with treatment [Emim][OAc] and 1.99 for the SBP treated with [Bmim][HSO_4] can be considered as low standard deviation (Table 5.8). Therefore low standard deviation demonstrates that the data lignin recovered (%) incline toward the mean. In addition this means there is no a huge difference between the data which was obtained in duplicate.

In Table 5.9 below, Anova test of mean lignin recovered (%) from cellulignin and SBP was performed to confirm what was observed from Figure 5.1 and Table 5.9. Anova test was used to verify whether there is any significant difference between treatment times; which means to confirm if there is any significant different between the values of lignin recovered (%) per each treatment time and the treatment groups (in overall any significant difference of lignin

recovered (%) for the samples treated with [Emim][OAc] and [Bmim][HSO_4] ionic liquids).

Table 5.9 Anova test of mean lignin recovered from cellulignin and SBP

	Source	Type III sum of squares	Df	Mean square	F	P	Partial eta squared
Cellulignin							
Time/min	Greenhouse-geisser	1319.038	1.749	754.351	328.8	0.000	0.994
Time * Group	Greenhouse-geisser	232.314	1.749	132.859	57.9	0.002	0.967
Error (Time)	Greenhouse-geisser	8.021	3.497	2.294			
SBP							
Time/min	Greenhouse-geisser	2778.520	1.679	1654.853	305.5	0.000	0.993
Time * Treatment	Greenhouse-geisser	618.093	1.679	368.129	67.9	0.002	0.971
Error(Time)	Greenhouse-geisser	18.185	3.358	5.415			

Where Df is the degree of freedom; P-value is the level of marginal significance representing a given event's probability of occurrence; F Anova values representing the variance of the group means (Mean Square Between). As shown in Table 5.9 the mean lignin recovered (%) from cellulignin treated with [Emim][OAc] and [Bmim][HSO4] with respect to time differed significantly beyond the 0.05 level. $P < 0.01$ with Greenhouse-Geisser adjustment means there is a difference which exist between the treatment times and the lignin recovered (%) from cellulignin. At each treatment time, the lignin recovered (%) is different and can be clearly observed from the results in Table 5.10. Partial eta squared above 0.5 represented a significant effect of time on lignin yield (Cohen.1988). This suggests that the percentage yields of the lignin extracted were not the same with respect to the different time of treatment for both ionic liquids. The mean lignin recovered (%) from SBP extracted using [Emim][OAc] and [Bmim][HSO_4] ILs with respect of time for the treatment differed significantly beyond the 0.001 level. $P < 0.001$ means

statically highly significant. This indicates that the recovery of lignin pattern for both ILs changes with time with a large effect.

5.4 Characterization

5.4.1 UV-VIS spectroscopy

5.4.1.1 Alkali lignin standards

Figure 5.2 below is the standard calibration curve of alkali lignin standards used during this study.

Figure 5.2 Alkali lignin standards calibration curve

From Table 5.4 it was observed that lignin represents only 29 % from the entire complex cellulignin. This means that if 0.5 g of cellulignin sample is treated, the maximum lignin recovery in term of total mass of lignin should be 0.145 g. Thus, from Figure 5.2, we can confirm that there is a strong linear relationship between the concentration of the lignin and the absorbance. Table 5.10 reports the different absorbance obtained with different concentrations of alkali standard.

Table 5.10 Alkali lignin standards concentrations and absorbance

Lignin concentrations (% m/v)	Abs [cm^{-1}]
0.01	0.32
0.02	0.49
0.03	0.49
0.05	0.94
0.1	1.91
0.125	2.02
0.15	2.73
0.2	3.03

0.23	3.17
0.25	3.88

5.4.1.2 Lignin extraction from the complex cellulignin

UV spectra of cellulignin treated with [Bmim][HSO_4] and [Emim][OAc] ionic liquids is shown in Figure 5.3. The absorbance of the extracted lignin peaks from Figure 5.3 (a) is seen to increase steadily from 3 to 10 minutes and thereafter a decrease in absorbance is observed. The optimum time of lignin extracted from the complex cellulignin using both ionic liquids [Emim][OAc] ionic liquid (Figure 5.3 a and b) is therefore 10 minutes with a maximum percentage yield of 68.00 % and 67.20 %. High absorbance peak at 10 minutes reaction time indicates, the presence of the most important functional group i.e. a free phenolic group in the extracted lignin, which corroborates with the absorbance band obtained from the ATR analysis (see Figure 5.4.2) and the existence of aromatic rings conjugated by $C_\alpha = C_\beta$ and the C=C linkages Sidiki *et al*. (2013)

