STUDY OF SELECTED IONIC LIQUIDS IN THE PRETREATMENT OF *PROSOPIS JULIFLORA* AND *GADAM* SORGHUM STALKS FOR BIOETHANOL PRODUCTION

BY

AJIAMBO FLORENCE

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DECLARATION

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AJIAMBO FLORENCE DATE

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DECLARATION BY SUPERVISORS:

We declare that this thesis has been submitted for examination with our approval as university supervisors.

Signature: _____

PROF. SAUL NAMANGO, DATE

DEPT. OF CHEMICAL & PROCESS ENG.,

SCHOOL OF ENGINEERING,

MOI UNIVERSITY

Signature: _____

DR. CHARLES NZILA, DATE

DEPT. OF MANUFACTURING, INDUSTRIAL

& TEXTILE ENG., SCHOOL OF ENGINEERING,

MOI UNIVERSITY

ABSTRACT

Production of bioethanol as an alternative energy source, from the abundant sources of lignocellulosic biomass requires that the latter undergo pretreatment processes. Conventional pretreatment methods tend to be harmful to the environment. A new pretreatment method by use of Ionic Liquids (ILs) has shown to be promising. This research work analyzed pretreatment of *Prosopis Juliflora* and *Gadam* Sorghum stalks using three different types of ILs (Imidazolium, Phosphonium and Pyridinium based). Regeneration of cellulose from the ILs was carried out and the performance of the ILs established through analysis of glucose concentrations upon hydrolysis. Inhibitor (furfural) production was also investigated. Finally recyclability of ionic liquids (IL) was established. Pretreatment was carried out on both biomasses at varied temperatures (80°C–140°C), pretreatment periods (40min – 160min) and a biomass loading of 6%wt using 1-butyl-3-methylimidazolium chloride ([BMIM]Cl), 1-butyl-4-methylpyridinium chloride ([4MBP]Cl) and trihexyltetradecylphosphonium chloride ([P66614]Cl). Simple acid hydrolysis was then done at a temperature of 130°C for a duration of 10 minutes. Finally glucose measurement was done using a UV – Vis Spectrophotometer at a wavelength of 520nm. Furans in the hydrolysate were also monitored at a wavelength of 284nm. The glucose yields varied according to the pretreatment time, temperature, type of IL and biomass. [P66614]Cl gave the least glucose yield overally (maximum of 7.19%) as compared to [4MBP]Cl (maximum of 61.63%) and [BMIM]Cl (maximum of 73.27%). Higher overall glucose yields were also observed with *Prosopis Juliflora* (73.27%) as compared to *Gadam* Sorghum (60.42%). This can be attributed to the higher cellulose content in its structure. The investigation on furfural inhibitor production showed a decrease of between 46.2% and 64% in furfural concentration when ILs were used for pretreatment as compared to dilute alkaline pretreatment. It was also established that using the Imidazolium based IL, it is possible to recycle the IL about six times without any deleterious effect on generation of fermentable sugars. The use of *prosopis juliflora* and *gadam* sorghum as biomass will go a long way in supplementing the dwindling petroleum oil reserves while easing food versus fuel competition. Recycling of the IL will facilitate cost effective use of ionic liquids for pretreatment hence reducing pretreatment cost.

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LIST OF ACRONYMS AND SYMBOLS

AFEX	Ammonia fibre explosion
ARP	Ammonia recycle percolation
ASL	Acid soluble lignin
[AMIM]Cl	1-Allyl-3-methylimidazolium chloride
[BMIM]Cl	1-butyl-3-methyl imidazolium chloride
[BMIM]I	1-butyl-3-methyl imidazolium iodide
[BMIM]Br	1-butyl-3-methyl imidazolium bromide
BMIM]PF6	1-butyl-3-methyl imidazolium hexafluorophosphate
[BMIM]Tf_2N	1-Butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide.
4MBPCl	1-butyl-4-methyl pyridinium chloride
[BMPy]BF4	1-butyl-4-methyl pyridinium tetrafluoroborate
CMC	Carboxyl Methyl Cellulose
CO_2	Carbon dioxide
EABL	East Africa Breweries Limited
EMIMCl	1-ethyl-3-methyl imidazolium chloride
GHG	Greenhouse gas
GIm	*Gadam* Imidazolium
GPh	*Gadam* Phosphonium
GPy	*Gadam* Pyridinium
GS	*Gadam* Sorghum
H_2O_2	Hydrogen Peroxide
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
HMF	Hydroxymethyl furfural
$IL_{(s)}$	Ionic Liquid(s)
JBEI	Joint BioEnergy Institute
LCB	Lignocellulosic Biomass
LHW	Liquid hot water
MTOE	Million tons of oil equivalents

NREL	National Renewable Energy Laboratory
P66614Cl	Trihexyltetradecylphosphonium chloride
PJ	*Prosopis* Juliflora
PIm	*Prosopis* Imidazolium
PPh	*Prosopis* Phosphonium
PPy	*Prosopis* Pyridinium
RTIL	Room Temperature Ionic Liquid
SAA	Soaking in aqueous ammonia
SC-CO_2	Supercritical carbon dioxide
SRS	Sugar Recovery Standard
TSILs	Task specific ionic liquids

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CHAPTER ONE

INTRODUCTION

1.1 Background

The search for sustainable methods to produce transportation fuels is driven by concerns associated with its supply and demand and its impact on climate change and greenhouse gas (GHG) emissions. Global petroleum demands have increased steadily from 57 x 10^6 barrels/day in 1973, to 160 x 10^6 barrels/day in 2004. By 2025, projected economic growth is anticipated to increase global demands for liquid fuels by approximately another 50% (Ragauskas *et al*., 2006). However, taking into account current production and existing reserves, the Hubbert's predicted 'peak oil' levels (Campbell and Leherrere, 1998) may be approached soon. Growing dependency on oil and the inability to protect supply lines from global political intrigues and ensuing price volatility is another reason for pursuing alternative fuels. In selecting alternative transportation fuels, due consideration must be given to those which serve to combat climate change and produce cleaner air. Since the industrial revolution, atmospheric CO_2 level has increased from approximately 275 to approximately 380 ppm owing to the burning of fossil fuels. Consequently, atmospheric temperatures have risen by 0.6 ± 0.2 °C during the twentieth century. If left unchecked, CO_2 level could easily surpass 550 ppm by the middle of this century (Zhang, 2008). Policies outlining strategic guidelines for reducing net greenhouse gas (GHG) emissions from traditional automotive fuels are currently being prepared and implemented.

The only current sustainable source of organic carbon is plant biomass. Biomass is defined as organic matter available on a renewable basis. Biomass includes forest and mill residues, agricultural crops and wastes, wood and wood wastes, animal wastes, livestock operation residues, aquatic plants, fast growing trees, and municipal and industrial wastes. Biofuels derived from plant biomass are currently the only sustainable class of liquid fuels (Lowe, 2008). They include bio-alcohols (ethanol, butanol etc), biodiesel, bio-oils and syngas derivatives.

First-generation biofuels mainly utilize plants rich in carbohydrates (i.e. sugar and starch) to produce ethanol or oils such as canola and soybean for biodiesel production. The problem with first-generation biofuels is that as their use increases, demand for the feedstock will intensify and ultimately clash with fundamental agricultural endeavors such as food and fiber production. Second-generation biofuels are derived from the inedible and/or unexploited part of the plant (lignocellulose) and can be sourced from plant residues or organic waste such as crop straw, forestry thinning or contents of landfill. Lignocellulosic materials are of interest as raw materials for bio-energy production since they are available in large amounts and are relatively cheap. Economical conversion of lignocellulosic biomass into liquid fuel is a great challenge currently.

Efficient conversion of lignocellulosic materials into fuel ethanol or other energy fractions has become a priority for producing reasonably priced and renewable energy (Zhao, *et al.,* 2009*).* The pretreatment of lignocelluloses is known as a key technology, enabling a fast enzymatic hydrolysis of cellulose. The underlying reason is the increased surface area accessible to water and cellulases – a transformation expected to improve

hydrolysis kinetics and conversion of cellulose into glucose (Zhang *et al.,* 2004). Different pretreatment methods – biological, physical, chemical and physico-chemical – are available. Unfortunately, each of these methods has some disadvantages:

The biological processing methods require a long residence time. The physical treatments are energy-demanding, expensive and do not remove the lignin. The chemical methods are costly and mainly suitable for high-value paper products. The physico-chemical pretreatment procedures are considered as very promising, but require high pressures/temperatures and the use of catalysts.

Some time ago, it was discovered that novel, non-volatile solvents called ionic liquids (ILs) are able to dissolve significant amounts of cellulose. Preliminary investigations suggest that celluloses regenerated from IL solutions are subjected to faster saccharification than the untreated substrates (Dadi *et al.,* 2008). These encouraging results indicate the possible utilization of ILs as alternative and non-volatile solvents in cellulose pretreatment processes (Zhao *et al.,* 2009).

1.2 Pretreatment

The goal of pretreatment is to make cellulose accessible to hydrolysis for conversion into sugars and subsequently into ethanol. Hydrolysis of cellulose/lignocellulose to monosaccharides is usually catalyzed by enzymes (Dadi *et al*., 2006). Enzymatic hydrolysis of lignocellulosic materials without pretreatment is generally inefficient, with less than 20% theoretical maximum yield (Liu and Chen, 2006). Most pretreatment options change the physical and chemical structure of the lignocellulose biomass and improve hydrolysis for conversion to constituent sugars (Kumar *et al*., 2009). According

to (Zhang, 2008), an effective lignocellulosic pretreatment and fractionation method should have several or all of the following features:

- Produces high monomeric sugar yields
- Generates highly digestible cellulose for rapid hydrolysis with lower enzyme use
- Fractionates lignocellulose components for better economy
- Minimizes production and/or release of sugar and lignin degradation products
- Low energy consumption and/or has potential for re-generating energy, chemicals and/ or enzymes
- Low capital and operational costs
- Broad substrate applicability for a variety of feedstocks

Besides cellulose release, most pretreatment processes liberate natural biomass inhibitors and often generate toxic degradation products which inhibit subsequent hydrolysis and fermentation processes (Mosier *et al*., 2005). Current pretreatment options strongly impact on the success and viability of other process variables such as enzyme loadings, mixing power, ethanol concentration and purification, waste treatment demands, and power generation (Wyman, 2007). A potential solution to these and other problems associated with traditional pretreatment options may rest with the green chemical solvents referred to as ionic liquids (ILs).

1.3 Ionic Liquids

Ionic liquid (IL) solvents are a promising new approach in the pretreatment of lignocellulosic material because of their ability to dissolve large amounts of cellulose at considerably mild conditions with close to 100% recovery (Thomas, 2005). ILs are a class of organic salts, comprised entirely of cations (usually organic) and anions (usually inorganic). Unlike molten salts such as sodium chloride which is an ionic liquid at high

temperatures, room temperature ionic liquids exist as liquids at relatively low temperatures.

The most common ILs are divided into four groups according to their cations, which include the following: quaternary ammonium ILs, N-alkylpyridinium ILs, N-alkyl-isoquinolinium ILs, and 1-alkyl-3-methylimidazolium ILs.

Today, ILs are recognized as one of the most promising green chemical solvents due to their desirable properties. Owing to their non-volatile and non-flammable properties, they are considered ideal replacements for conventional environmentally harmful molecular solvents which are used in catalytic and organic reactions. They have a wide liquidus range (for example [BMIM]Cl has a melting point of 41 °C and decomposition temperature of 254 °C (Huddleston *et al.* , 2001). The asymmetric nature of the ions prevents compact packing of the ions, and makes them very useful in reactions which require both high and low temperatures. Other beneficial properties of ILs include their high thermal stability, high ionic conductivity, large electrochemical window, miscibility, water stability, density, viscosity, polarity and refractive index. Referred to as “designer solvents”, ionic liquid solvent’s chemical and physical properties can be adjusted and set by using different anion and cation combinations. ILs miscibility with water is controlled by the choice of anion and cation. Water interacts mainly with the anion via the formation of hydrogen bonds. The cation contributions are secondary, acting as a weak hydrogen bond donor (Murugesan and Linhardt, 2005). Examples of water immiscible anions include $[PF_6]^-$ and $[(CF3SO2)N]^-$, while water miscible anions are epitomized by $[CH3COO]^-$, $[CF3COO]^-$, $[NO3]^-$, BR^-, I^- and Cl^-. ILs miscibility with organic solvents

also varies according to the design of the cation and anion components. For example, [BMIM][Tf_2N] is miscible with dichloromethane and ketone but immiscible with alkanes and ether, [BMPy][BF_4] is miscible with benzene, toluene and styrene but immiscible with higher alkyl benzenes (Van e*t al.* , 2007). Variability in miscibility properties of different ILs in diverse media imparts a great advantage to process design, mainly because the ILs can be recovered and recycled through extraction.

Since it was first reported that Ionic Liquids (ILs) can dissolve cellulose, there has been extensive research to explore if this ability to dissolve cellulose can be used in the context of LCB pretreatment (Swatloski et al., 2002). Significant research effort has been directed in understanding the role of imidazolium based ionic liquids in the dissolution of microcrystalline cellulose (Swatloski et al., 2002). However, there have been no detailed studies in the use of non imidazolium based ILs and the possibility of their application in pretreatment of LCB.

1.4 Statement of the Problem

The goal of pretreatment is to make cellulose accessible to hydrolysis for conversion into sugars and subsequently into ethanol. Hydrolysis of lignocellulosic materials without pretreatment is generally inefficient, with less than 20% theoretical maximum yield. Most pretreatment options change the physical and chemical structure of the lignocellulose biomass and improve hydrolysis for conversion to constituent sugars.

Besides cellulose release, most pretreatment processes liberate natural biomass inhibitors and often generate toxic degradation products which inhibit subsequent hydrolysis and

fermentation processes. Current pretreatment options strongly impact on the success and viability of other process variables such as enzyme loadings, mixing power, ethanol concentration and purification, waste treatment demands, and power generation. A potential solution to these and other problems associated with traditional pretreatment options may rest with the green chemical solvents ionic liquids (ILs).

1.5 Justification and Significance of the Study

Lignocellulosic materials are of interest as raw materials for bio-energy production since they are available in large amounts and are relatively cheap. The demand for sorghum in Kenya has shot up dramatically following the decision by the East Africa Breweries Limited (EABL) to use the produce for manufacture of one of its beer brands. With this increased campaign for more sorghum to be grown, adequate supplies of sorghum stalk biomass could be available since the brewer utilizes only the grains in the beer making process. As such, the sugar rich stalks can be used for bioethanol production. *Prosopis juliflora* is the other lignocellulosic material readily available in dry and semi arid areas of Kenya.

Having a lower environmental impact, pre-treatment methods using ionic liquids (ILs) offer many advantages over other methods. ILs require a relatively low operation temperature and also necessitate lower equipment and energy costs compared to other methods. Moreover, bench-scale experiments indicate that ILs can be recycled, making the pretreatment process more environmentally friendly. In addition, low fermentation inhibitors production has been reported with the use of ionic liquids for pretreatment.

1.6 Objectives

General Objective

To rate Ionic Liquids (ILs) as pretreatment agents for *gadam* sorghum stalks and *prosopis juliflora* (mathenge plant) biomass.

Specific Objectives

i. Establish the quantity of cellulose, hemicelluloses and lignin content in the *prosopis juliflora* (mathenge plant) and *gadam* sorghum stalks before pretreatment with ionic liquid.
ii. To determine efficiency of three given ionic liquids in pretreatment of biomass via glucose yield.
iii. To assess effect of cation type, pretreatment period and temperature on pretreatment process.
iv. To establish concentration levels of furfural produced during pretreatment with Ionic liquids.
v. To determine recyclability of Ionic Liquids as pretreatment agents.

1.7 Scope of the Study

This research is limited to the pre-treatment of:-

1. *Prosopis Juliflora* (mathenge plant) fibrous stem collected from Baringo county
2. *Gadam* Sorghum stalks collected from Malaba, Nangéni in Bungoma county.

The research also is limited to the use of the following ionic liquids:

1. 1-butyl -4-methypyridinium chloride (4MBPCl),
2. trihexyltetradecylphosphonium chloride (P66614Cl)
3. 1-butyl -3-methylimidazolium chloride ([$BMIM^+$][Cl^-])

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Energy availability, supply and use play a central role in the way societies organize themselves, from individual welfare to social and industrial development. By extension, energy accessibility and cost is a determining factor for the economical, political and social interrelations among nations. Considering energy sources, human society has dramatically increased the use of fossil fuels in the past 50 years in a way that the most successful economies are large consumers of oil. However, geopolitical factors related to security of oil supply, high oil prices and serious environmental concerns (Wyman, 1996) have led to a push towards decreased consumption.

Given this reality, nations around the world are investing in alternative sources of energy including bioethanol. The leading nations in bioethanol production are Brazil and the USA as depicted in Table 2.1 below, (Carere et al., 2008). Asian countries altogether account for about 14% of world's bioethanol production.

Table 2.1 Leading Bioethanol Producers In the World

Country/group of countries	Ethanol produced in:	
	Million liters	MTOE
Brazil	19,000	10.44
Canada	1,000	0.55
China	1,840	1.01
India	400	0.22
USA	26,500	14.55
European Union	2,253	1.24
Others	1,017	0.56
World (Total)	52,000	28.57

*Source: Data from OECD-FAOA glink-Casimo database (2007). MTOE: Million tons of oil equivalents.

Bioethanol production from sugarcane and starch rich feed stocks such as corn, potato, etc., is considered first generation process and it has already been developed. However, the long-term viability of this process is in question because it will require significantly increased amounts of cultivatable land and significant hike in food prices that will ultimately lead to food insecurity (Mitchell, 2008). Estimates clearly point to the fact that

first generation ethanol production process can not sufficiently meet the global energy needs. Therefore, second generation processes to produce bioethanol are gaining momentum. The second generation processes will use lignocellulosic materials for this purpose and the biosphere clearly has sufficient supplies of lignocellulosic materials. The production of ethanol from lignocellulosic biomass [corn stover, wheat straw, sugarcane bagasse, rice straw, rice hull, corn cob, oat hull, corn fiber, woodchips and cotton stalk; energy crops such as switch grass and Alfa Alfa, and various weeds such as *Saccharum spontaneum*, *Lantana camara*, *Eichhornia crassipes* (water hyacinth), etc.] has become one of the best alternatives, because these sources have widespread abundance and the cost of their procurement is relatively cheap.