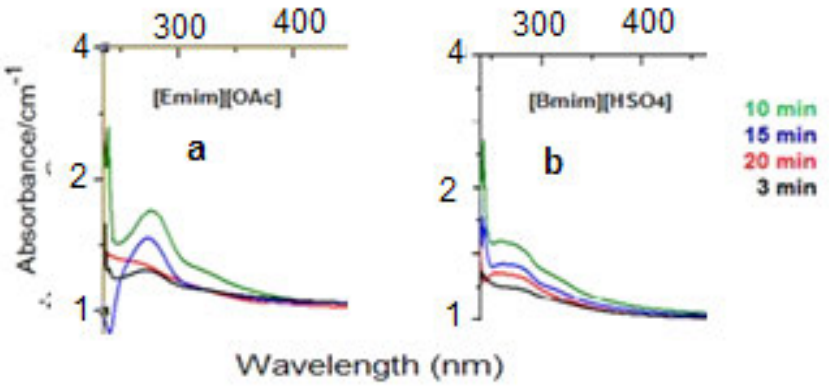


Figure 5.3: UV spectra of the extracted lignin from cellulignin treated with ILs: [Emim][OAc] (a); and [Bmim][HSO_4] (b) : 10 min ; 15 min ; 20 min ; 3 min

5.4.1.3 Lignin extraction from sugarcane bagasse pellets

UV spectra of the extracted lignin from sugarcane bagasse pellets treated with [Bmim][HSO_4] and [Emim] [OAc] ionic liquids is shown in Figure 5.4

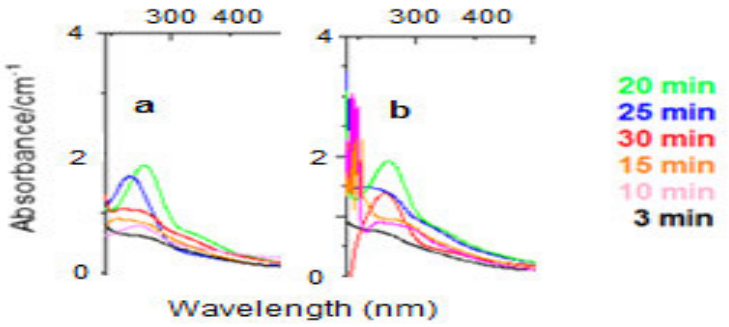


Figure 5.4: UV spectra of the extracted lignin from sugarcane bagasse pellets treated with ILs: [Emim][OAc] **(a)** and [Bmim][HSO4] **(b)** 20 min ; 25 min; 30 min
15 min; 15 min; 3 min

From 3 to 20 min, the absorbance peaks increased proportionally as time increases and thereafter decreases after 30 min treatment time.

Treatment time of 20 minutes can be considered as the optimum time required for maximum lignin yield (67.25 %) as shown in Figure 5.4. The wavelength of maximum absorbance (λ_{max}) for absorption spectra was not consistent. A drift from 280 nm was observed. Figure 5.4 (b) was more consistent in term of λ_{max} in comparison to Figure 5.4 (a). From the UV analyses, shorter time for lignin extraction (68 %) from the complex cellulignin (10 min) compared to the sugarcane bagasse pellets (20 min), 67.20 % owing to the fact that the complex cellulignin has a less compact structure compared to sugarcane bagasse pellets by the removal of hemicellulose during the LHW process. In a similar investigation by Ko *et al.* (2015) verified that hemicellulose was mostly depolymerized, and its degradation products are dissolved in the liquid phase during LHW process. Therefore lignin and cellulose are retained completely in the solid portion.

- **Attenuated total reflectance infrared (ATR-IR) spectroscopy**

Figures 5.5 and 5.6 show the experimental results obtained during ATR-IR for the spectra scanned from 4000 cm^{-1} to 500 cm^{-1}. For the samples treated with ILs and lignin standard, the absorption peaks around the region of 3500-3000 cm^{-1} is attributed to the O-H stretching of the hydroxyl groups in the lignin and carbohydrate molecules (Li *et al*. 2012; Kumar *et al*. 2013). The presence of the O-H groups between 3100 and 3400 cm^{-1} was due to the absorption of moisture in the treated samples. In contrast, the O-H stretching was absence in the spectra of the untreated samples which is attributed to its storage conditions in a desiccator prior to analyses. All spectra (Figure 5.5 and 5.6) show lignin patterns although some difference in the intensities and widths of absorption bands are observed, all spectra have a strong wide band in the 3500 – 3100 cm^{-1} wavenumber range as mentioned above. The band is caused by the presence of alcoholic and phenolic hydroxyl groups involved in hydrogen bonds (Nikitin, V.M. 1961).

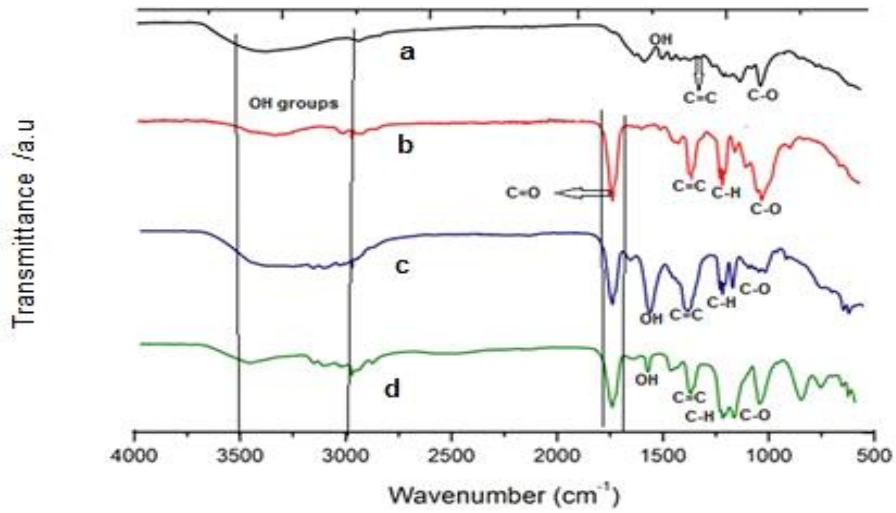


Figure 5.5: ATR spectra of lignin standard (a); untreated cellulignin (b); cellulignin residue sample after treating with [Emim][OAc] (c) and cellulignin residue sample after treating with [Bmim][HSO_4] (d).

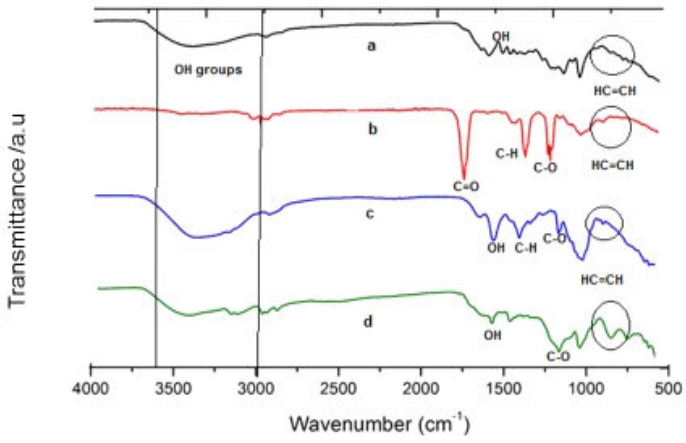


Figure 5.6. ATR spectra of lignin standard (a); untreated SPB (b); SPB residue sample after treating with [Emim][OAc] (c) and SBP residue sample after treating with [Bmim][HSO_4] (d).

In Figures 5.5 and 5.6, the spectra of the untreated samples (cellulignin before ILs treatment and untreated pellets) showed similar peaks situated in the region of 1750

cm^{-1} mostly attributed to the C=O stretching vibration of acetyl and ester groups from hemicellulose and the p-coumaric acids of lignin by linkage as reported by Sun *et al*. (2005).A band positioned around 1640 cm^{-1} corresponds to the O-H bending of water absorbed into cellulose fiber structure and is present in all samples Zhao *et al.* (2010).

From Figure 5.5, the peaks observed in ATR spectra (treated cellulignin with ILs and lignin standard) at 1750 cm^{-1}, and 1473 cm^{-1} were assigned to the characteristic vibrations of aromatic structures in lignin due the presence of C=C aromatic skeletal vibration situated in the region of 1618 cm^{-1} as reported by Capraru *et al*. (2009).The peak at 1345 cm^{-1} is related to C-H deformation of lignin as reported by Labbe *et al*. (2005). The peak at 1125 cm^{-1} observed in Figures (5.6 and 5.7) is attributed to the aromatic C-H stretch in plain deformation of syringyl units. The peaks at 765 cm^{-1} arises from CH=CH bending associated with the syringyl units.

The peak located around the region of 1730 cm^{-1} is for the most part attributed to the C=O stretching vibration of the acetyl and uronic ester groups, from gelatin, hemicellulose or the ester linkage of the carboxylic gathering of ferrulic and p-coumaric acids of lignin as well as hemicellulose (Sain *et al.* 2006). The prominent peak around the region of 1750 cm^{-1} while the cellulignin residue is treated with [Bmim][HSO_4] ionic liquid is expected to C=C aromatic skeletal vibration. The absorption peak at 1513 cm^{-1} is related to the C=C in the aromatic ring presence in lignin was reported by Wyeth *et al*. (2003). The vibration peak detected at 1382 cm^{-1} is predominantly attributed to the bending vibration of the C-H and the C-O bonds in the polysaccharide aromatic rings. The absorption peak at 1254 cm^{-1}

represents the C-O out of plane stretching vibration of the aryl/ phenol group in lignin Troedec *et al*. (2008).