Even though the lignocellulosic biomass is abundant, the commercialization of the process to produce bioethanol from it is limited due to insufficient data, especially the research data related to minimization of production cost. Bioethanol production from lignocellulosic materials relies on technologies that will efficiently hydrolyze cellulosic biomass to fermentable sugars. This process of ethanol production generally involves hydrolysis of pretreated lignocellulosic biomass to fermentable sugars followed by fermentation of such sugars to ethanol as illustrated in Figure 2.1.

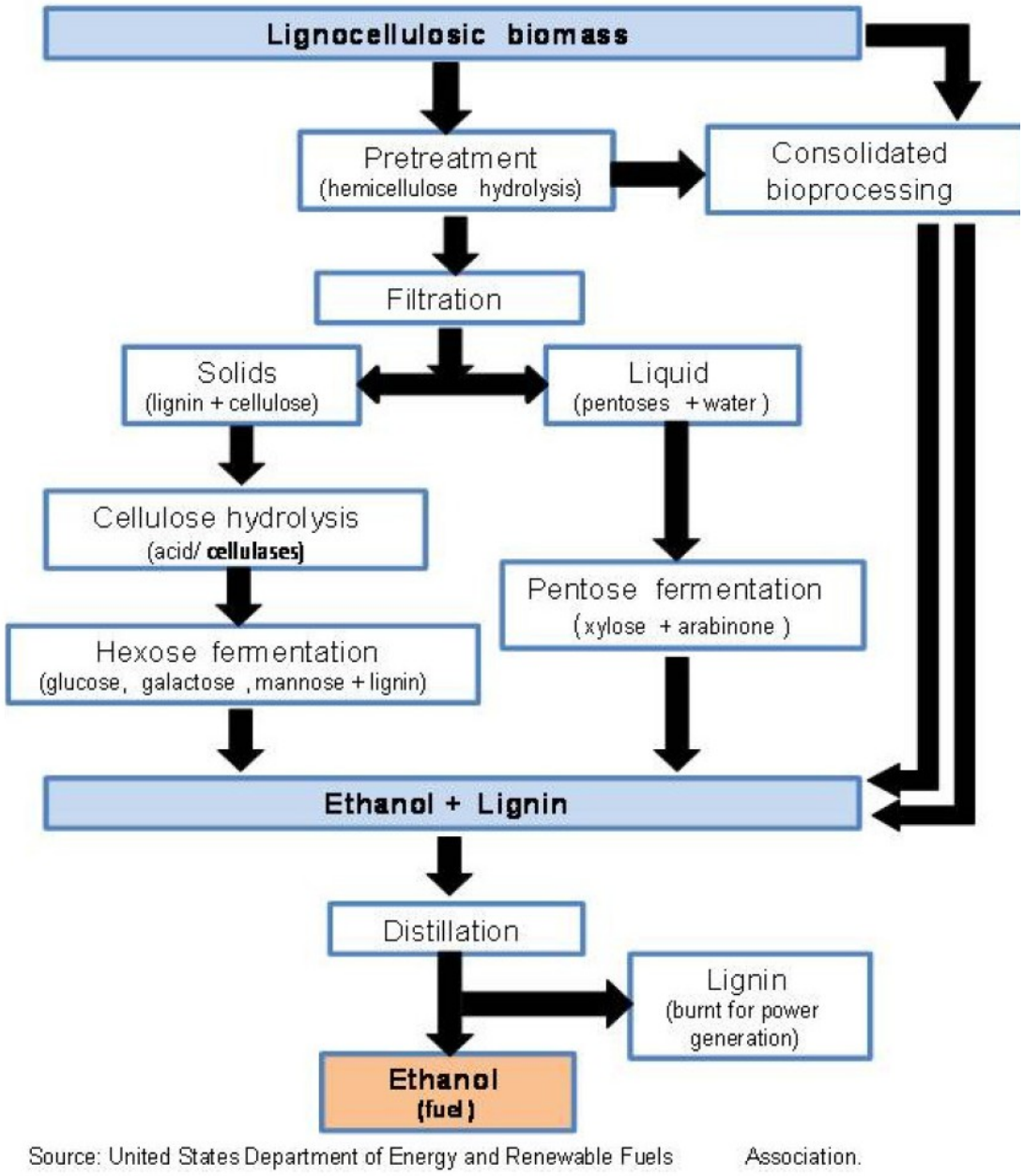


Figure 2.1 Ethanol production process from LCB.

Although biotechnology presents important opportunities to achieve very low costs, pretreatment of naturally resistant cellulosic materials is essential so as to achieve high yields from biological operations. This operation is projected to be the single, most expensive processing step, representing about 20% of the total cost. Achieving

fermentable levels of sugars from lignocellulosic biomass requires relatively harsh pretreatment processes. The pretreatment process has pervasive impact on the overall operation because the process depends on the choice of lignocellulosic source, the size reduction via grinding, chemical treatment, acid hydrolysis, neutralization and fermentation. Recent advances in the process technologies have made it possible to use simultaneous saccharification and fermentation.

2.2 Lignocellulosic Biomass

.2.1 Composition

Biomass as a carbon based material is composed of a mixture of organic molecules containing carbon and hydrogen. It usually possesses atoms of oxygen and nitrogen whilst including small quantities of other elements, such as metals. The carbon used to construct biomass is absorbed from the atmosphere as carbon dioxide (CO_2) by plant life, using energy from the sun. Therefore biomass is the most abundant renewable resource available. The term “lignocellulosic biomass” is often used to describe the material that composes the plant cell wall, i.e. cellulose, hemicelluloses and lignin.

The major constituents of lignocellulosic biomass are polymeric carbohydrates (cellulose and hemicellulose) and lignin (Zhang & Zhao, 2010). The percentage compositions established are usually as shown in Figure 2.2 below: cellulose (40–50 wt %), hemicelluloses (25 wt%) and lignin (25 wt%). These percentages of the three components may vary from species to species as well as across different parts in the same plant. Moreover, changeability of these components can be influenced by geography or environmental factors

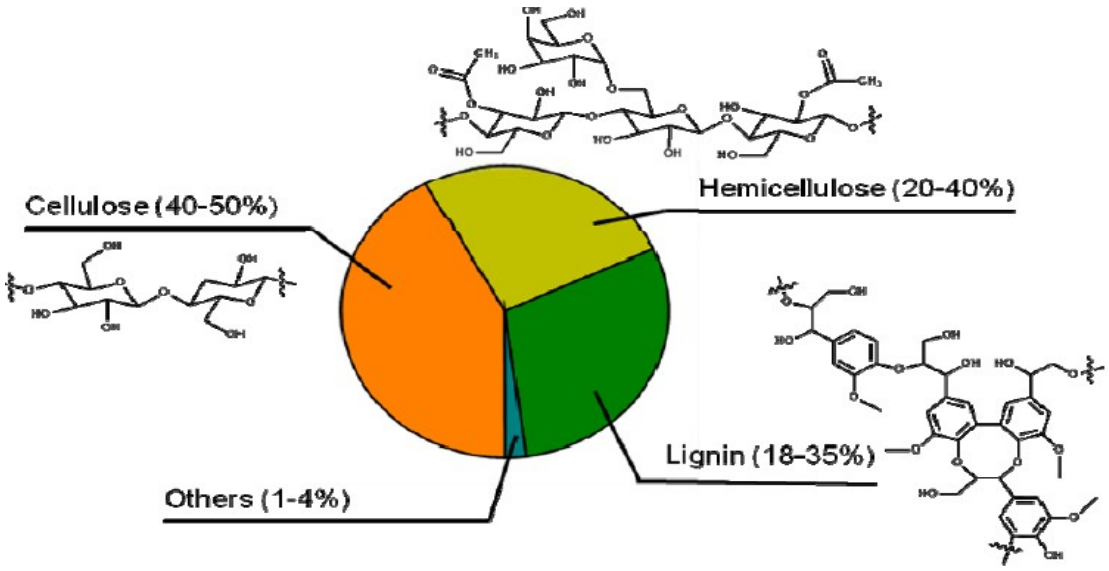


Figure 2.2 Approximate distribution of the components of LCB

Cellulose is a major structural component of cell walls, and it provides mechanical strength and chemical stability to plants. Solar energy is absorbed through the process of photosynthesis and stored in the form of cellulose. (Raven *et al*., 1992) Hemicellulose is a copolymer of different C5 and C6 sugars that also exist in the plant cell wall. Lignin is a polymer of aromatic compounds produced through a biosynthetic process and forms a protective layer for the plant walls. In nature, the above substances grow and decay during the year. It has been estimated that around $7.5x10^{10}$ tonnes of cellulose are consumed and regenerated every year (Kirk and Othmer, 2001). It is thereby the most abundant organic compound in the world.

Apart from the three basic chemical compounds that lignocellulose consists of; water is also present in the complex. Furthermore, minor amounts of proteins, minerals and other components can be found in the lignocellulose composition as well.

The composition of lignocellulose highly depends on its source. There is a significant variation of the lignin and (hemi) cellulose content of lignocellulose depending on whether it is derived from hard-wood, softwood, or grasses. Table 2.2 below summarizes the composition of lignocellulose encountered in the most common sources of biomass.

Table 2.2 Composition of lignocelluloses in several sources on dry basis

Lignocellulosic materials	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwoods stems	40–55	24–40	18–25
Softwood stems	45–50	25–35	25–35
Nut shells	25–30	25–30	30–40
Corn cobs	45	35	15
Grasses	25–40	35–50	10–30
Paper	85–99	0	0–15
Wheat straw	30	50	15
Sorted refuse	60	20	20
Leaves	15–20	80–85	0
Cotton seed hairs	80–95	5–20	0
Newspaper	40–55	25–40	18–30
Waste papers from chemical pulps	60–70	10–20	5–10
Primary wastewater solids	8–15	NA	24–29
Swine waste	6.0	28	NA
Solid cattle manure	1.6–4.7	1.4–3.3	2.7–5.7
Coastal Bermuda grass	25	35.7	6.4
Switchgrass	45	31.4	12.0

(*Source: Sun and Cheng, 2002)*

.2.2 Internal Structure

Lignocellulosic biomass has a complex internal structure. It is comprised of a number of major components that have, in their turn, also complex structures. To obtain a clear picture of the material, an analysis of the structure of each main component is made.

2.1.1 Cellulose

Cellulose is the main structural constituent in plant cell walls and is found in an organized fibrous structure. The structure of cellulose is shown in Figure 2.3. This linear polymer consists of D-glucose subunits linked to each other by β-(1, 4)-glycosidic bonds.

Cellobiose is the repeat unit established through this linkage, and it constitutes cellulose chains. The long-chain cellulose polymers are linked together by hydrogen and van der Waals bonds which cause the cellulose to be packed into microfibrils. Hemicelluloses and lignin cover the microfibrils.

Fermentable D-glucose can be produced from cellulose through the action of either acid or enzymes breaking the β-(1, 4)-glycosidic linkages. Cellulose in biomass is present in both crystalline and amorphous forms. Crystalline cellulose comprises the major proportion of cellulose, whereas a small percentage of unorganized cellulose chains form amorphous cellulose. Cellulose is more susceptible to enzymatic degradation in its amorphous form.

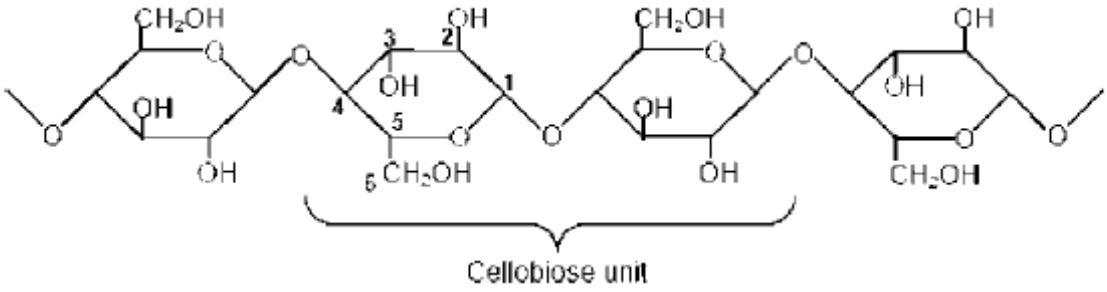


Figure 2.3 Structure of a cellulose chain

2.1.2 Hemicellulose

The main feature that differentiates hemicellulose from cellulose is that hemicellulose has branches with short lateral chains consisting of different sugars. These monosaccharides include pentoses (xylose, rhamnose, and arabinose), hexoses (glucose, mannose, and galactose), and uronic acids (e.g., 4-*o*-methylglucuronic, D-glucuronic, and D-galactouronic acids). The backbone of hemicellulose is either a homopolymer or a

heteropolymer with short branches linked by β-(1,4)-glycosidic bonds and occasionally β-(1,3)-glycosidic bonds.

Also, hemicelluloses can have some degree of acetylation, for example, in heteroxylan. In contrast to cellulose, the polymers present in hemicelluloses are easily hydrolyzable. These polymers do not aggregate, even when they cocrystallize with cellulose chains.

The most common type of polymers that belongs to the hemicellulose family of polysaccharides is xylan. As shown in Figure 2.4, the molecule of a xylan involves 1-4 linkages of xylopyranosyl units with α-(4-O)-methyl-D-glucuronopyranosyl units attached to anhydroxylose units. The result is a branched polymer chain that is mainly composed of five carbon sugar monomers, xylose, and to a lesser extent six carbon sugar monomers such as glucose.

Important aspects of the structure and composition of hemicellulose are the lack of crystalline structure, mainly due to the highly branched structure, and the presence of acetyl groups connected to the polymer chain (Kirk and Othmer, 2001).

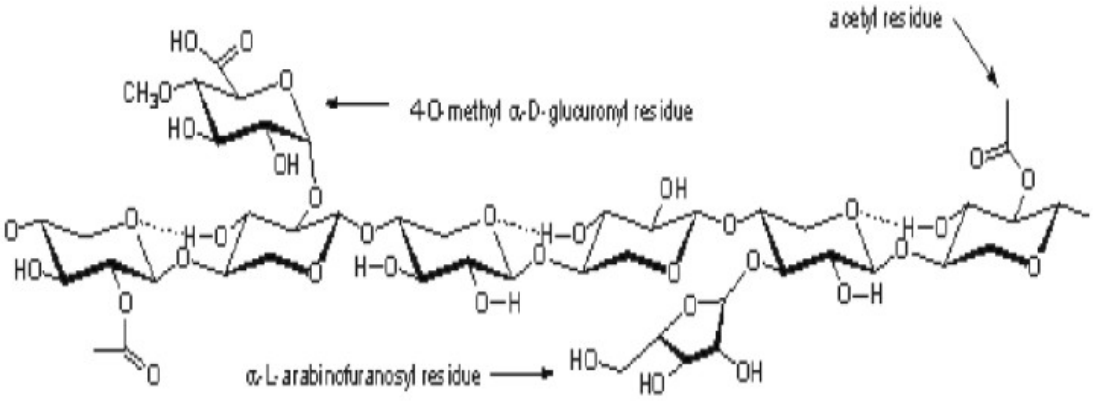


Figure 2.4 Overview of the hemicellulose backbone of arborescent plants

2.1.3 Lignin

Lignin is a complex, large molecular structure containing cross-linked polymers of phenolic monomers. It is present in the primary cell wall, imparting structural support, impermeability, and resistance against microbial attack. Three phenyl propionic alcohols exist as monomers of lignin as illustrated in Figure 2.5 below: coniferyl alcohol (guaiacyl propanol), coumaryl alcohol (*p*-hydroxyphenyl propanol), and sinapyl alcohol (syringyl alcohol). Alkyl-aryl, alkyl-alkyl, and aryl-aryl ether bonds link these phenolic monomers together. In general, herbaceous plants such as grasses have the lowest contents of lignin, whereas nutshells and softwoods have the highest lignin contents as listed in Table 2.2.

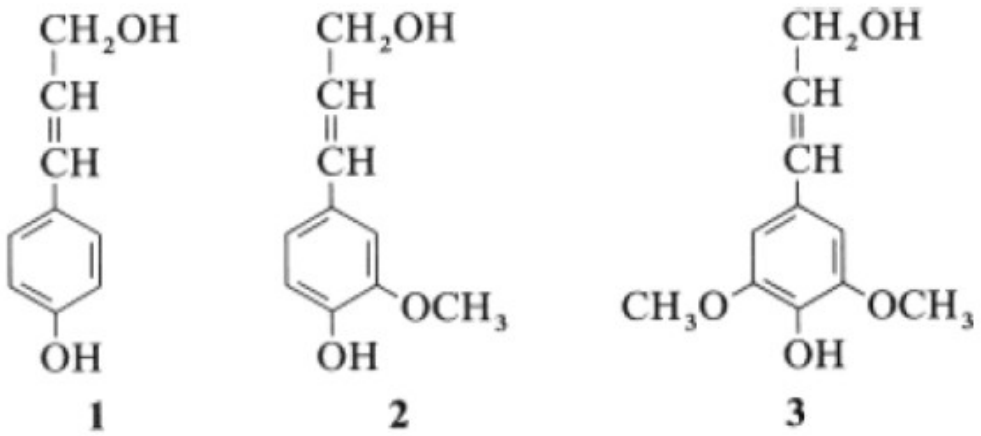


Figure 2.5 Dominant building blocks of the 3-D polymer lignin

.2.3 Feedstock Choice

The lignocellulosic materials chosen for the study are majorly *Prosopis Juliflora* (Mathenge plant) and *Gadam* Sorghum stalks represented in Figures 2.6 and 2.7 respectively.

2.1.1 *Prosopis Juliflora* (Mesquite/Mathenge Plant)

Figure 2.6 *Prosopis Juliflora*

Prosopis juliflora (Sw.) DC is an evergreen tree native to South America, Central America and the Caribbean. In the United States, it is well known as mesquite. It is fast growing, nitrogen-fixing and tolerant to arid conditions and saline soils. It is an invasive multipurpose dry land tree or shrub belonging to the fabaceae family and is therefore a softwood (Chandra et. al., 2007). *Prosopis juliflora* is an evergreen tree with a large crown and an open canopy, growing to a height of 5-10m and the root system includes a deep taproot. It has dark green compound leaves and the inflorescence is small, green-yellowish spikes without any particular fragrance or attractiveness, though relished by bees. Flowering begins at the age of 3-4 years. The bisexual, pea like flowers are cross-pollinated by wind and insects. The seed is disseminated and pretreated by the agency of animals that feed on the pods. The fruit is a non-dehiscent pod with ovoid, hard, darkbrown seeds, with mucilaginous endosperm surrounding the embryo. Under the right conditions, *Prosopis juliflora* can produce a variety of valuable goods and services: construction materials, charcoal, soil conservation and rehabilitation of degraded and saline soils. Concern about deforestation, desertification and fuelwood shortages in the

late 1970s and early 1980s prompted a wave of projects that introduced *Prosopis juliflora* and other hardy tree species to new environments across the world. *Prosopis juliflora* has survived where other tree species have failed and in many cases become a major nuisance.

The first documented introductions of *Prosopis juliflora* and *Prosopis pallida* to Kenya was in 1973 for the rehabilitation of quarries near the coastal city of Mombasa, with seed sourced from Brazil and Hawaii (Johansson, 1985 cited in Choge et al, 2002). The same species were introduced into the semi-arid districts of Baringo, Tana River and Turkana districts in the early 1980s with the intention of ensuring self-sufficiency in wood products, making the environment habitable and safeguarding the existing natural vegetation from overexploitation by the rising human populations (Choge et al, 2002).
Uses of prosopis include timber (building materials, floor tiles, furniture, handicrafts), charcoal, firewood, human food (toasted seeds, beverage and processed food), animal feed (fodder, bee forage flowers are good for honey production), gum production, tannin extraction, possible medicinal values and wind breaks of agricultural crops (Pasiecznik *et al*., 2001; Aboud *et al*., 2005; Choge *et al*., 2007). Fruit pods are high in sugar and protein content and are a rich food source for livestock like sheep, goats and cattle. Prosopis pods are palatable feeds and good sources of energy for ruminants due to their digestible carbohydrate content. They can replace part of the diet grains (Sawal *et al*., 2004).

On average, the chemical composition of prosopis julifora is 49.4% cellulose, 18% hemicellulose, 4.3% ash, 28.3% lignin and extractives (Naseeruddin, et al., 2013).

2.1.2 *Gadam* or *Sila* Sorghum

Figure 2.7 *Gadam Sila* Sorghum

The demand for sorghum in Kenya has shot up dramatically following the decision by the East Africa Breweries Limited (EABL) to use the produce for manufacture of one of its beer brands. The type of sorghum being supplied to the EABL is known as gadam or sila sorghum, which matures in three months and grows in areas that receive average rainfall. The agriculture business model is particularly important in Kenya because the crop does well in areas that are drought prone and which suffer the biggest impact of hunger when the amount of rainfall reduces. The gadam and sila sorghum are currently being grown across parts of Eastern province, Nyanza and Western Kenya.

East African Breweries (EABL) has extended its commercial sorghum growing project to Siaya in an effort to secure more supplies for its low-cost beer brands. The project is expected to boost earnings of farmers in the region as the company provides a reliable market for their crop since the company's demand for the sorghum is huge. EABL has in the past few years increased its partnership with sorghum growers in various parts of the

country as it seeks to reduce reliance on the relatively expensive barley, part of which is imported.

The growth of products made from local sorghum means the company will have access to cheaper raw material whose supply can be better managed. With this increased campaign for more sorghum to be grown, adequate supplies of sorghum stalk biomass could be available since the brewer utilizes only the grains in the beer making process. As such, the sugar rich stalks can be used for bioethanol production.

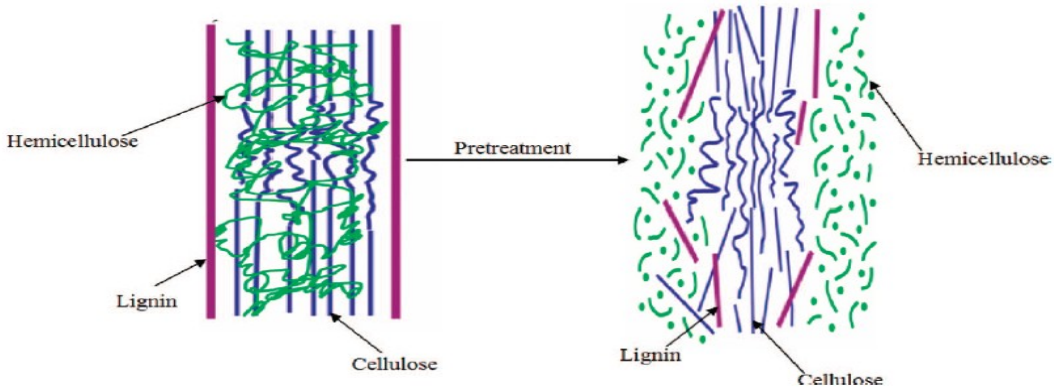
On average, the chemical composition of gadam sorghum is 35.87%, cellulose, 26.04% hemicelluloses, 7.52% ash and 30.57% lignin & extractives (Cardoso et. al., 2013).

2.2 Pretreatment Methods

The inherent properties of native lignocellulosic materials make them resistant to enzymatic attack. The aim of pretreatment is to change these properties in order to prepare the materials for enzymatic degradation. Since lignocellulosic materials are very complicated, their pretreatment is not simple either.

Pretreatment is a crucial process step for the biochemical conversion of lignocellulosic biomass into e.g. bioethanol. It is required to alter the structure of cellulosic biomass to make cellulose more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars (Mosier *et al.*, 2005). Pretreatment has been recognised as one of the most expensive processing steps in cellulosic biomass-to-fermentable sugars conversion and several recent review articles provide a general overxview of the field

(Alvira *et al*. 2009; Carvalheiro *et al.*, 2008; Hendriks and Zeeman, 2009; Taher-zadeh and Karimi, 2008).



(Source: (Hsu et al, 1980)

Figure 2.8 Effects of pretreatment on lignocellulosic biomass

Pretreatment involves the alteration of biomass so that (enzymatic) hydrolysis of cellulose and hemi-cellulose can be achieved more rapidly and with greater yields. Possible goals include the removal of lignin and disruption of the crystalline structure of cellulose (Figure 2.8). The following criteria lead to an improvement in enzymatic hydrolysis of lignocellulosic material:

- Increasing of the surface area and porosity of the lignocellulosic material
- Modification of lignin structure
- Removal of lignin
- Partial depolymerisation of hemicelluloses
- Removal of hemicelluloses
- Reducing the crystallinity of cellulose

In an ideal case the pretreatment employed leads to a limited formation of degradation products that inhibit enzymatic hydrolysis and fermentation, and is also cost effective. However, these are actually the most important challenges of current pretreatment technologies.

Pretreatment must meet the following requirements:

- Improve the formation of sugars or the ability to subsequently form sugars by hydrolysis,
- Avoid the degradation or loss of carbohydrate,
- Avoid the formation of byproducts that are inhibitory to the subsequent hydrolysis and fermentation processes, and
- Be cost-effective.

.3.1 Mechanical Pretreatment Methods

2.1.1 Milling

Reduction of particle size is often needed to make material handling easier and to increase surface area/volume ratio. This can be done by chipping, milling or grinding. Mechanical pretreatment is usually carried out before a following processing step, and the desired particle size is dependent on these subsequent steps. For mechanical pretreatment factors like capital costs, operating costs, scale-up possibilities and depreciation of equipment are very important.

2.1.2 Ultrasonic Pretreatment

The method of ultrasonication for lignocellulosic biomass has been investigated at laboratory scale; it is a well known technique for treatment of sludge from waste water

treatment plants. The experiments show the effect of the pretreatment of pure cellulose on its enzymatic hydrolysis, using a model compound (CarboxyMethyl Cellulose, CMC). The issue of lignocellulose pretreatment is not addressed in the experiments. The experimental results showed that when a suspension of cellulose is provided with energy by irradiation, the reaction rate of the subsequent enzymatic hydrolysis is increased by approximately 200% (Imai *et al.*, 2004). The mode of action is not investigated. Presumably, it is the hydrogen bonds of the cellulose crystalline structure that break if treated with enough energy. The energy provided in this case is 130 kJ/g CMC, which is significantly higher than the energy of the hydrogen bond 0.12 kJ/g cellulose (21 kJ/mol cellulose (Bochek, 2003)).

.3.2 Chemical Pretreatment Methods

To this group belong the pretreatments that are purely initiated by chemical reactions for disruption of the biomass structure.

2.1.1 Liquid Hot Water

Liquid hot water (LHW) processes are biomass pretreatments with water at high temperature and pressure. Other terms are hydrothermolysis, hydrothermal pretreatment, aqueous fractionation, solvolysis or aquasolv (Mosier *et al.*, 2005). Solvolysis by hot compressed water contacts water with biomass for up to 15 min at temperatures of 200–230 °C. Between 40% and 60% of the total biomass is dissolved in the process, with 4–22% of the cellulose, 35–60% of the lignin and all of the hemicellulose being removed. Over 90% of the hemicellulose is recovered as monomeric sugars when acid was used to hydrolyze the resulting liquid. In addition, acetic acid is formed during the treatment and

acts as a catalyst for polysaccharide hydrolysis. This results in the formation of monomeric sugars that may further decompose to furfural (inhibitor of fermentation). Variability in results was related to the biomass type with high lignin solubilization impeding recovery of hemicellulose sugars (Mok and Antal Jr, 1992).

2.1.2 Weak Acid Hydrolysis

Dilute acid treatment is one of the most effective pretreatment methods for lignocellulosic biomass. In general there are two types of weak acid hydrolysis:

- High temperature and continuous flow process for low-solids loading (T ˃ 160°C, 5-10 wt% substrate concentration)
- Low temperature and batch process for high-solids loading (T ≤ 160°C, 10-40 wt % substrate concentration)

Dilute (mostly sulphuric) acid is sprayed onto the raw material and the mixture is held at 160-220 °C for short periods up to a few minutes. Hydrolysis of hemicellulose then occurs, releasing monomeric sugars and soluble oligomers from the cell wall matrix into the hydrolysate. Hemicellulose removal increases porosity and improves enzymatic digestibility, with maximum enzymatic digestibility usually coinciding with complete hemicellulose removal (Chen *et al*., 2007). As an alternative to inorganic acids, organic acids (e.g. maleic acid, fumaric acid) can be used for dilute acid pretreatment (Kootstra et al, 2009).

The treatment offers good performance in terms of recovering hemicellulose sugars but there are also some drawbacks. The hemicellulose sugars might be further degraded to furfural and hydroxymethyl furfural, strong inhibitors to microbial fermentation.

Furthermore, acids can be corrosive and neutralization results in the formation of solid waste. The method is especially suitable for biomass with low lignin content, as almost no lignin is removed from the biomass.

2.1.3 Strong Acid Hydrolysis

Concentrated strong acids such as H_2SO_4 and HCl have been widely used for treating lignocellulosic materials because they are powerful agents for cellulose hydrolysis (Sun and Cheng, 2002), and no enzymes are needed subsequent to the 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. To date, several companies are in the process of commercialising strong acid hydrolysis of lignocellulosic biomass for microbial fermentation purposes (BlueFire Ethanol, 2010; Biosulfurol, 2010).

2.1.4 Alkaline Hydrolysis

The major effect 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 and Holtzapple, 2000). It is reported that alkaline hydrolysis mechanism is based on saponification of intermolecular ester bonds crosslinking xylan hemicelluloses and other components such as lignin (Sun and Cheng, 2002).

Usually lime (calcium hydroxide) or sodium hydroxide is used. By using these components salts are formed that may be incorporated into the biomass and they need to be removed or recycled (González *et al*., 1986). Process conditions are relatively mild but reaction times can be long. These mild conditions prevent condensation of lignin, resulting in high lignin solubility, especially for biomass with low lignin content such as softwood and grasses. Due to the mild conditions, degradation of sugars to furfural, HMF and organic acids is limited. The addition of air or oxygen to the reaction mixture greatly improves the delignification, especially highly lignified materials (Chang and Holtzapple, 2000).

Pretreatment of biomass with aqueous ammonia at elevated temperatures reduces lignin content and removes some hemicellulose while decrystallising cellulose. Ammonia pretreatment techniques include the ammonia fibre explosion-method (AFEX), ammonia recycle percolation (ARP) and soaking in aqueous ammonia (SAA).

With ARP the biomass is pretreated with aqueous ammonia in a flow-through column reactor. The liquid flows at high temperature through the reactor column, which has been previously packed with biomass. To prevent flash evaporation the reactor system must be slightly pressurized (e.g. 2.3 MPa) (Kim *et al*., 2003; Kim and Lee, 2005). After reaction, the solid fraction, rich in cellulose and hemicellulose, is separated from the liquid. This liquid fraction is sent into a steam-heated evaporator for ammonia recovery and lignin and other sugar separation. Ammonia is then recycled to the reactor inlet whereas the separated fraction is sent into a crystallizer. After crystallization, a washing step is carried out in order to extract the sugars that have been retained in the solid matrix.

Soaking in aqueous ammonia (SAA) at low temperature efficiently removes the lignin in the raw material by minimizing the interaction with hemicellulose. As a result an increase of surface area and pore size is achieved. Thus, retained hemicellulose and cellulose can be hydrolyzed to fermentable sugars by most commercial xylanase and cellulase mixtures. The cost of ammonia and especially of ammonia recovery drives the cost of the pre-treatment (Holt-zapple *et al.*, 1991 & 1994). However, biomass pretreatment economics are also strongly influenced by total sugar yields achieved.

2.1.5 Organosolv

Organosolv processes use an organic solvent or mixtures of organic solvents with water for removal of lignin before enzymatic hydrolysis of the cellulose fraction. In addition to lignin removal, hemicellulose hydrolysis occurs leading to improved enzymatic digestibility of the cellulose fraction. Common solvents for the process include ethanol, methanol, acetone, and ethylene glycol. Temperatures used for the process can be as high as 200 °C, but lower temperatures can be sufficient depending on e.g. the type of biomass and the use of a catalyst (Ghose *et al.*, 1983). Possible catalysts include inorganic or organic acids (Sun and Cheng, 2002).

The solvent itself can be an inhibitor for the enzymatic hydrolysis and fermentation step. Therefore, the solvent must be partly removed prior to fermentation. Removal and recovery of the solvent is required for reducing its cost and environmental impact as well. Benefits of organosolv pretreatment include:

- The production of a high-quality lignin, which might facilitate higher-value applications of lignin such as production of (platform) chemicals.
- Potentially lowering the enzyme costs by separation of lignin before the enzymatic hydrolysis of the cellulose fraction. In addition to improved accessibility of the cellulose fibres, absorption of cellulase enzymes onto lignin is also minimized by actual removal of lignin beforehand.

2.1.6 Oxidative Delignification

Delignification of lignocellulose can also be achieved by treatment with an oxidising agent such as hydrogen peroxide, ozone, oxygen or air. The lignin polymer will be converted into e.g. carboxylic acids. Since these acids formed will act as inhibitors in the fermentation step, they have to be neutralized or removed. In addition to an effect on lignin, oxidative treatment also affects the hemicellulose fraction of the lignocellulose complex. A substantial part of the hemicellulose might be degraded and can no longer be used for sugar production.

Hydrogen peroxide

An oxidative compound commonly used is hydrogen peroxide (H_2O_2). Dissolution of about 50% of lignin and most of the hemicellulose has been achieved in a solution of 2% H_2O_2 at 30 °C. The yield of enzymatic hydrolysis followed can be as high as 95%.

Ozonolysis

Ozone treatment focuses on lignin degradation by attacking and cleavage of aromatic rings structures, while hemicellulose and cellulose are hardly decomposed. It can be used to disrupt the structure of many different lignocellulosic materials, such as wheat straw, bagasse, pine, peanut, cotton straw and poplar sawdust (Sun and Cheng, 2002).

Wet oxidation

Wet oxidation operates with oxygen or air in combination with water at elevated temperature and pressure (McGinnis *et al.*, 1983). It was presented as an alternative to steam explosion which had become the most widely used pretreatment method (Ahring *et al*, 1999). Industrially, wet air oxidation processes have been used for the treatment of wastes with a high organic matter by oxidation of soluble or suspended materials by using oxygen in aqueous phase at high temperatures (150-350 °C) and high pressure (5-20 MPa) (Jorgensen *et al*., 2007).

Compared to other pretreatment processes, wet oxidation has been proven to be efficient for treating lignocellulosic materials because the crystalline structure of cellulose is opened during the process (Panagiotou and Olsson, 2007).

About a 65% degree of delignification could be achieved with wheat straw (Klinke *et al*., 2002). Wet oxidation of wood material has been shown to dissolve mainly the hemicellulose. One reported advantage of the wet oxidation process is the lower production of furfural and 5-hydroxymethylfurfural, which are potential inhibitors in the fermentation step.

2.1.7 Room Temperature Ionic Liquids

Room Temperature Ionic Liquids (RTIL) are salts that are in the liquid phase at temperature as low as room temperature. There is a vast variety of different RTIL, however, they share a common characteristic, which is that they are usually comprised of an inorganic anion and an organic cation of very heterogeneous molecular structure. The

difference in the molecular structure renders the bonding of the ions weak enough for the salt to appear as liquid at room temperature (Van Rantwijk, 2003).

There is an indication that mainly due to their polarity and in general their unique properties, they can function as selective solvents of lignin or cellulose. That would result in separation of lignin and increase of cellulose accessibility under ambient conditions and with no use of acid or alkaline solution. The formation of inhibitor compounds could also be avoided.

.3.3 Combined Chemical and Mechanical Pretreatment

These methods combine mechanical and chemical action. To this group belong the following pre-treatments:

2.1.1 Steam Explosion

Steam explosion (uncatalysed or catalysed) is one of the most applied pretreatment processes owing to its low use of chemicals and limited energy consumption. With this method high-pressure saturated steam is injected into a batch or continuous reactor filled with biomass. During the steam injection, the temperature rises to 160-260 ºC. Subsequently, pressure is suddenly reduced and the biomass undergoes an explosive decompression with hemicellulose degradation and lignin matrix disruption as a result.

Results of steam-explosion pretreatment depend on residence time, temperature, particle size and moisture content (Sun and Cheng, 2002). Studies have been carried out to try to improve the results of steam explosion by addition of chemicals such as acid or alkali (Cara *et al.*, 2008, Stenberg *et al.*, 1998, Zimbardi *et al*., 2007). Limitations of steam

explosion include the formation of degradation products that may inhibit downstream processes (Garcia-Aparicio *et al.*, 2006).