The band detected at 1164 cm^{-1} is due to the O-H stretching of the secondary alcohol. In the region of 1110 cm^{-1} the observed band is due to the C-O-C stretching. The peak observed at 1048 cm^{-1} is due to the O-H stretching of the primary alcohol was reported by Pappas *et al*. (1999). The absorption peak at 898 cm^{-1} represents the β-glycosidic linkages which arise from the polysaccharide component. The absorption peak at 1605 cm^{-1} is associated with the aromatic C-Ph vibration present in lignin Corrales *et al*. (2012).

- **Thermogravimetric analysis (TGA)**

5.4.3.1 TGA of the complex cellulignin

The thermal analysis of the untreated cellulignin (CL) and the CL residue after pretreatment using [Emim][OAc] and [Bmim][HSO_4] ionic liquids was investigated in the temperature range from 100 to 800 ^oC at the heating rate of 10 ^oC/ minute under nitrogen flow. The thermogram curve of the CL as shown in Figure 5.9 exhibited two degradation steps. Initially, a sharp degradation step is observed between 300 ^oC to 350 ^oC which is attributed to the decomposition of lignin structure and finally a slight degradation step is observed between 350 to 500 ^oC, which is due to the decomposition of cellulose (Deepa *et al*. 2011). Those two observations confirmed that hemicellulose was removed during the liquid hot water process. A steady decomposition of the lignin is also observed which extends to the whole temperature range.

Approximately 2.48 mg of each sample was used during the TGA analysis. From The TGA curves of the treated CL using [Emim][OAc] and [Bmim][HSO_4] ionic liquids (Figure 5.7), it can be observed that all samples showed a weight loss (0.25 mg) around 100 oC, which is attributed to loss of moisture on the surface of samples. The TGA curve of sample treated with [Bmim][HSO_4] exhibited a broad decomposition peak between 240 to 450 ^{o}C, which can be attributed to the degradation of lignin units (Buranov and Mazza. 2008). More so the TGA curve of the cellulignin treated using [Emim][OAc] showed three thermal events observed from 260 to 350 ^{o}C ,

400 ^{o}C to 450 ^{o}C lastly from 500 to 600 ^{o}C, which is attributed to the steady decomposition of lignin in the entire range.

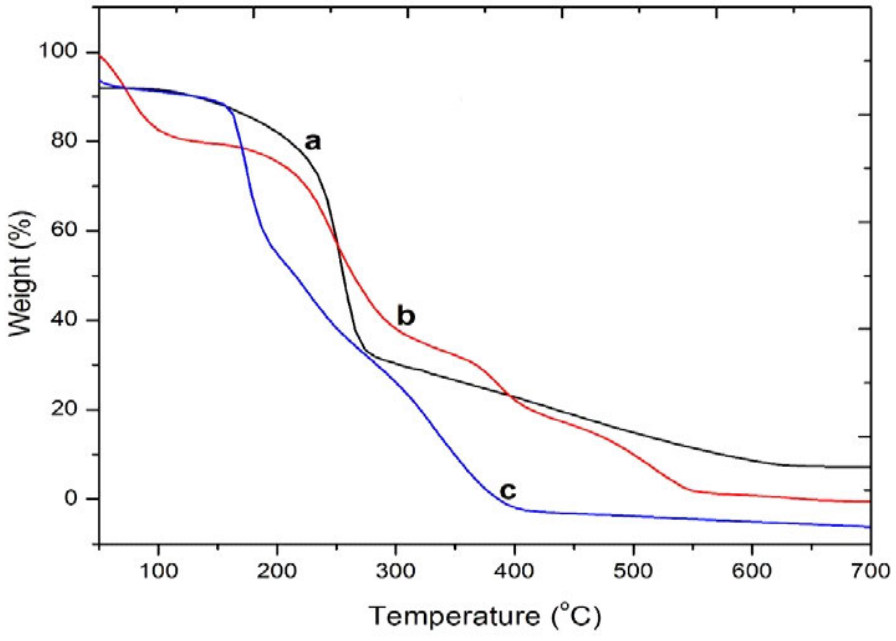


Figure 5.7: Combined TGA graphs of untreated cellulignin (a); cellulignin residue sample after treating with [Bmim][HSO_4] (b) ionic liquid and cellulignin residue sample after treating with [Emim][OAc] ionic liquid