2.1.2 Ammonia Fibre Explosion (AFEX)

In the AFEX process, biomass is treated with liquid ammonia at high temperature and pressure (Tey-mouri *et al.*, 2005). After a few seconds, pressure is swiftly reduced. A typical AFEX process is carried out with 1-2 kg ammonia/kg dry biomass at 90 °C for 30 min. It reduces the lignin content and removes some hemicellulose while decrystallising cellulose. The cost of ammonia and especially of ammonia recovery drives the cost of the pre-treatment (Holtzapple *et al.*, 1991 & 1994), although ammonia is easily recovered due to its volatility.

2.1.3 CO_2 Explosion

This method is similar to steam and ammonia fibre explosion; high pressure carbon dioxide (CO_2) is injected into the batch reactor and then liberated by an explosive decompression. It is believed that CO_2 reacts to carbonic acid (carbon dioxide in water), thereby improving the hydrolysis rate. Yields of CO_2 explosion are lower than those obtained with steam or ammonia explosion, but they are higher than those reached with enzymatic hydrolysis without pretreatment (Sun and Cheng, 2002).

Carbonic acid may offer the benefits of acid catalysts without the use of an acid like sulphuric acid. The pH of carbonic acid is determined by the partial pressure of CO_2 in water, and can be neutralized by releasing the reactor pressure.

An alternative use of CO_2 in pretreatment is extraction with supercritical CO_2. Carbon dioxide becomes supercritical under relatively mild conditions (Tc=304K and Pc=73 bar). Supercritical carbon dioxide (SC-CO_2) has been widely used as an extraction solvent. Recently, SC-CO_2 extraction is being considered as possible pretreatment route for lignocellulosic material.

2.1.4 Mechanical/Alkaline Pretreatment

Combined mechanical/alkaline pretreatment consists of a continuous mechanical pretreatment (e.g. milling, extrusion, refining) of lignocellulosic biomass with the aid of an alkali. The resulting fractions consist of a soluble fraction (containing lignin, hemicellulose and inorganic components) and a cellulose-enriched solid fraction.

As opposed to the acid-catalysed methods, the general principle behind alkaline pretreatment methods is the removal of lignin whereas cellulose and part of the hemicelluloses remain in the solid fraction. The solid fraction is submitted to enzymatic hydrolysis for the production of C6- and C5 sugars and this pretreatment method is especially suitable in combination with fermentation routes in which both C6- and C5 sugars can be converted to products.

By performing extrusion and chemical pretreatment in one step the accessibility of cellulose for enzymes is improved, resulting in higher delignification values and improved enzymatic hydrolysis. In addition, the moderate operation temperatures of this process prevent the formation of degradation and oxidation products.

The combination of alkaline pretreatment with mechanical action increases the efficiency of the pretreatment compared to alkaline pretreatment, but the use of expensive chemicals remain necessary, and recycling and waste treatment is an important issue.

.3.4 Biological Pretreatment

In this group of pretreatments microorganisms such as white, brown and soft rot-fungi are employed to degrade hemicellulose and lignin. Advantages of biological pretreatments are low energy requirement and mild operation conditions. Nevertheless, the rate of biological hydrolysis is usually very low, so this pretreatment requires long residence times (Cardona and Sanchez, 2007; Sun and Cheng, 2002; Tengerdy and Szakacs, 2003).

As outlined in the above pretreatment methods, most have several disadvantages. They are tailored to specific types of lignocelluloses (Mosier et al., 2005) and some of the solvents cause the degradation of lignocelluloses upon dissolving. These unwanted by-products can inhibit a subsequent hydrolysis or fermentation step (Larsson et al., 1999; Weil et al., 1994).

2.3.5 Summary

The following drawbacks are also notable with the different methods: (a) the biological processing methods require a long residence time; (b) the physical treatments are energy-demanding, expensive and do not remove the lignin; (c) the chemical methods are costly and mainly suitable for high-value paper products; (d) the physico-chemical pretreatment procedures are considered as very promising, but require high pressures/temperatures and the use of catalysts.

Cellulose solvents should have most of the features listed below (Olivier-Bourbigou et al., 2010):

- be able to dissolve cellulose at low temperatures,
- be non volatile, non toxic and chemically stable,
- not decompose cellulose,
- be easy to regenerate,
- be recyclable,
- be cost effective and easily processed
- be non-toxic to enzymatic and microbial fermentation.

As indicated in the list above, solvent use and recovery increases the total cost of pretreatment. Some pretreatment methods are too slow (even weeks), while other methods cannot be considered as “green” since hazardous or toxic compounds are released (Zhu 2008). Therefore, more efficient pretreatment procedures are required. One potential alternative is the use of ionic liquids.

2.1 Ionic Liquids

.4.1 General Properties

Ionic liquids (ILs) are a group of new organic salts that are liquids at relative low temperatures (below 100°C). As the name suggest they are completely ionic with most ionic liquids consisting of an organic cation and an inorganic anion. Many ionic liquids are also liquids at room temperature making it an ideal solvent to work with. Compared with traditional molecular solvents, ionic liquids express very interesting properties like broad liquid regions, high thermal stabilities and negligible vapour pressures (Brennecke

& Maginn, 2001). Ionic liquids are also called green solvents because no toxic or explosive gases are formed when used (Anderson et al., 2002). Depending on which anion and cation is chosen, their physical and chemical properties such as melting points, viscosity, hydrophobicity and hydrolysis stability are affected (Huddleston et al., 2001). Therefore, optimal ionic liquids for a special application can be designed. Simply by making changes to the structure of either the anion or the cation, or both, properties such as solubility, density, refractive index and viscosity can be adjusted to meet the requirements of the user or experiment. The tuning of properties is possible by varying the length and branching of the alkyl groups that are incorporated to the cation. There is also the potential for task-specific ionic liquids (TSILs) to be produced.

When observing the physical properties of ionic liquids, studies have shown that the melting point is influenced by the charge distribution on the ions, H-bonding ability, symmetry of the ions and van der Waals interactions. When comparing the melting points between NaCl and 1-propyl- 3-methylimidazolium chloride, 803 °C and 60 °C respectively, it is clear that the reduction in the melting temperature is caused by replacing the small inorganic cations by bulky asymmetric organic cations. Many ionic liquids undergo supercooling which make their melting point rather uncertain. Many RTILs (room temperature ionic liquid) can be supercooled to more than 100 K below the melting point and in many cases they then form a glass (March et al., 2004).

Most of the known ionic liquids are denser than water, with values between 1-1.6 g/cm^3. The density decreases with increasing length of the alkyl chain in a systematic manner (March et al., 2004). In most cases ionic liquids are viscous, similar to that of oils. Their

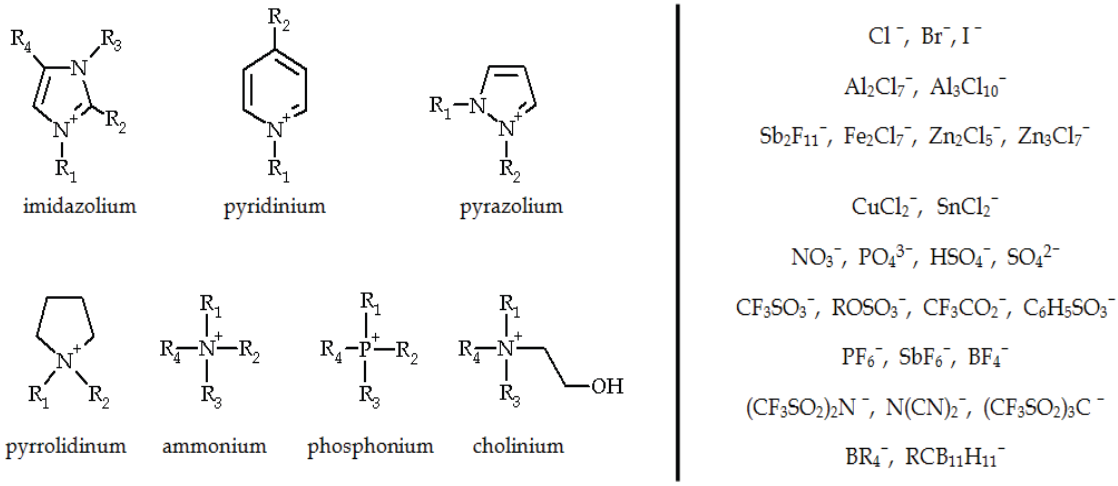
high viscosity is a disadvantage because it will negatively affect the mass transfer and power requirements for mixing heterogeneous liquid-liquid systems. An increase in length of one or both alkyl chains in the cation does not automatically result in a direct increase of the viscosity.

The first published report of dissolution of cellulose in an ionic liquid ([Nethylpyridinium][Cl] in the presence of a nitrogen base) dates back to 1934 (Graenacher 1934).

Today, there are more than 20 ionic liquids which are known to dissolve cellulose (Barthel & Heinze, 2006). Ionic liquids are also able to dissolve other biomaterials (Fort et al., 2006), such as lignin and lignocelluloses (Kilpeläinen et al., 2007). Dissolved cellulose can be precipitated and separated from lignin and hemicelluloses by the addition of anti-solvents, such as water (Fort et al., 2006). Recent patents also prove that the pretreatment of lignocellulose using ionic liquids (ILs) is of industrial interest, and the commercialisation of such processes is underway (Myllymäki & Aksela, 2008; Zhu, 2008).

The ability of ionic liquids to dissolve cellulose depends on the nature of the native cellulose (its degree of polymerization and crystallinity) on the operating conditions (temperature, reaction time, initial concentration of cellulose in the IL) and the presence of impurities (water). The use of a nondried ionic liquid can affect the solubility of cellulose, so much that severely dried ionic liquid is needed to achieve an optimal dissolution (Vitz et al., 2009).

Figure 2.9 below shows the cations and anions that make up ionic liquids. However, the ionic liquids which are most commonly considered for extraction and synthesis are those with cations based on an imidazolium or pyridinium ring with one or more alkyl groups attached to the nitrogen or carbon atoms.



(Source: Olivier-Bourdigou et al., 2010)

Figure 2.9 Main cations and anions described in literature

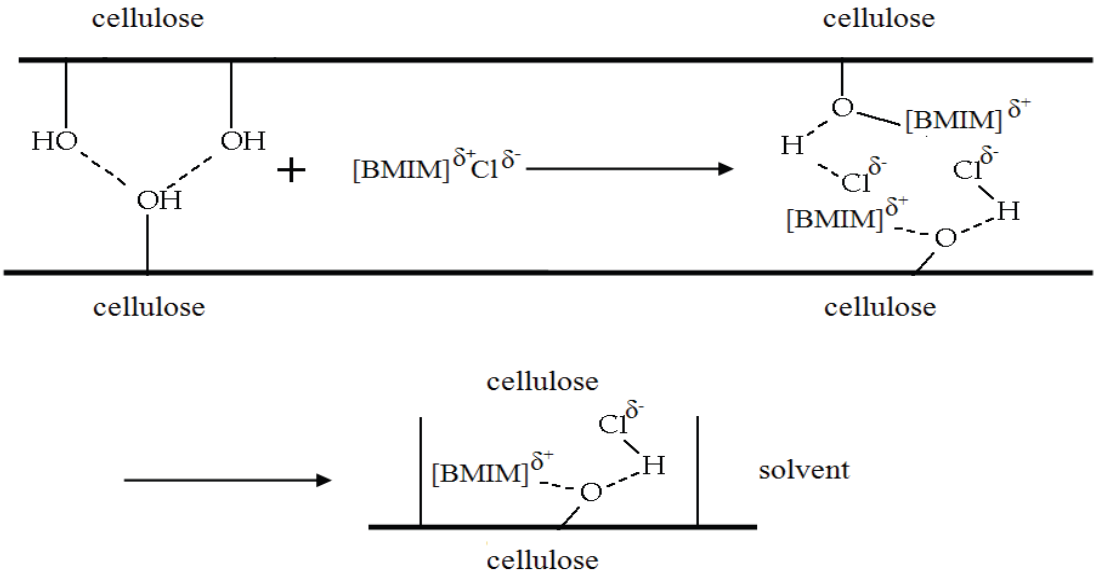
.4.2 Dissolution Process in Ionic Liquids

The main properties of ILs affecting the dissolution and functional modification of cellulose and carbohydrates are their polar characteristics, the basic form of the ionic liquid anions and their ability to generate H-bonds. Figure 2.10 and 2.11 below give the most important contribution which is the hydrogen´s bonding ability that the IL anion has (chlorine forms an H-bond with the hydroxyl groups of the biomolecule) (Olivier-Bourbigou et al., 2010).

In a typical process when dissolving cellulose, the reaction medium has to be heated. Therefore, thermal stability is also an important aspect. Depending on the purity of the IL, the melting points of IL can differ. In order to dissolve cellulose, the ionic liquid should satisfy the following three conditions:

- the anion must be a good hydrogen bond acceptor;
- the cation should be a moderate hydrogen bond donator because the cation has the most moderate activated hydrogen for forming hydrogen bonding with oxygen atoms of the hydroxyls of cellulose and
- The size of the cation should not be too large (Zhang et al., 2010). The IL solutions become increasingly viscous as the length of the dissolved cellulose oligomer increases (Moulthorp et al., 2005).

The ionic parameters, such as cation and anion size, as well as hydrogen bond basicity, play important roles in the ability of ionic liquids to dissolve cellulose. Smaller cations can be more efficient for the dissolution of cellulose. For example, with the same anion, [EMIM]+ had a higher capacity for the dissolution of cellulose than [BMIM]+ because [EMIM]+ is smaller than [BMIM]+ . The ability of ILs to form hydrogen bonds with cellulose decreases when the cation is too large. Anion size may have the similar effects; the ability of ILs to dissolve cellulose decreased due to the different anion in the following order: [BMIM]Cl > [BMIM]I and [BMIM]Br (partial solubility) > [BMIM]PF6 (unable to dissolve cellulose). This is consistent with the increasing thermochemical radii of these anions as follows: $Cl^- < Br^- < I^- < PF^-$.



(Source : Feng & Chen, 2008)

Figure 2.10 Dissolution mechanism of cellulose in [BMIM][Cl]

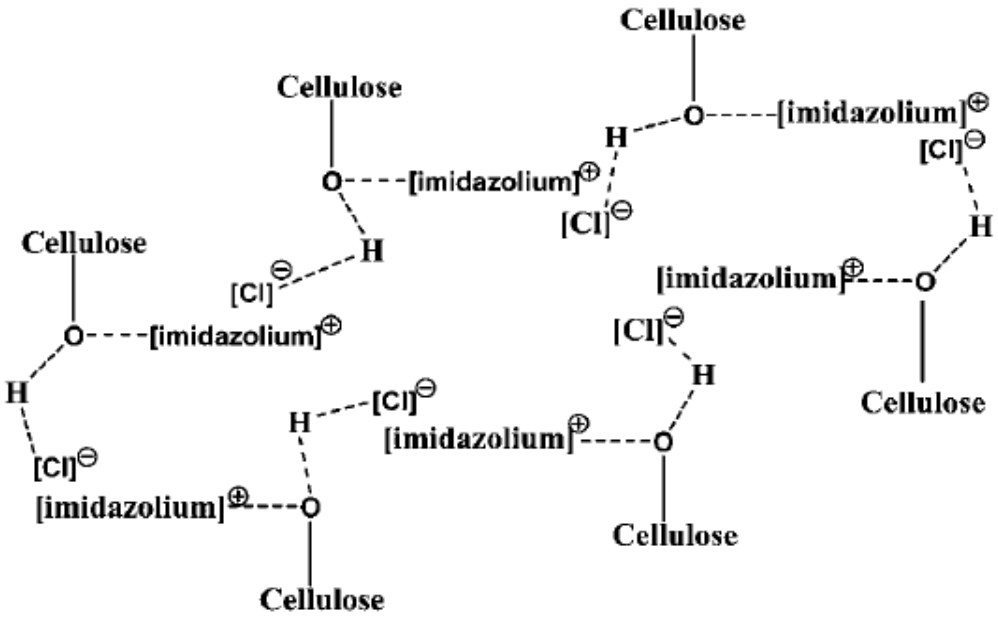


Figure 2.11 Hydrogen bonding between IL anions and cellulose.

(Zhang et al., 2005) speculated that the small cation [AMIM]+ favours the attack on the oxygen atom of the hydroxyl group in cellulose and that the less electronic chemical structure caused by the alkyl group also enhances the interaction between cations in ionic liquid and oxygen atoms of the hydroxide group in cellulose. Above the critical temperature, the ions in [AMIM][Cl] dissociate to individual [AMIM]+ and Cl- ions. The free Cl- ions associate with the cellulose hydroxyl proton and the free cations complex with the cellulose hydroxyl oxygen. This leads to the disruption of hydrogen bonds in cellulose and to the dissolution of cellulose. (Fukaya et al., 2008) discovered the same mechanism occurring in their studies.

Studies by (Zhang et al., 2010) showed that the hydrogen bonding of hydroxyls in cellulose with the [CH3COO-] anion and [AMIM] + cation is the major force behind cellulose dissolution in an ionic liquid. The relatively small acetate anion favours the formation of a hydrogen bond with hydrogen atoms in hydroxyls, while the aromatic protons in the bulky cation imidazolium (especially H_2) prefer to associate with the oxygen atoms of hydroxyls where there is less steric hindrance.

The carbohydrate dissolving ionic liquids usually contain anions such as Cl^-, $HCOO^-$ and OAc^-, which form strong hydrogen bonds with carbohydrates when being dissolved. For this reason, these ionic liquids are more likely to denature the enzymes and thereby prevent a further enzymatic modification of dissolved carbohydrates in ionic liquids. To overcome this problem, development over the past two years has been in the form of synthesizing new glycol-substituted ionic liquids that are capable of dissolving carbohydrates but not denature lipase (Zhao et al., 2008).

When using ionic liquids as cellulose solvents, it is important to investigate if any structural changes occur in the cellulose molecule during the cellulose dissolution, i.e. if any derivatisation or depolymeration of cellulose takes place. Ionic liquids, such as 1-butyl-3- methylimidazolium chloride, 1-methyl-3-methylpyridinium chloride and N-benzyl-N,N-dimethyltetradecylammonium chloride, were found to be non-derivative solvents for cellulose (Heinze et al., 2005). When dissolving dry fibre sludge (fibre sludge is pure cellulose) from a pulp mill in 1-butyl-3-methylimidazolium chloride, the IL was found to be non-derivative (Holm, 2010, unpublished data).

.4.3 Cellulose Regeneration and Ionic Liquid Recovery

(Zavrel et al., 2009) reported that a structural change of cellulose was observed when dissolved cellulose was precipitated with water. This could potentially enhance subsequent hydrolysis. The cellulose regenerated from ionic liquids was essentially found to be amorphous and porous, which was much more prone to degradation by cellulases (Dadi et al., 2006). The celluloses regenerated by rapid precipitation of the dissolved cellulose dopes with an anti-solvent (water) have demonstrated a great improvement on enzymatic hydrolysis kinetics (Kuo & Lee, 2009).

Glucose degradation products observed with acid pretreatment or hydrolysis include hydroxymethyl furfural (HMF) and furfural (Dadi et al., 2006). In the presence of water, HMF produces levulinic and formic acid that inhibit downstream fermentation. For example, if a [BMIM][Cl] pretreated fibre sludge sample is left standing for a long period

of time (weeks) before analysis, 5-HMF and levulinic acid can be found in the solution (Holm, 2010 unpublished data).

Following on from this, the dissolved cellulose in the ionic liquid can be precipitated from its solution by the addition of a non-solvent (anti-solvent), such as water, methanol, ethanol or acetone (Swatloski et al., 2002; Kosan et al., 2008). When the anti-solvent (e.g. water) is added to the solution the ions in the ionic liquid are extracted into an aqueous phase (Mäki- Arvela et al., 2010). The resulting regenerated cellulose can then be separated by filtration or centrifugation while the ionic liquid can be recovered through distillation of the antisolvent. The recovery of ionic liquids still requires much more research, although studies on biphasic systems with regards to the recovery have already been performed.

The regenerated cellulose can differ from the native cellulose in both the macro- and microstructure while the degree of crystallinity can be changed due to the changes in the regeneration conditions. The regenerated cellulose can have the same degree of polymerisation and polydispersity as the native cellulose but this depends on the operating conditions in the treatment (Olivier-Bourdigou et al., 2010). The regenerated cellulose demonstrated improved enzymatic hydrolysis kinetics (Dadi et al., 2006).

During the cellulose regeneration, careful attention should be taken when removing ionic liquid residues as their presence has detrimental effect on the cellulase activity depending on the amount of ionic liquid remaining. Such an inactivation may affect the final concentration levels of the total sugars reduction and glucose after the completion of hydrolysis (Zhao et al., 2009).

Since it was first reported that Ionic Liquids (ILs) can dissolve cellulose, there has been extensive research to explore if this ability to dissolve cellulose can be used in the context of LCB pretreatment (Swatloski et al., 2002). Extensive studies have been conducted in an effort to explore and further understand the use of ionic liquids as solvents for biomass dissolution; with easy recovery of the cellulose upon the addition of an anti-solvent.

From the aforementioned review, it is clear that ILs provide higher advantages than other pretreatment methods. In addition, significant research effort has been directed in understanding the role of imidazolium based ionic liquids in the dissolution of microcrystalline cellulose (Swatloski et al., 2002). However, there have been no detailed studies in the use of non-imidazolium based ILs and the possibility of their application in pretreatment of *Gadam* Sorghum and *Prosopis Juliflora*.

This study focuses mainly on the use of Ionic liquids with a purpose to explore non-imidazolium based ILs (Phosphonium and Pyridinium Based) as well in the pretreatment of *Gadam* Sorghum and *Prosopis Juliflora*.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

The research was carried out through literature review and experimentation. Experimentation majorly consisted of the pretreatment of the lignocellulosic biomasses with the different ionic liquids at varied conditions (temperature and time) in order to determine optimal conditions. The chemical composition analysis of the biomass materials before pretreatment was carried out at Moi University, in the laboratory of the Department of Chemical and Process Engineering. Analysis of the pretreated biomass after hydrolysis for glucose concentrations was carried out at Moi University also, in the laboratory of the Department of Chemical and Process Engineering.

By incorporating the use of classical OFAT design, coupled with a systematic and simultaneous monitoring of the effect of altering various parameters on the overall yield of glucose; the entire experimental domain was exhausted. The following variables had a direct impact on the results that were attained from this study: biomass type, pretreatment temperature and pretreatment period. The biomass loading was held at a constant of 6wt % while the particle size was maintained within the range 400 to 450 μm. The remaining variables - biomass type, pretreatment temperature and pretreatment period - were adjusted with the progression of the experiments.

3.2 Experimental Materials

The materials used in the experiments for the study were:

- *Prosopis Juliflora* (Mathenge plant) obtained from Marigat Area in Baringo County. The composition of this biomass is depicted by Table 4.1 in Section 4.1
- *Gadam* sorghum stalks obtained from Bungoma County. The composition of this biomass is depicted by Table 4.2 in section 4.1
- Commercial ionic liquid 1-butyl-4-methypyridinium chloride (4MBPCl), supplied by Sigma Aldrich whose properties are tabulated in Table 3.1 below.

Table 3.3 Properties of 1-butyl – 4 – methylpyridinium chloride

PROPERTY	**VALUE/STATE**
Molecular Formula	$C_{10}H_{16}ClN$
Physical State at room temperature	Solid
Nature	Hygroscopic
Melting Point (◦C)	117
Molecular Weight (g/mol)	185.69
Viscosity (cP at 25°C)	1557

Source: (National Centre for Biotechnology Information, 2016)

- Commercial ionic liquid trihexyltetradecylphosphonium chloride (P66614Cl) supplied by Sigma Aldrich whose properties are tabulated in Table 3.2 below.

Table 3.4 Properties of Trihexyltetradecyl phosphonium

PROPERTY	**VALUE/STATE**
Molecular Formula	$C_{32}H_{68}ClP$
Physical State at room temperature	Liquid
Nature	Hydrophobic
Melting Point (◦C)	-50
Molecular Weight (g/mol)	519.3
Density (g/cm^3 at 25°C)	0.8819
Viscosity (cP at 25°C)	1824

Source: (National Centre for Biotechnology Information, 2016)

- Commercial ionic liquid 1-butyl -3-methylimidazolium chloride ([BMIM$^+$][Cl$^-$]), supplied by Sigma Aldrich whose properties are tabulated in Table 3.3 below.

Table 3.5 Properties of 1-butyl – 3 – methylimidazolium chloride

PROPERTY	VALUE/STATE
Molecular Formula	$C_8H_{15}ClN_2$
Physical State at room temperature	Solid
Nature	Hygroscopic
Melting Point (◦C)	70
Molecular Weight (g/mol)	174.67
Viscosity (cP at 25◦C)	1033

Source: (National Centre for Biotechnology Information, 2016)

- Pure D(+) Glucose standard produced by Sigma Aldrich
- Pure furfural standard produced by Sigma Aldrich
- Glycerol for use in the oil bath
- Distilled water
- Hydrochloric Acid (1M)
- Sulphuric acid

3.3 Experimental Equipment

1. Weighing Balance, sensitive to 0.1 g
2. Electric mill used for size reduction of the biomass. This is done to increase the surface area for action of ionic liquid on the biomasses.
3. Mechanical sieve plates as shown in Image 3.1 below (Ranging between 50 μm to 5.6mm) and were used to obtain fractions of the biomass with a particle size range of 450-500μm.

Image 3.1 Mechanical sieve plates

4. Oven as represented in Image 3.2 below was used to dry the biomass to a moisture content of below 10%.

Image 3.2 Oven

5. Autoclave.
6. Soxhlet apparatus.
7. Dessicator.

8. Oil bath (Fabricated) for pretreatment at a contolled temperature. A cost effective oil bath was fabricated as shown in Images 3.3 – 3.5 below. This was done using a corrosion-resistant stainless steel for the working chamber, a temperature control which can regulate temperature up to 400^{o}C , a contactor which acts as a switch to cut out power when the required temperature is achieved, a temperature probe which is the sensing element (transducer) and an immersion heater (with specification of 2000Watts).

Image 0.3 Fabricated Oil Bath

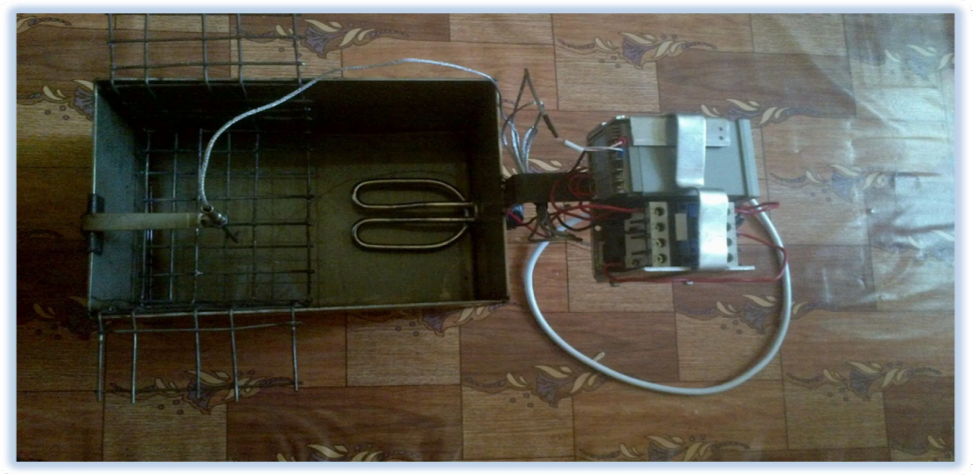


Image 3.4 Plan View of the Fabricated Oil Bath

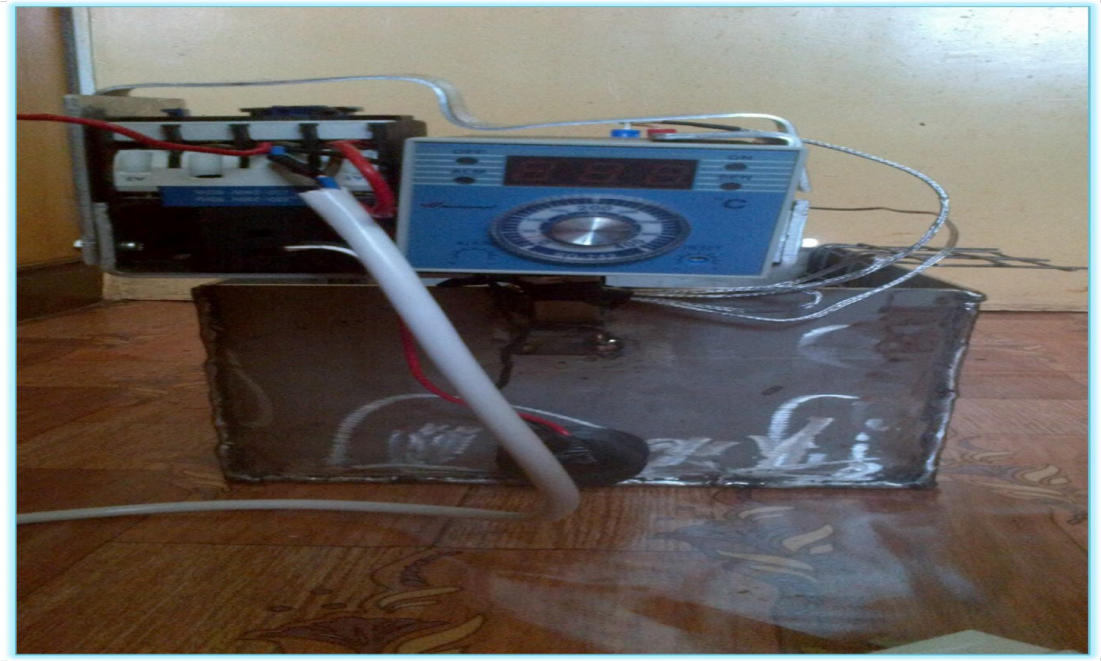


Image 3.5 Control System of the Oil Bath *(Temperature range of 0-400 ^{O}C)*

9. Basic Laboratory glassware: test tubes, boiling tubes, beakers, measuring cylinders and flasks.
10. Sample bottles/tins for storing the samples.
11. Separating funnel and nylon filter paper: used for separation of ILrich phase and solid rich phase. Also used for biomass and wash solution separation.
12. Water bath as shown by Image 3.6 below and used during hydrolysis at a controlled temperature.

Image 3.6 Water bath *(Temperature range of 0-100 ^{o}C)*

13. UV–VIS Spectrophotometer: Represented by Image 3.7 below and used for glucose and furfural concentration determination.

Image 3.7 Shimadzu UV- VIS Spectrophotometer

14. Quartz Cuvet.

15. Distillation unit.

3.4 Experimental Procedures

.4.1 Experimental Design

Three process variables were major in this work; pretreatment duration, pretreatment temperature and cation type. As a result of this, several variable combinations needed to be designed in order to establish the near optimum combination. The experimental matrices tabulated in Table 3.4 and Table 3.5 below were designed and used to execute the experimental runs.

Table 3.6 Experimental Matrix for Imidazolium IL

Biomass	Ionic liquid	Temp (◦C)	Time(mins)	Response (Glucose Yield)	Number of Specimens
Gadam Sorghum & Prosopis Juliflora	Imidazolium	80	40		6
			80		6
			120		6
			160		6
Gadam Sorghum & Prosopis Juliflora	Imidazolium	100	40		6
			80		6
			120		6
			160		6
Gadam Sorghum & Prosopis Juliflora	Imidazolium	120	40		6
			80		6
			120		6
			160		6
Gadam Sorghum & Prosopis Juliflora	Imidazolium	140	40		6
			80		6
			120		6
			160		6
Total Number of Specimens					**96**

Table 3.7 Experimental Matrix for Phosphonium and Pyridinium ILs

Biomass	Ionic liquid	Temp (◦C)	Time(mins)	Response (Glucose Yield)	Number of Specimens
Gadam Sorghum & Prosopis Juliflora	Phosphonium/ Pyridinium	80	40		4
			80		4
			120		4
			160		4
Gadam Sorghum & Prosopis Juliflora	Phosphonium/ Pyridinium	100	40		4
			80		4
			120		4
			160		4
Gadam Sorghum & Prosopis Juliflora	Phosphonium/ Pyridinium	120	40		4
			80		4
			120		4
			160		4
Gadam Sorghum & Prosopis Juliflora	Phosphonium/ Pyridinium	140	40		4
			80		4
			120		4
			160		4
Total Number of Specimens					**64**

The Experimental Design was then executed as illustrated in Figure 3.1 below.

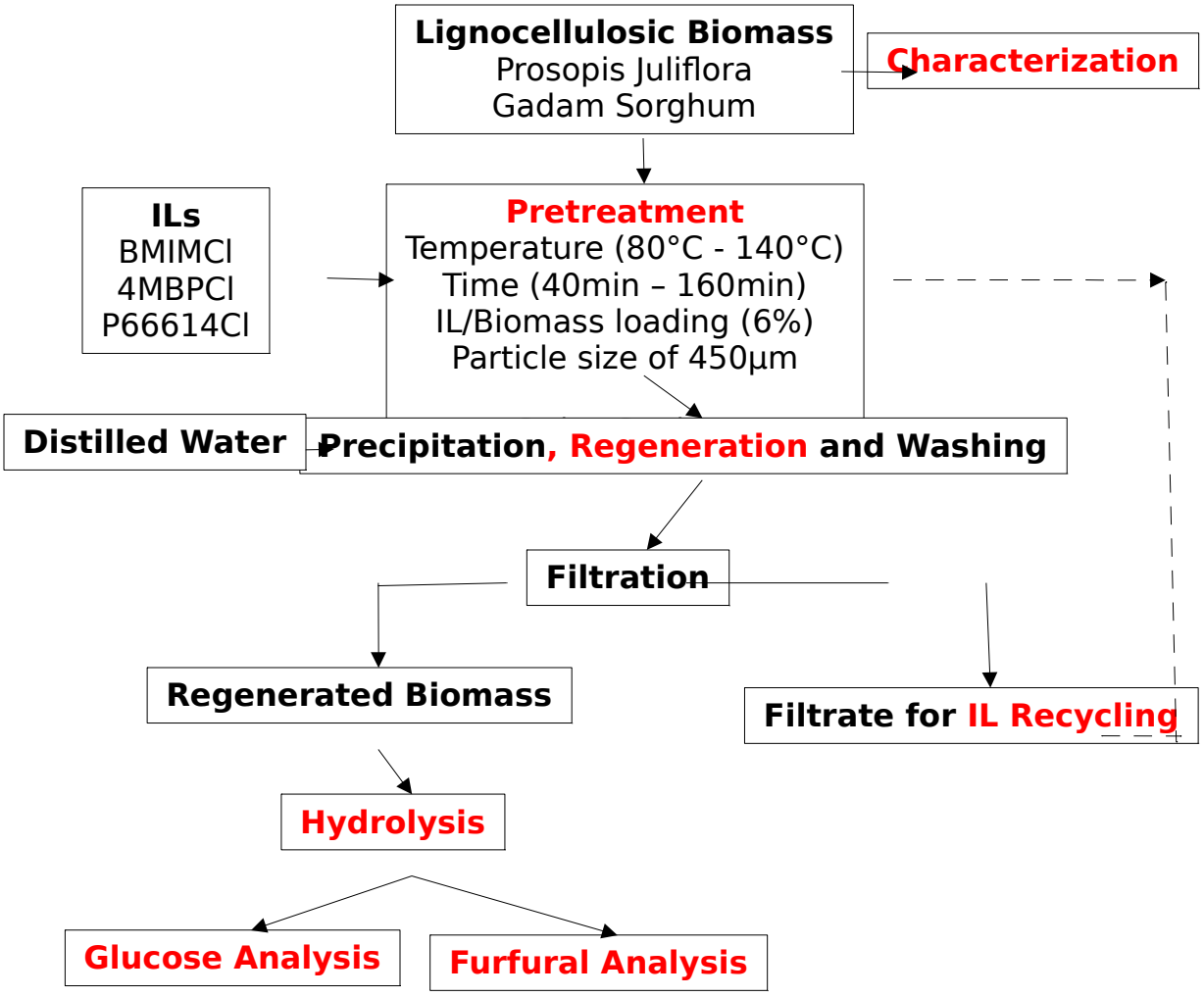


Figure 3.12 Overall Scheme of the Experimental Process

.4.2 Experimental Preparation and Analysis

Each of the lignocellulosic biomass was collected and size reduced mechanically using an electric mill and sieved to obtain fractions with a particle size range of 0.2-0.5mm. A particle size of approximately 450µm was obtained and used for all the experiments. Size reduction was done to increase the surface area for action of ionic liquids on the biomass.