5.4.3.2 TGA of SBP

The thermogravimetric analysis of the untreated pellets, and pellets treated with both [Emim][OAc] and [Bmim][HSO_4] ionic liquids is shown in Figure 5.8. It can be observed that all the samples showed a weight loss (0.3 mg) above 100˚C, which is attributed to the loss of moisture in samples. Equally important, a subtle difference was also observed among the three samples. For instance, the untreated SBP showed three thermal events when it was treated with [Emim][OAc] ionic liquid: the weight loss around 150-260 ^{o}C correspond to the degradation of hemicellulose, while the broad exothermic peak around 260-500 ^{o}C corresponds to the decomposition of the aromatic rings of lignin. Although the samples treated with [Emim][OAc] showed three thermal events, the degradation temperature was, however, lower when compared to the

untreated pellets. For example, the degradation of hemicellulose occurred around 150-200 ^{o}C while the degradation of lignin occurred around 200-350 ^{o}C. Which means that above 200 ^{o}C only cellulose and lignin are only present in SBP sample when it is treated with [Emim][OAc]. In contrast, the sample treated with [Bmim][HSO_4] showed two thermal events. It was observed that there was no hemicellulose degradation peaks. More so, a slight decrease in the thermal stability of lignin was observed, as the degradation peak appeared at lower temperature (120 - 375 ^{o}C). This studies reported by Prado *et al*. (2012) that treatment of plant fibers with [Bmim][HSO_4] using microwave radiation decrease the concentration of hemicellulose in the samples. Although this may be considered positive, it could, however, also cause slight decomposition of lignin in the sample.

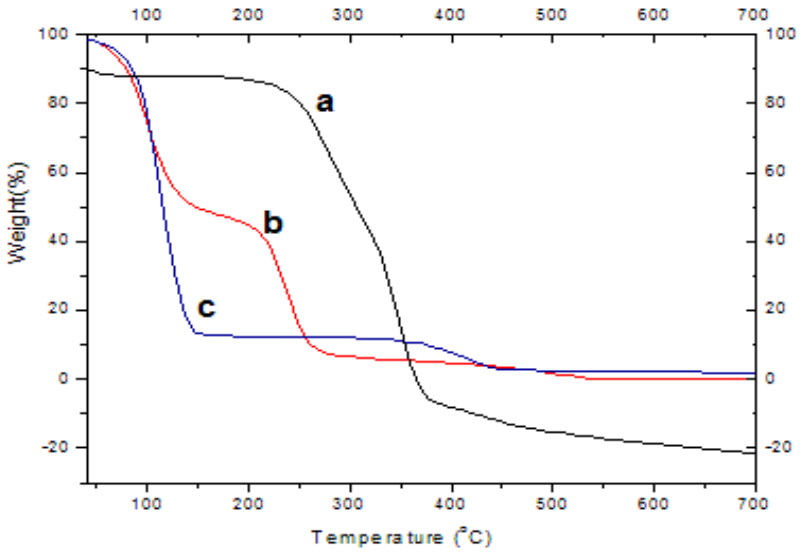


Figure 5.8: Combined TGA graphs of untreated SBP (a); SBP treated using [Emim][OAc] (b) ionic liquid and SBP treated using [Bmim][HSO_4] (c) ionic liquid.

In a research conducted by Perez *et al*. (2015) on agave bagasse the onset of degradation temperature was observed to decrease for ionic liquids treated samples when compared to that for the untreated samples. A similar trend was also observed for thermal decomposition temperature stage, in both sample (SBP and cellulignin) cases, the lowest value corresponds to ionic liquid pretreated sample at 140 ^{o}C. The results indicated that ionic pretreatment reduced the activation energy that is needed to decompose woody biomass by deconstructing the tight plant cell wall structures. The temperature region between 220 and 300 ^{o}C is mainly attributed to thermal depolymerisation of hemicellulose, while lignin decomposition extends to the whole temperature range, from 200 ^{o}C until 500 ^{o}C due to different activities of the chemical bonds present on its structure and the degradation of cellulose taken place. From Figures 5.7 and 5.8 it was observed that lignin was the most difficult to decompose, which happened slowly under the

whole temperature range from ambient to 500 ^{o}C. Couhert *et al.* (2009) also confirmed that the decomposition of pure components differs from real biomass because the pyrolysis reactions are less hindered by interaction with other components.

Ionic liquid treated samples are considered to be thermally more stable in terms of decomposition temperatures after pretreatment that leads to a downshift in degradation temperatures.This is useful in application of lignin where high thermal stability is required.

5.4.4 X-ray diffraction (XRD) analysis

X-ray diffraction analysis was performed using different samples of cellulignin and sugarcane bagasse pellets and the corresponding combined XRD diffractograms are given in Figures 5.9 and 5.10, respectively.