Drying was then carried out at 100°C to eliminate/reduce the moisture content of the biomass in an oven to 9.12%.

By incorporating the use of statistical experimental design, coupled with a systematic and simultaneous monitoring of the effect of altering various parameters on the overall yield of glucose; the entire experimental domain was exhausted. That said, the following variables had a direct impact on the results that were attained from this study: particle size, biomass loading, biomass type, pretreatment temperature and pretreatment duration. Out of these variables, the biomass loading was held at a constant of 6wt% (6% biomass weight against 94% ionic liquid weight), while the particle size was maintained within the range 400 to 450 μm. The remaining variables - biomass type, pretreatment temperature and pretreatment duration - were adjusted with the progression of the experiments.

The characterization of each of the biomasses was first done to determine the compositional analysis prior to pretreatment with the three classes of ionic liquids. The percentage glucose yield was obtained for each of the sample runs and finally inhibitor concentration levels determined. The variables were majorly the pretreatment temperature, time and type of ionic liquid. The biomass loading was kept constant at 6%wt for all the samples.

In each case, three replications were employed and averages obtained. The analysis of the results was done using Microsoft 2007 Excel program.

3.1.1 Determination of Water-Soluble Extractives

The National Renewable Energy Laboratory (NREL) - Laboratory analytical procedures (LAP 010) was applied.

- The soxhlet apparatus was washed and dried then assembled.
- Using an electronic weighing balance, 6.19g of sample was measured and added to the tared extraction thimble and covered using cotton wool. The thimble was then inserted into the soxhlet apparatus.
- 190ml of distilled water was measured into the receiving flask which was then placed on the soxhlet apparatus. The heating mantle was adjusted to provide three siphon cycles per hour.
- The contents were refluxed for 9 hours to facilitate extraction process after which the heating mantle was turned off and glassware allowed to cool to room temperature.
- The thimble was left in the Soxhlet apparatus for ethanol extraction. However, residual water from the Soxhlet tube was removed.
- The extract solution was retained in a beaker and vacuum evaporation using a rotary vacuum evaporator was carried out.

3.1.2 Determination of Ethanol-Soluble Extractives

The National Renewable Energy Laboratory (NREL) - Laboratory analytical procedures (LAP 010) was applied.

- With the thimble in the case above still inserted on the Soxhlet extractor, 190ml of absolute ethanol was measured into the receiving flask which was then placed on the soxhlet apparatus. The heating mantle was adjusted to provide three siphon cycles per hour.

- The contents were refluxed for 6 hours to facilitate extraction process after which the heating mantle was turned off and glassware allowed to cool to room temperature.
- Ethanol was removed from the extractives by Vacuum evaporation

3.1.3 Determination of Lignin and Structural Carbohydrates

The National Renewable Energy Laboratory (NREL) - Laboratory analytical procedures (LAP 004) was applied.

- Using an electronic weighing balance, 0.3g biomass sample was measured into a tared flask. 3ml of 72% H_2SO_4 was then added into the biomass sample in the flask and thoroughly mixed.
- The flask was then placed in a water bath set at 30°C and incubated for one hour. Using the stir rod, the sample was stirred every 5 to 10 minutes without removing the sample from the bath. Stirring was essential to ensure even acid to particle contact and uniform hydrolysis.
- Upon completion of the 60-minute hydrolysis, the flask was removed from the water bath. The acid was then diluted to a 4% concentration by addition of 84.00mL distilled water. The sample was mixed by shaking the flask several times to eliminate phase separation between high and low concentration acid layers.
- Incubation was then carried out in a water bath set at 95°C for two hours.

Filtration was done into beakers and the filtrate neutralized to a pH of 5 using $CaCO_3$. $CaCO_3$ pellets were added to the filtrate while stirring.

The pH was tested using universal indicator – A few drops of the filtrate was put on a white tile then a drop of universal indicator was added to the filtration on the tile. The resulting color was compared with colours on a colour chart to determine the pH.

The filter paper containing the residue was stored in a refrigerator awaiting ashing [for acid insoluble lignin determination].

- The neutralized filtrate was put into 2 test tubes, labeled, sealed and stored in a refrigerator
- A SRS [Sugar Recovery Standard] was also prepared and hydrolyzed under similar conditions as the biomass.
- SRS was prepared by dissolving 0.3 g of D[+] glucose using similar procedures for biomass.
- Hydrolysis of extractives – free biomass was carried out using similar procedures applied to biomass hydrolysis.

3.1.4 Dissolution with Ionic Liquid and Regeneration of Cellulosic Materials

This procedure was carried out according to R. Pezoa et al, (2010) article on Use of Ionic Liquids in The Pretreatment of Forest and Agricultural Residues for The Production of Bioethanol.

1 0.03g of each biomass sample was weighed using an analytical balance and transferred into test tubes. 0.47g of ionic liquid was added to the test tubes containing the biomass substrates thus formimg a biomass/IL loading of 6 % (w/w).

2 The test tubes containing the samples were stirred and heated in an oil bath at different temperature conditions; 80 °C, 100^{o}C, 120^{o}C and 140^{o}C for different durations (40min, 80min, 120min, 160min). This helped to determine the *Reaction time effect, Biomass type effect* and *Temperature effect.*

3 After incubation, the reaction mixtures were cooled down to 60 °C and then 4.0 ml deionized water as an anti-solvent was added to precipitate and regenerate the dissolved cellulose, while stirring in a mixer.

4 Next, the precipitated material was filtered through 125mm filter paper using a funnel and washed with deionized water in order to ensure that excess ionic liquid had been removed. Then prior to acid hydrolysis, the precipitates were dried at 25 °C for 24h as shown in the Image 3.8 below.

Image 3.8 Pretreated solids being dried at room temperature

5 Simple acid hydrolysis was then carried out and the hydrolysates analyzed for glucose using a Shimadzu UV-Vis Spectrophotometer at a wavelength of 520nm (Addison, 2001).

6 The concentration of glucose in the samples was then calculated based on a standard curve obtained using a standard glucose solution as shown in Appendix 1.

3.1.5 Furfural Determination

This procedure was carried out according to Zhang C et al, 2010 article on Rapid Method for Determination of Furfural and 5-Hydroxymethyl Furfural in Pre-Extraction Stream of Biomass Using UV Spectroscopy.

1 Various IL/Biomass mixtures of 6wt% were prepared.

2 The test tubes containing the samples were stirred and heated in an oil bath at temperatures of 100°C and 120°C for 80 minutes.

3 After incubation, the reaction mixtures were cooled down to 60 °C and then 4.0 ml deionized water as an anti-solvent was added to precipitate and regenerate the dissolved cellulose, while stirring in a mixer.

4 Next, the precipitated material was filtered through 125mm filter paper using a funnel and washed with deionized water in order to ensure that excess ionic liquid had been removed. Then prior to acid hydrolysis, the precipitates were dried at 25 °C for 24h.

5 Simple acid hydrolysis was then carried out and the hydrolysates analyzed for furfural using a Shimadzu UV-Vis Spectrophotometer at a wavelength of 284nm (Akinwale, 2010).

6 The concentration of furfural in the samples was then calculated based on a standard curve obtained using a standard furfural solution as shown in Appendix 2.

3.1.6 Ionic Liquid Recycling

This procedure was inferred from the Joint BioEnergy Institute (JBEI) approach. In this technology, adding an alkali solution to extract sugars after the acid catalysed hydrolysis of biomass yields two aqueous phases: an ionic liquid-rich phase at the top and a sugar/alkaline rich phase at the bottom.

1 0.037g of each biomass sample was weighed using an analytical balance and transferred into test tubes. 0.576g of imidazolium ionic liquid was added to the test tubes containing the biomass substrates thus formimg a biomass/IL loading of 6 % (w/w).

2 The test tubes containing the samples were stirred and heated in an oil bath at 100°C for 120min.

3 Hydrolysis was then performed by adding 2ml of 4M hydrochloric acid into the test tube containing the biomass solutions after which these were immersed into an oil bath at a temperature of 105°C for 30 minutes with 2ml distilled water being added into the biomass solution at intervals of 10 minutes.

4 The resulting mixture was then transferred into centrifuge tubes and centrifuged at maximum speed (about 14,000rpm) using a Hettich centrifuge for 10 minutes to separate the solid residue from the aqueous solution.

5 A volume of 3ml of 15% (w/v) NaOH was then added to the supernatant after which the mixture was agitated by shaking before being centrifuged again for 10 minutes.

6 The upper [BMIM]Cl phase and the lower sugar/NaOH phases were separated with a pipette with the upper IL – Rich phase being preserved for recycling.

7 The lower NaOH phase containing fermentable sugars (glucose) was analyzed for glucose using a Shimadzu UV Vis Spectrophotometer at a wavelength of 520nm (Addison, 2001).

8 The concentration of glucose in the samples was then calculated based on a standard curve obtained using a standard glucose solution as shown in Appendix 1.

9 0.037g of biomass was again weighed and mixed with the upper IL – Rich phase after which the steps 2 to 8 above were repeated.

10 Step 9 was redone until the concentration of glucose achieved was almost similar to that of hydrolysed unpretreated biomass samples.

3.1.7 Acid Hydrolysis for Control Experiment

For the control experiment, biomass was hydrolysed without being pretreated and the monomeric sugar (glucose) produced determined.

Method used was according to Chandel et al, 2007.

1 To the unpretreated biomass (0.03g of sample) 2ml of 2 w/v% sulphuric acid was added.
2 The reaction mixture was heated in an oil bath at a temperature of 130°C for 10 minutes.
3 The cellulosic hydrolysate was then separated from the solids by filtration.

4 The hydrolysate was finally analyzed by the UV-visible spectrophotometer to give the glucose concentration.

3.1.8 Acid Hydrolysis of Regenerated Cellulose

Hydrolysis was carried out after pretreatment to determine the effect of ionic liquids pretreatment on biomass. This helped in determining the effect of biomass pretreatment by comparing the amount of sugars (glucose) formed.

Method used was according to Chandel et al, 2007.

5 To the regenerated biomass, 2ml of 2 w/v% sulphuric acid was added.

6 The reaction mixture was heated in an oil bath at a temperature of 130°C for 30 minutes.

7 The cellulosic hydrolysate was then separated from the pretreated solids by filtration.

8 The hydrolysate was finally analyzed by the UV-visible spectrophotometer to give the glucose concentration.

CHAPTER FOUR

RESULTS, ANALYSIS AND DISCUSSION

4.1 Characterization of the Biomass

The two biomasses used in the present study were collected, processed mechanically and the chemical compositions determined according to the standard methods were obtained. These results were tabulated and compared to those obtained from established references as shown in Tables 4.1 and 4.2 below.

Table 4.8 Compositional Analysis of *Prosopis Juliflora*

Components	Percentage Composition	
	Prosopis (Experimental Results)	Prosopis (Naseeruddin, et al., 2013)
Moisture	11.25	-
Ash	5.70	4.30
Cellulose	34.00	49.40
Hemicellulose	22.75	18.00
Extractives	14.50	28.30
Acid Soluble Lignin	11.80	
Total	**100.00**	**100.00**

Table 4.9 Compositional Analysis of *Gadam* Sorghum

Components	Percentage Composition	
	Gadam Sorghum (Experimental Results)	Gadam Sorghum (Cardoso, Tardin & Tavares,2013)
Moisture	9.12	-
Ash	4.97	7.52
Cellulose	29.68	35.87
Hemicellulose	15.75	26.04
Extractives	32.20	30.57
Acid Soluble Lignin	8.28	
Total	**100.00**	**100.00**

Gadam sorghum belongs to the grass family. *Prosopis julifora* on the other hand, belongs to the Fabaceae family; it is therefore a softwood (Chandra et. al., 2007). The results obtained showed that *prosopis juliflora* was found to posses relatively higher percentage of cellulose (34%) compared to *gadam* sorghum (29.68%).

4.2 Effect of Pretreatment Variables on Glucose Yield

The control experiment was carried out and it involved the unpretreated biomass being hydrolysed and glucose yields determined in order to compare with those obtained with pretreated biomass. Results of the control experiment are shown in Table 4.3 below:-

Ionic liquid pretreatment appears to have great influence on the glucose yield after acid hydrolysis. The glucose yield was obtained after the regeneration process of each biomass followed by acid hydrolysis.

Results for the effect of pretreatment with the various ionic liquids of both biomass samples (*gadam* sorghum and *prosopis juliflora*) on glucose yield by heating at various pretreatment temperatures and times at 6%wt biomass loading were tabulated as shown in Tables 4.4 and Table 4.5 respectively.

Table 4.10 Percentage Glucose Yield for Unpretreated Biomass **(Control Experiment)**

Biomass	Extractive Glucose Yield
Gadam Sorghum	2.730%
Prosopis Juliflora	4.050%

Table 4.11 Percentage Glucose Yield for *Gadam* Sorghum Pretreatment

Ionic Liquid Type	Pretreatment Time (min)	Pretreatment Temperature (◦C) and Glucose Yield			
		80	100	120	140
1-butyl - 3- methyl Imidazolium Chloride	40	8.070%	9.570%	6.450%	9.360%
	80	10.440%	12.360%	10.500%	4.250%
	120	12.260%	24.100%	10.720%	2.950%
	160	7.850%	14.330%	4.180%	2.820%
Trihexyltetradecyl phosphonium Chloride	40	0.721%	3.044%	2.670%	1.357%
	80	1.346%	1.716%	3.020%	2.877%
	120	1.914%	2.537%	3.930%	3.590%
	160	1.638%	1.649%	2.600%	2.750%
1-butyl - 4- methyl pyridinium Chloride	40	6.342%	7.040%	7.491%	15.098%
	80	7.460%	9.730%	18.516%	23.078%
	120	8.036%	18.392%	31.964%	26.208%
	160	40.531%	45.035%	51.289%	60.422%

Table 4.12 Percentage Glucose Yield for *Prosopis Juliflora* Pretreatment

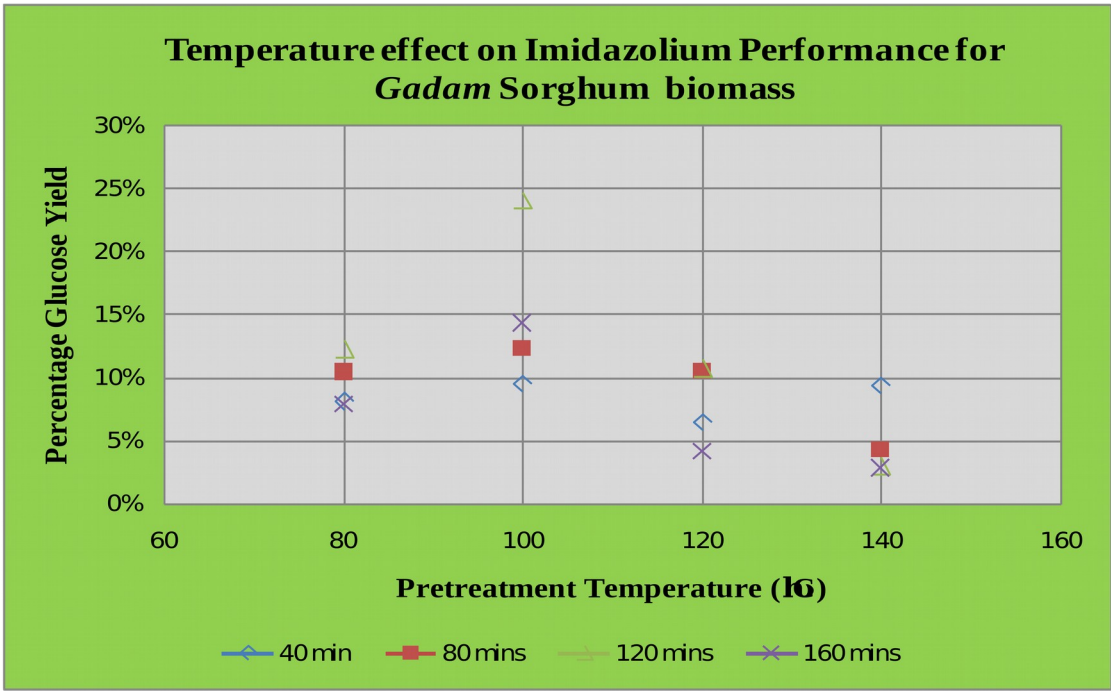
Ionic Liquid Type	Pretreatment Time (min)	Pretreatment Temperature (◦C) and Glucose Yield			
		80	100	120	140
1-butyl - 3- methyl Imidazolium Chloride	40	34.240%	16.120%	2.700%	1.880%
	80	46.880%	16.810%	4.480%	2.990%
	120	59.650%	73.270%	6.650%	4.170%
	160	31.500%	71.940%	5.500%	3.050%
Trihexyltetradecyl phosphonium Chloride	40	1.016%	1.641%	4.828%	2.611%
	80	1.642%	1.650%	6.457%	2.668%
	120	1.747%	2.267%	7.186%	3.878%
	160	1.052%	2.862%	6.530%	2.147%
1-butyl - 4- methyl pyridinium Chloride	40	6.540%	3.385%	9.348%	26.241%
	80	8.870%	17.669%	11.931%	32.921%
	120	33.862%	39.279%	19.621%	44.790%
	160	42.686%	51.014%	53.903%	61.630%

.2.1 Effect of Temperature on Pretreatment

By and large, it was observed that an increase in temperature accelerates the swelling and dissolution rates of both biomass substrates in the various ionic liquids. This phenomenon is largely attributed to a possible destabilization effect, caused by an increase in temperature, on the hydrogen bonds in the three dimensional cellulose structure (Zavrel et. al., 2009). By decreasing temperature, longer durations are required for an efficient swelling and dissolution of biomass (Xie & Shi , 2010). Across the temperature range that was used in this study, the least substrate swelling and dissolution was encountered at 80℃ even at prolonged pretreatment durations of 160 minutes. As from a temperature of 120℃, the intensity of the colour of the ionic liquid-biomass mixture increased. This served as an indication that the dissolution rate of the lignocellulosic biomass had increased.