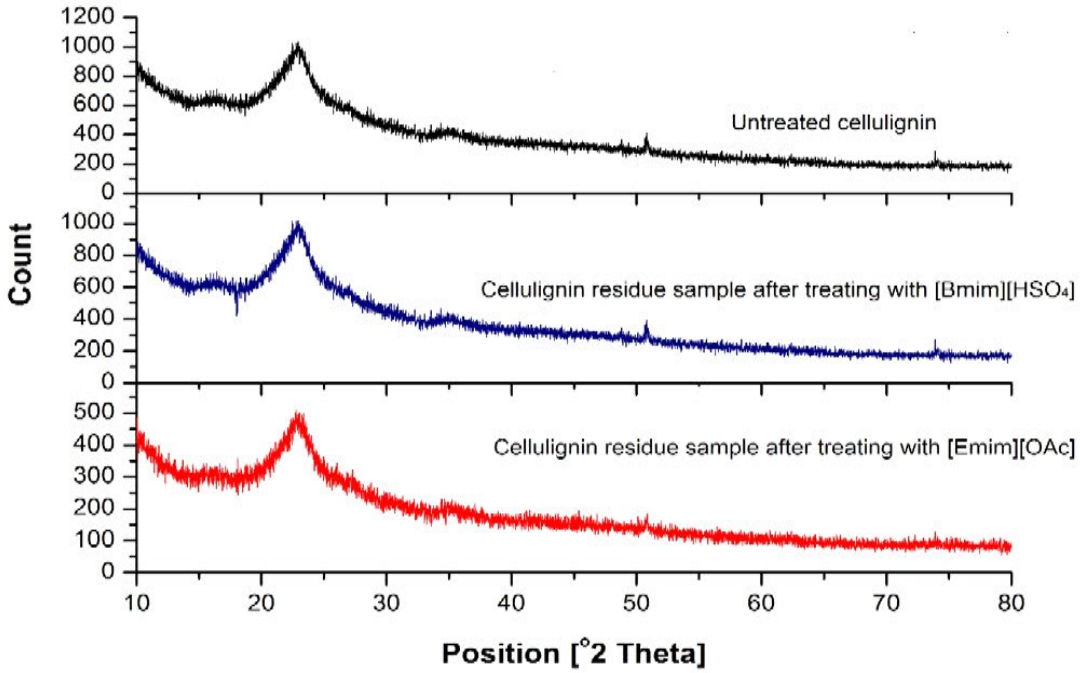


Figure 5.9: Combined XRD patterns of untreated cellulignin, cellulignin residue sample after treating with [Bmim][HSO_4] and [Emim][OAc] ionic liquids.

Figure 5.9 shows characteristic cellulose peaks around 2θ = 22.5 °. The XRD profiles are similar suggesting that all three samples contain cellulose. There was no change in the relative intensity with respect to the crystalline peaks.

Figure 5.10 below shows the combined XRD patterns of untreated SBP, SBP treated with [Bmim][HSO_4] and [Emim][OAc] ionic liquids.

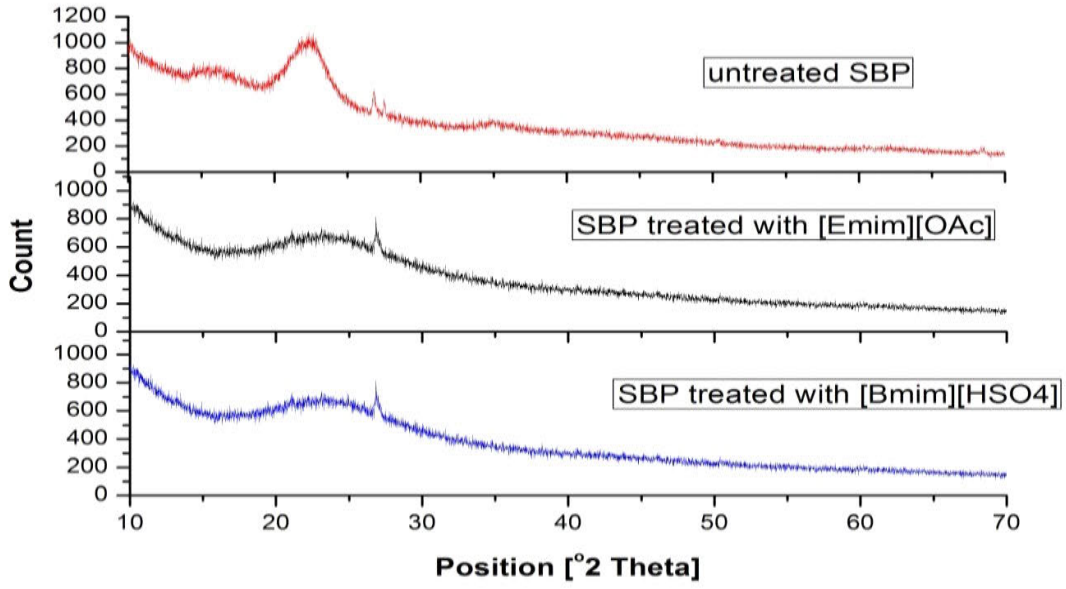


Figure 5.10 Combined XRD patterns of untreated SBP, SBP treated with [Bmim][HSO_4] and [Emim][OAc] ionic liquids.

From Figure 5.10, it can be observed that the XRD profile of the untreated SBP shows characteristic cellulose peak (crystalline phase) around 2θ = 22.5° which is more pronounced. The crystalline phase is assigned to cellulose I to

the planes of (110), (200), respectively Zhouyang *et al*.(2017) reported that when treating with [Bmim][HSO_4] and [Emim][OAc] ionic liquids, shows a decline in the crystalline phase which results in broad peaks of lignin; which confirms the removal of cellulose. Similar diffraction peak of lignin was observed by Sivasangar *et al.* (2013) and the peaks were associated to the amorphous structure of lignin based on the molecular chain arrangements, presence of branches and bonding between the molecules.

CHAPTER 6

CONCLUSION AND RECOMMENDATION

During this study, a comparison of lignin yield from sugarcane bagasse pellets and cellulignin was investigated using two environmentally friendly pretreatment methods namely: liquid hot water and ionic liquids and only ionic liquids. In the liquid hot water process the sugarcane bagasse pellets were treated with water at high temperature followed by enzymatic hydrolysis. The lignin yield from sugarcane bagasse pellets after liquid hot water and enzymatic hydrolysis was found to be 37.8 %. The sugarcane bagasse pellets treated directly with ionic liquids using either of two ionic liquids, namely,1-ethyl-3-methylimidazolium acetate ([Emim][OAc]) or 1-butyl-3-methylimidazolium hydrogen sulphate ([Bmim][HSO_4]) under microwave digestion system at varying time intervals (3 minutes; 10 minutes; 15 minutes; 20 minutes; 25 minutes and 30 minutes) was also part of research.

Also the sugarcane bagasse pellets were treated with [Emim][OAc] and [Bmim][HSO_4] for 20 minutes reaction time, the maximum yields of the extracted lignin were found to be 67.20 % (m/v) and 48.94 % (m/v), respectively. On the other hand when the complex cellulignin was treated with [Emim][OAc] or [Bmim][HSO_4] at 10 minutes

reaction time under microwave digestion system, the maximum yields of the extracted lignin were found to be 68.00 % (m/v) and 32.04 % (m/v), respectively. [Emim][OAc] was the better ionic liquid compare to [Bmim][HSO_4] during this research work. However for both ionic liquids ([Emim][OAc] or [Bmim][HSO_4]) the sugarcane bagasse pellets gave an overall higher lignin yields, 68.00 % and 48.94 %, respectively.

The ionic liquids pretreatment method should be considered since it eliminates the use of high energy input.

Based on the methods used for lignin extraction namely:

- Sugarcane bagasse pellets treated with liquid hot water followed by enzymatic hydrolysis
- Sugarcane bagasse pellets treated with ionic liquids
- Complex cellulignin treated with ionic liquids the best result was obtained for complex cellulignin treated with ionic liquids (68%). But the results for sugarcane bagasse pellets was 67.25 % which is an economically more feasible method since it doesn't require high energy input. Although the cost of ILs is relatively high, they can be recycled.

As a recommendation for further analysis the recovery of the ionic liquids should be investigated since ionic liquids are very expensive and recycling ILs can minimize the cost of the project.

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APPENDIX

1. Preparation of citrate buffer

The citric acid buffer solution was prepared by weighing 210 g of citric acid monohydrate transferred into 1000 ml volumetric flask and adding 50 g of NaOH. The salt was dissolved with deionized water and adjusted up to the calibration mark.

The pH value was adjusted to 4.3. This stock solution was diluted to 0.05 M buffer with a pH value between 4.8 and 5.0 for all experiments.

A 1: Photography of citrate buffer (colorless) and DNS reagent (yellow)

2. Preparation of 3, 5-dinitrosalicylic (DNS) acid reagent

Approximately 5 g of 3, 5-dinitrosalicylic acid was dissolved using 2 M NaOH solution in 500 mL volumetric flask. Thereafter 150 g of predissolved potassium sodium tartrate tetrathythydrate was added. The mixture was therefore top up with deionized water up to the calibration mark. Since the DNS acid solution is photosensitive, it was kept in a brown glass bottle and shielded by aluminum foil at 4 ^{o}C. Figure A 1 is a photograph of citrate buffer and DNS reagent.

3. Preparation of glucose stock solutions for cellulignin

Different concentrations (10 g/L; 5 g/L; 2.5 g/L; 1.25 g/L and 0.625 g/L) of glucose stock solutions were prepared individually into a 50 ml volumetric flask. For example 10 g/L stock solution preparation procedure: approximately 0.5 g of glucose hydrated salt was weighed on analytical balance then transferred into 50 ml volumetric,

dissolved with deionized water and diluted up to the calibration mark. Solution ready for UV analysis at 280 nm.

4. Preparation of lignin standards

Alkali lignin standard purchased from Sigma Aldrich was used during this research. From the compositional analysis of sugarcane bagasse pellets (SBP), 25 % (m/v) of lignin was obtained. Therefore working with 0.5 g of SBP sample will expecting a total mass of lignin extracted or recovery to be 0.125 g. Different alkali lignin standard solutions were prepared from 0.01 to 0.3 % (m/v). Example: preparation of 0.125 % (m/v). Approximately 0.125 g of alkali lignin standard weighed on the analytical balance was dissolved in 100 ml mixture of 95: 5 dioxane and water. Thereafter 1 ml of the aliquot was diluted to 10 ml with dioxane/water (50:50) v/v ready for UV analysis.