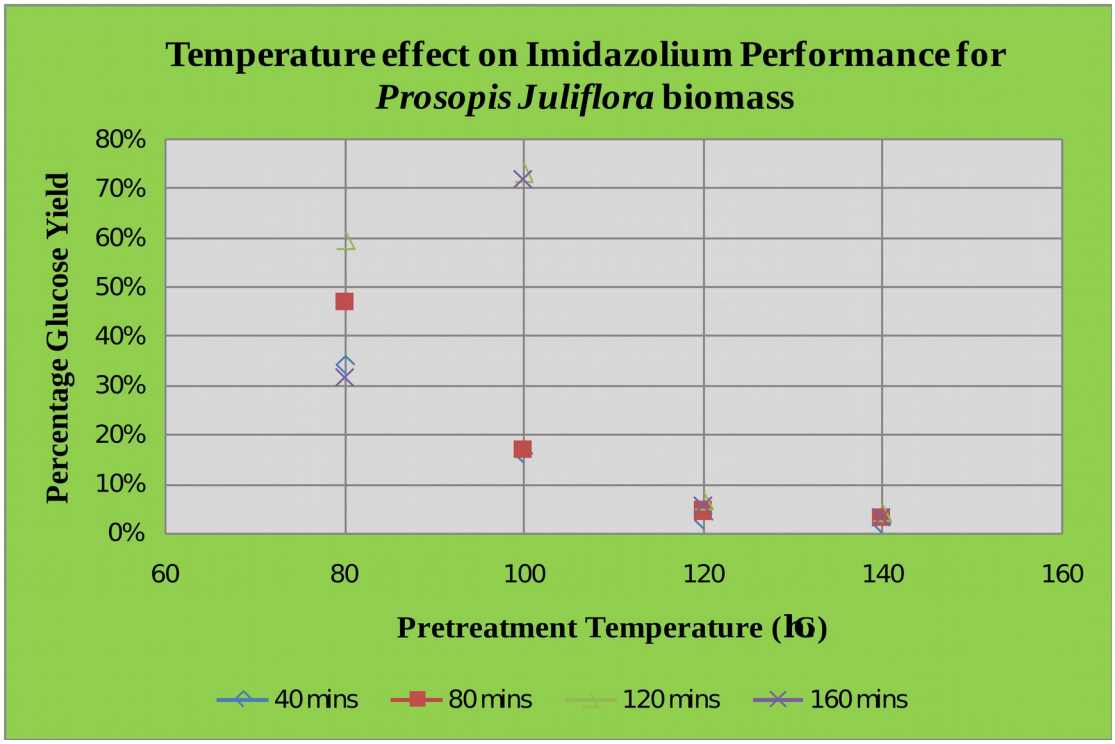
Notably, an increase in the pretreatment temperature instigated a subsequent decrease in the viscosity of the ionic liquids. A decline in the viscosity of the ionic liquids favours the mixing of the lignocellulosic particles with the ionic liquids during pretreatment (Yoon et. al., 2012). In turn, this increases particle swelling and dissolution (Tan & Lee, 2012).
Inevitably, there is a direct correlation between some of the physico-chemical properties of ionic liquids, such as viscosity and melting point, and temperature. In turn, this governs the condition(s) of the pretreatment process in ionic liquids. 1-butyl-3-methyl imidazolium chloride had the lowest viscosity of all the ionic liquids used in this study. It also resulted into the highest average glucose yield at a lower temperature of 100℃.

As shown in Figures 4.1 and 4.2, when 1-butyl-3-methyl imidazolium chloride was used in pretreatment, the highest average glucose yield was obtained at a temperature of 100℃. When the temperature was increased to 120℃, the glucose yield generally decreased by over 15% for *gadam* sorghum and over 73% for *prosopis julifora*. Similarly, when trihexyltetradecylphosphonium chloride was used in pretreatment (as shown in Figures 4.3 and 4.4), the highest average glucose yield was obtained at a temperature of 120℃. An increase of the temperature to 140℃ resulted into a general decrease in the average glucose yield by over 5% for *gadam* sorghum and over 45% for *prosopis julifora*.



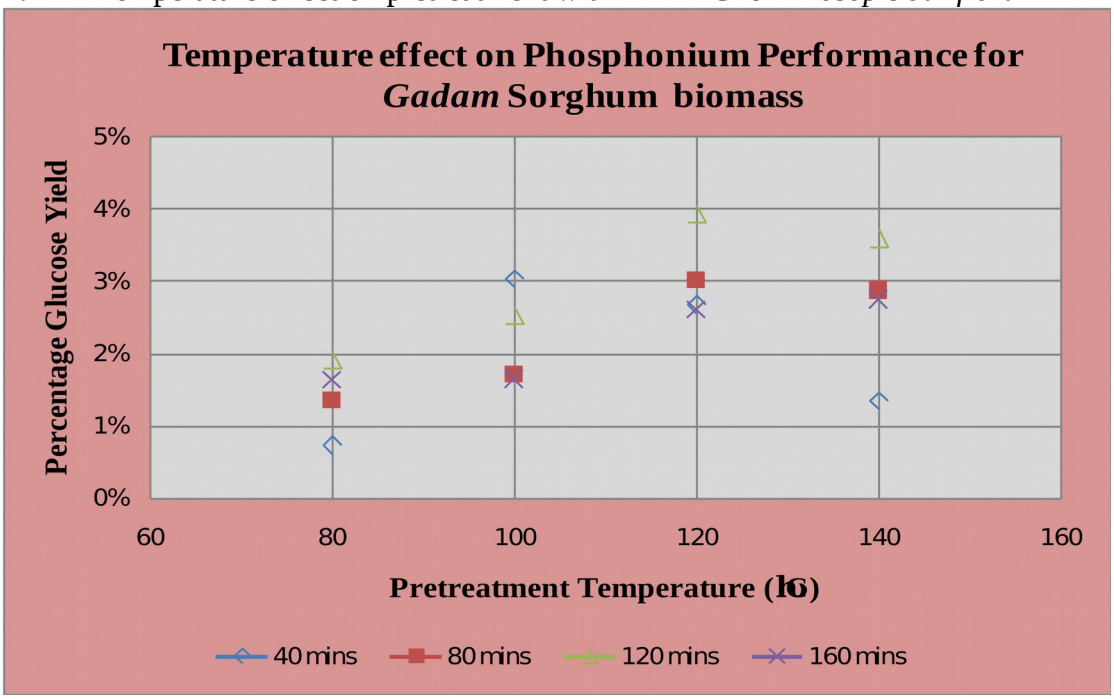
(Source: Table 4.4)

Figure 4.13 Temperature effect on pretreatment with BMIMCl on *Gadam* Sorghum



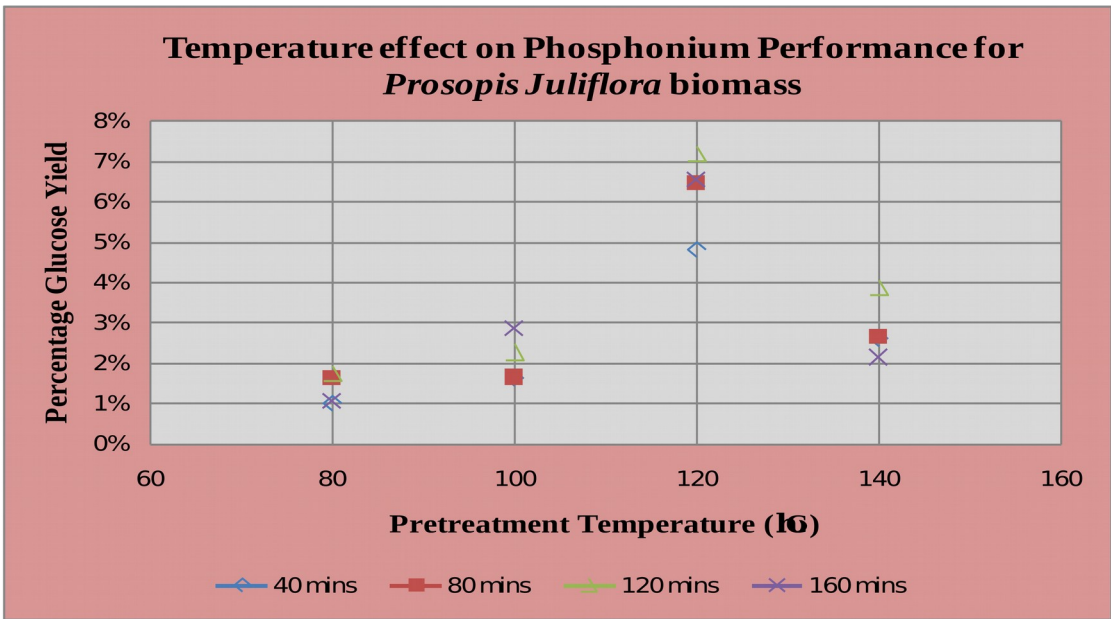
(Source: Table 4.5)

Figure 4.14 Temperature effect on pretreatment with BMIMCl on *Prosopis Juliflora*



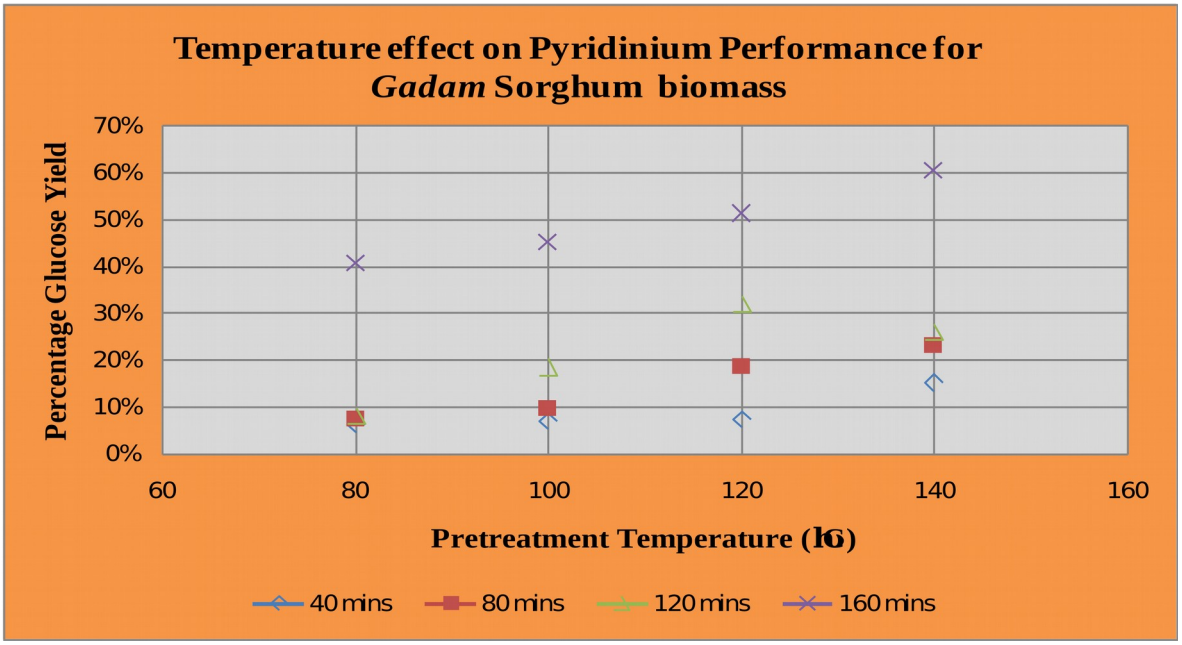
(Source: Table 4.4)

Figure 4.15 Temperature effect on pretreatment with P66614Cl on *Gadam* Sorghum



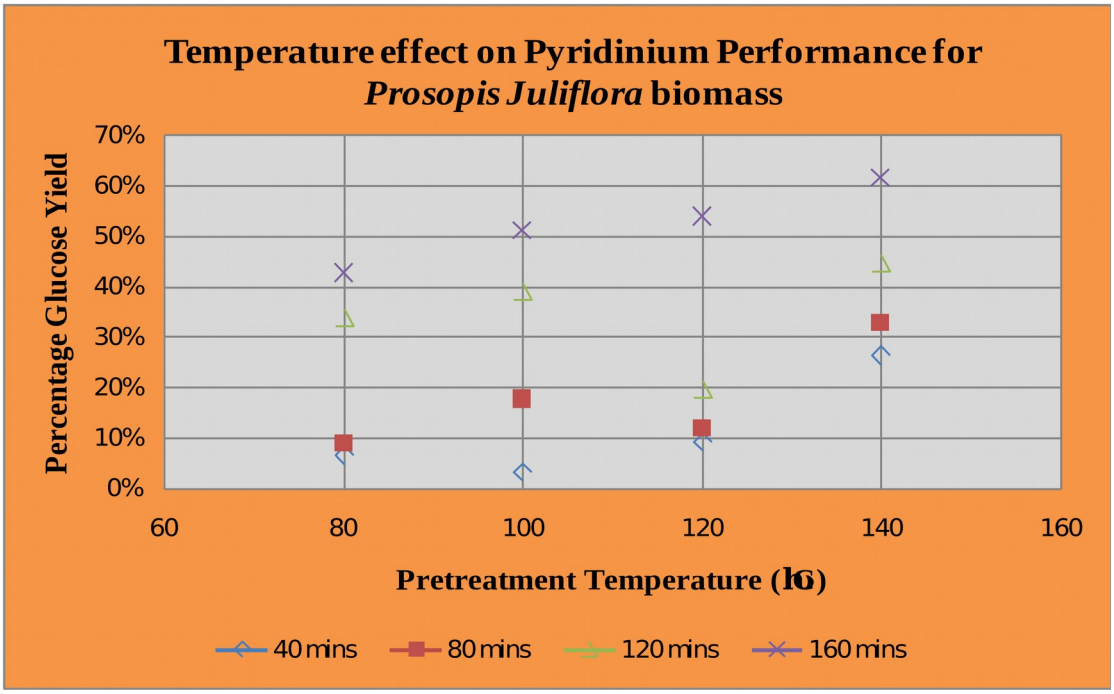
(Source: Table 4.5)

Figure 4.16 Temperature effect on pretreatment with P66614Cl on *Prosopis Juliflora*



(Source: Table 4.4)

Figure 4.17 Temperature effect on pretreatment with 4MBPCl on *Gadam* Sorghum



(Source: Table 4.5)

Figure 4.18 Temperature effect on pretreatment with 4MBPCl on *Prosopis Juliflora*

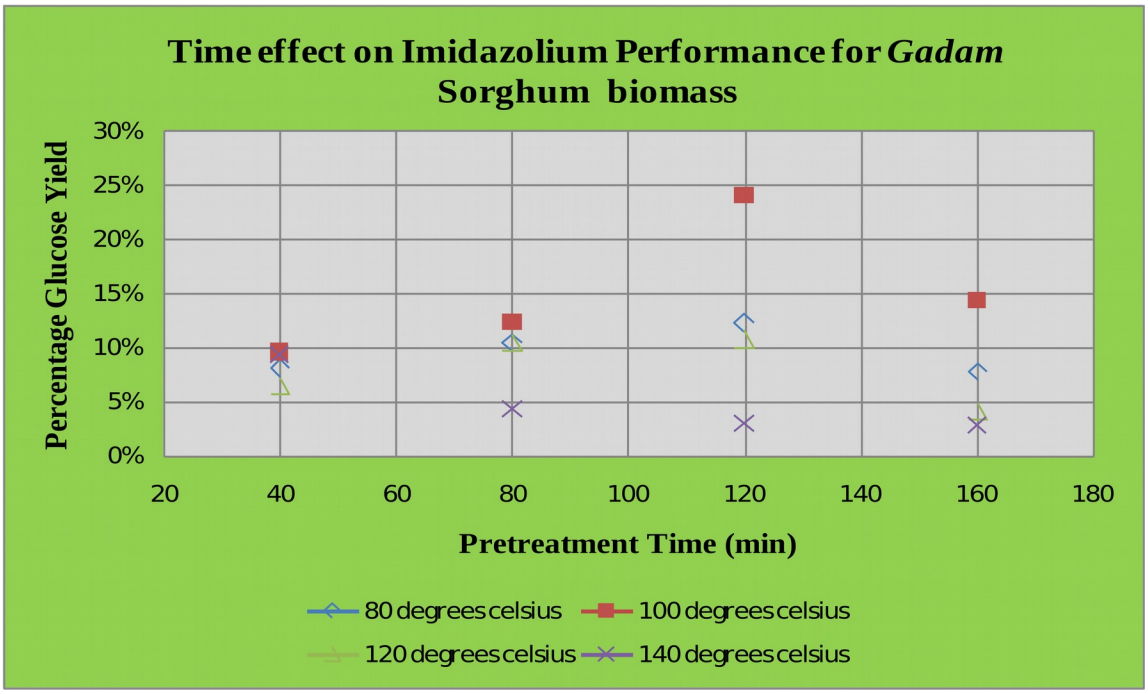
Samples pretreated using 1-butyl-4- methylpyridinium chloride showed a consistent increase in the average glucose yield with a subsequent increase in temperature. This indicates a significantly high thermal stability of the pyridinium based ionic liquid hence making it an excellent medium for high temperature biomass dissolution reactions.

Ionic liquid dissolution follows solubility equilibria between indissolved solids and their dissolved products in solution. Decrease in dissolution might be caused by depolymerization of dissolved cellulose after long hours of heating. The dissolved cellulose could have been broken down into shorter fragments, which were subsequently hydrolysed into its sugar monomers. Increase in dissolution ability after the decrease indicates that chemical equilibrium of the dissolution process had shifted to favoring cellulose dissolution again subsequent to the degradation of dissolved cellulose due to prolonged heating.