Conference Attendance:

Comparison of lignin yield from sugarcane bagasse using liquid hot water and ionic liquids and ionic liquids only

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Highlights

- Liquid hot water and ionic liquid pretreatment yielded higher yields of lignin (68 m/v %).
- Microwave digestion was effective in isolating the lignin.
- Optimized pretreatment time for only ionic liquids was 20 minutes.
- Ionic liquid 1-ethyl-3-methylimidazolium acetate was more effective than 1-butyl-3-methylimidazolium hydrogen sulphate.

1. Introduction

Globally the high cost and diminishing reserves of crude oil coupled with global warming effects have resulted in research for alternate energy and chemical sources. Lignocellulosic material is one of the largest renewable resources containing hemicellulose, lignin and cellulose. Extraction of the different components of the lignocellulosic material can lead to the valorization of the biomass and reduce dependence on crude oil.

There are many pretreatment methods for the separation of cellulose, lignin and hemicellulose, namely: acid hydrolysis, base hydrolysis, steam explosion, mechanical and biochemical methods. All of these methods are environmentally harmful due to the release of volatile organic compounds that contribute to global warming effects. A new class of solvents known as ionic liquids are suitable for pretreatment since they have properties such as: low vapour pressure, recyclability, solubility in a range of organic compounds and liquid at room temperature that make them attractive for biomass pretreatment.

Sugarcane bagasse (SCB) is a renewable lignocellulosic resource in South Africa obtained after the sugar milling process. The annual production of SCB is approximately 6 million tons per annum produced by 14 sugar mills that are located on the north coast of KwaZulu-Natal (Paterson – Jones, 1989).

In this work, lignin yields from sugarcane bagasse pellets was investigated using liquid hot water (LHW) and ionic liquids (ILs) and only ionic liquids. The LHW process was applied to sugarcane bagasse pellets at 200 °C, for 30 minutes in a high pressure reactor for removal of hemicelluloses. The complex cellulignin residue was treated with the ILs: 1-ethyl-3-methylimidazolium acetate ([Emim][OAc]) or 1-butyl-3-methylimidazolium hydrogen sulphate ([Bmim][HSO_4]), using microwave digestion at varying time intervals. Direct Ionic liquid treatment was done on sugarcane bagasse pellets with ILs: [Emim][OAc] or [Bmim][HSO_4] using microwave digestion.

2. Methods

The sugarcane bagasse was first ground to 3-5 mm, dried to 10-12 % moisture and pelletized using a pellet mill. 1.0 kg of pelletized bagasse was used for the LHW hydrolysis at a temperature of 200 ^oC in a 3.0 L high pressure fixed bed reactor for 30 minutes with a volume flow of 250 ml/min of water. Hemicellulose dissolved in the water and a solid residue (cellulignin) was collected from the reactor. Compositional analysis of the sugarcane bagasse pellets, cellulignin and the complex cellulignin after enzymatic hydrolysis was done using NREL procedures.

For the extraction of lignin, a ratio 1:10 of SBP (0.5 g) and ionic liquid (5 g) was weighed in duplicate and transferred into 65 mL Teflon vessels, transferred to a microwave oven with parameters: power (80 Watt); ramp time (10 minutes); temperature (180 ˚C) and different hold times of 3, 10, 15 and 20 minutes for each run. After each run the extracted lignin was transferred to a 100 mL beaker rinsed thoroughly with 10 mL solution of 1-4 dioxane-water 95:5 (v/v), transferred into a 50 mL volumetric flask and diluted with a mixture of 1-4 dioxane-water 50:50 (v/v) for UV analysis.

3. Results and discussion

The amounts of glucose released in g/L during enzymatic hydrolysis of cellulignin using enzyme Cellic CTec2 or Metaplus/Rapidas was 27.5 g/L and 3.8 g/L after 6 hours of

hydrolysis. The effectiveness of the combined enzyme Metaplus/Rapidas was lower than the Cellic ® CTec2 enzyme.

The IL [Emim][OAc] gave the highest lignin yield (68 %) for the LHW and IL method at 10 min. For the sugarcane bagasse pellets the highest yield was 59 98% at a reaction time of 25 min. The IL: [Emim][OAc] was the better of the two ILs used.

4. Conclusions

Liquid hot water and IL extraction of lignin from sugarcane bagasse pellets was successfully used to extract lignin. Although the highest yield was obtained for the LHW and IL method, the preferred method would be IL treatment on the sugarcane bagasse pellets since it eliminates the use of high energy input.

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