.2.2 Effect of Time on Pretreatment

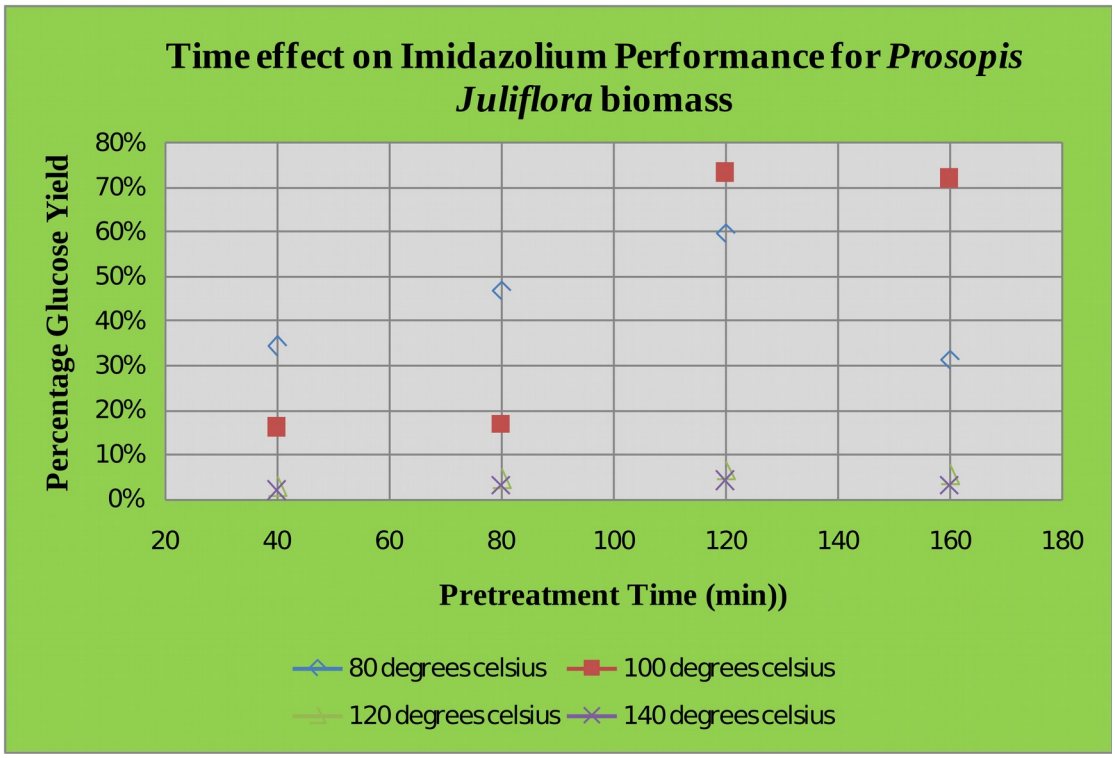
It can generally be concluded that the percentage glucose yield increases with an increase in the pretreatment duration across the selected temperature ranges. However, it should also be noted that prolonged pretreatment could lead to a decrease in the total reducing sugars. More particularly, this was observed across the selected temperature ranges for a majority of the samples which had been pretreated using 1–butyl-3-methyl imidazolium and trihexyltetradecylphosphonium chloride; in which there was a decrease in the glucose yield after a pretreatment duration of 120 minutes. This decline in glucose yield could be attributed to depolymerization of the biomass components (Yoon et. al., 2012).

Figures 4.7–4.12 below represent the glucose yield behavior with time for the two biomass across the studied temperature ranges.



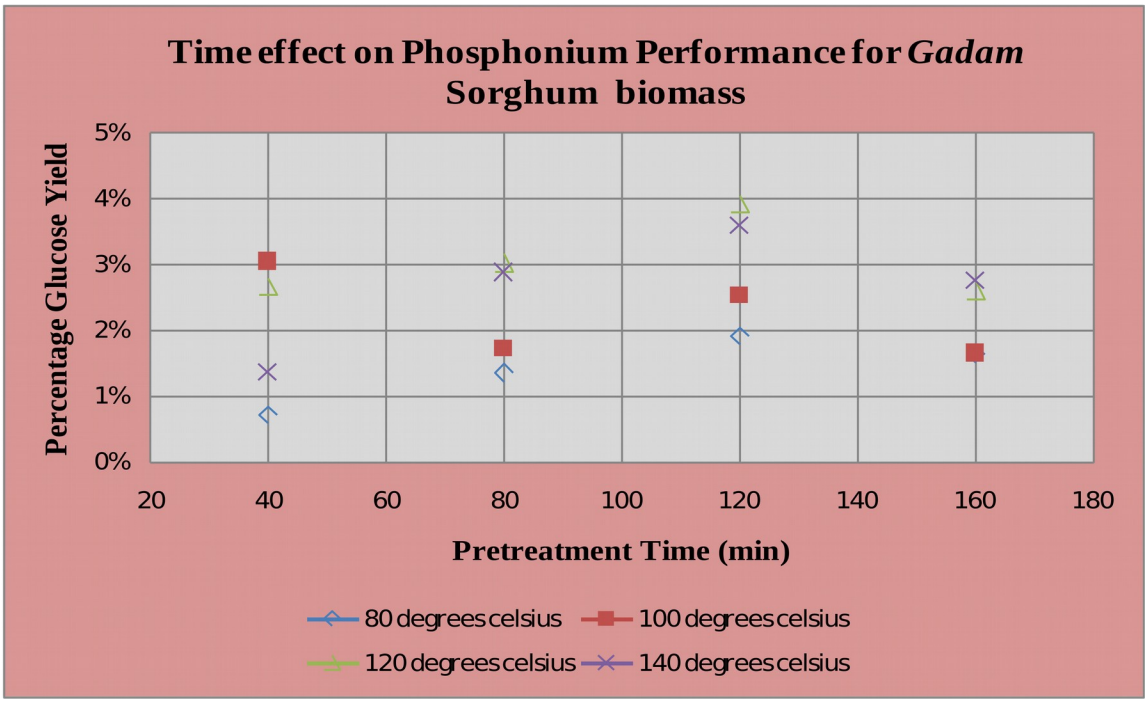
(Source: Table 4.4)

Figure 4.19 Time effect on pretreatment with BMIMCl on *Gadam* Sorghum



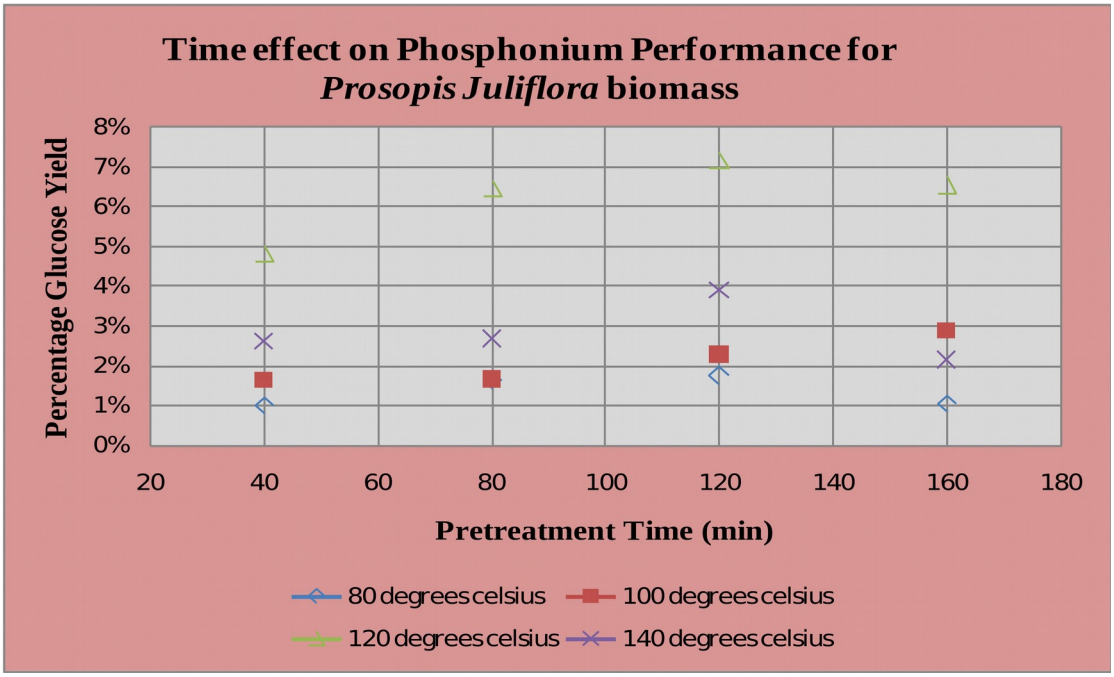
(Source: Table 4.5)

Figure 4.20 Time effect on pretreatment with BMIMCl on *Prosopis Juliflora*



(Source: Table 4.4)

Figure 4.21 Time effect on pretreatment with P66614Cl on *Gadam* Sorghum



(Source: Table 4.5)

Figure 4.22 Time effect on pretreatment with P66614Cl on *Prosopis Juliflora*

Use of trihexyltetradecylphosphonium chloride for pretreatment exhibits a similar trend as that observed with 1-butyl-3-methyl imidazolium chloride with the maximum average glucose yield obtained after a duration of 120 minutes for both *Prosopis Juliflora* and *Gadam* Sorghum biomass after which there was a decrease in the glucose yield. Figures 4.9 and 4.10 above illustrate this trend. The decline in glucose yield could be attributed to depolymerization of the biomass components (Yoon et. al., 2012).

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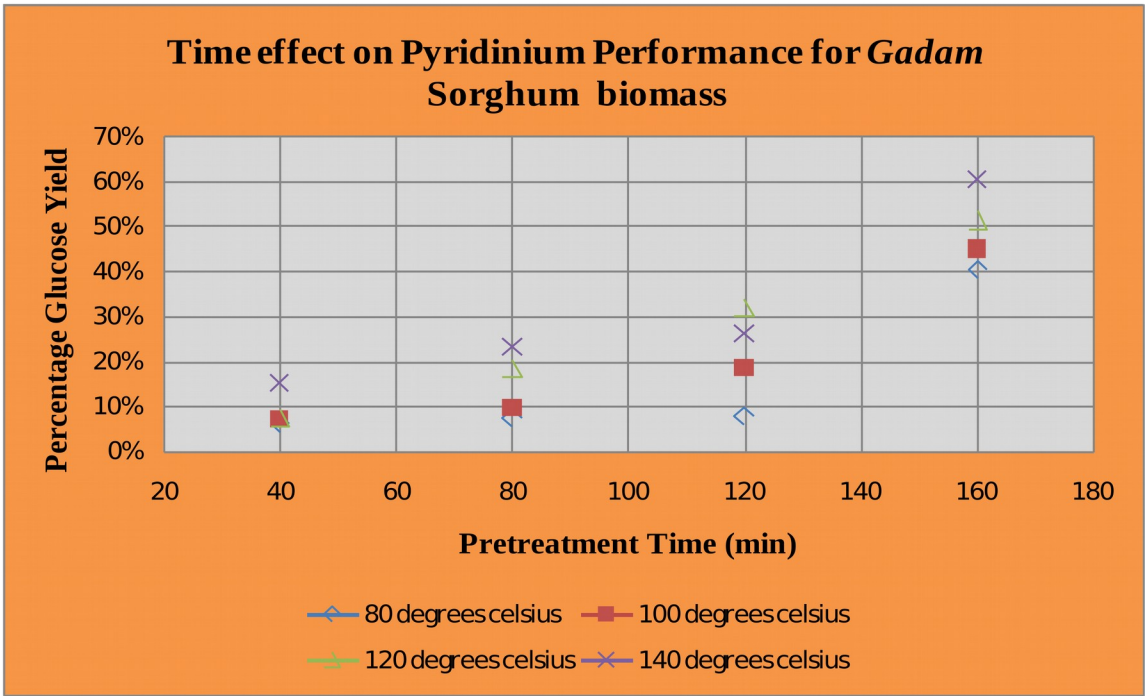
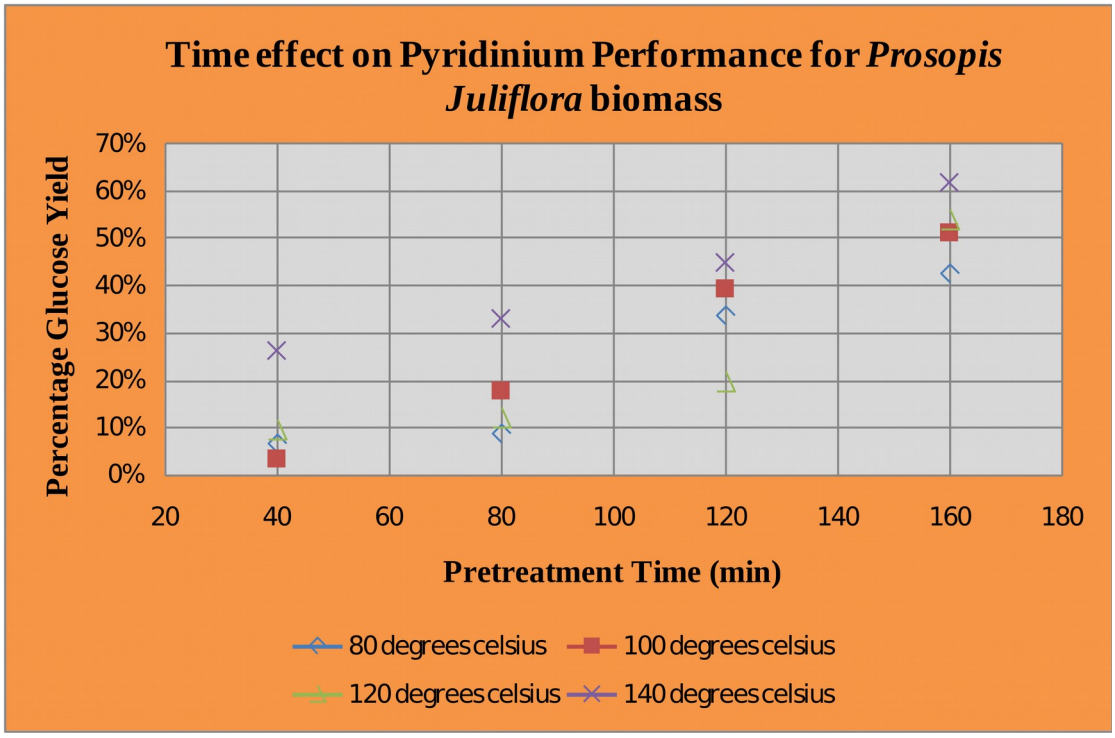


(Source: Table 4.4)

Figure 4.23 Time effect on pretreatment with 4MBPCl on *Gadam* Sorghum



(Source: Table 4.5)

Figure 4.24 Time effect on pretreatment with 4MBPCl on *Prosopis Juliflora*

Samples pretreated using 1-butyl-4- methylpyridinium chloride showed a consistent increase in the average glucose yield with a subsequent increase in pretreatment duration as shown in Figures 4.11 and 4.12.

The prolonged pretreatment time coupled with high temperatures could possibly have disrupted the cellulose chains in *gadam* sorghum and *prosopis juliflora* into soluble oligosaccharides (Nguyen et. al., 2010). Furthermore, through various research efforts, it has been affirmed that when higher temperatures are applied in biomass pretreatment for elevated duration, cellulose degradation becomes more pronounced (Kimon et. al., 2011).

.2.3 Effect of Cation on Pretreatment

Ionic liquids have different physical and chemical properties which impact on the pretreatment process. The following properties have an impact on the efficiency of an ionic liquid in pretreatment:

- The type of anion of the ionic liquid
- The size of the cation
- Hydrophobicity or Hydrophilicity of the ionic liquid
- The length of the alkyl substituent on the cation

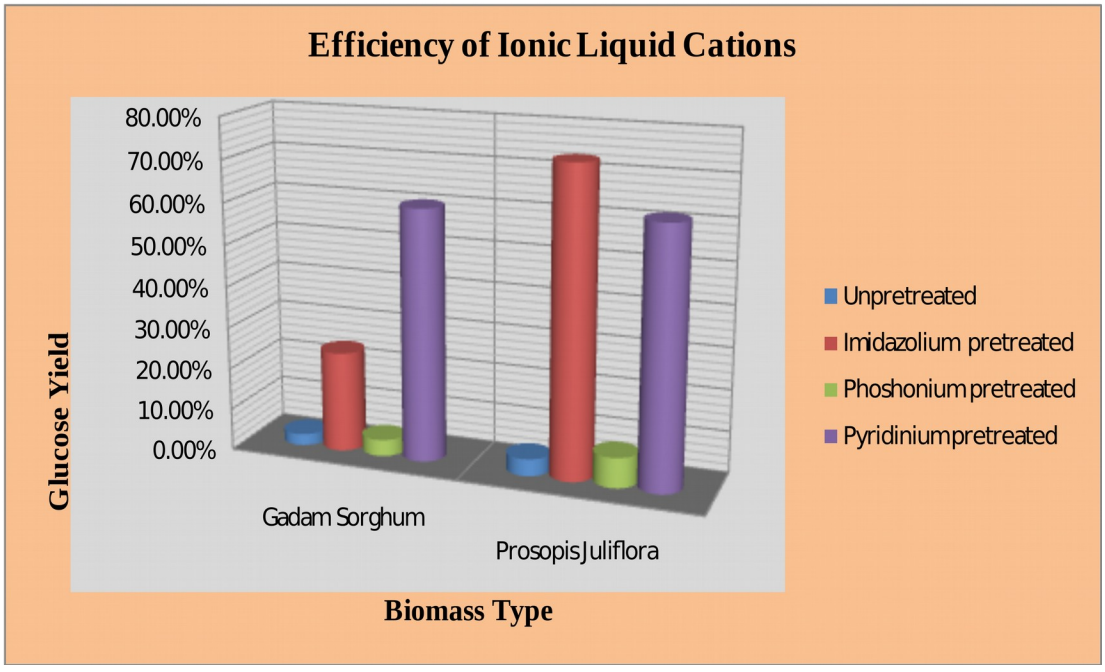
Ionic liquids have the ability to dissolve carbohydrates and lignin since they can effectively disrupt the intricate network of non-covalent interactions between these polymers. The fundamental interaction between the anion of the ionic liquid with the substrate carbohydrate is more prevalent in comparison to the interaction between the cation and the carbohydrate (Remsing et. al., 2006). All the ionic liquids that were used in this study had a chloride anion. Therefore, the dissolution of carbohydrates in ionic

liquids results from the formation of hydrogen bonds between the chloride anion of the ionic liquid and the hydroxyl protons of the cellobiose units from the carbohydrates.

The cations of the ionic liquids also impact on the dissolution process, though to a lesser extent. The cations mainly interact with the cellulose hydroxyl oxygen groups (Wang, et. al., 2011).

Table 4.13 Maximum Glucose Yield Achieved for each pretreatment method

[redacted]



(Source Table 4.6)

Figure 4.25 Glucose Extraction Efficiency Comparison of Ionic Liquids Cations

In reference to Figure 4.13 above, a higher glucose yield was reported for samples which had been pretreated with ionic liquids prior to hydrolysis than those samples which were unpretreated. An increase in glucose yield of between 1.44 times when phosphonium IL was used on *Gadam* Sorghum (from 2.73% unpretreated to 3.93%) and 22 times when pyridinium IL is used on *gadam* sorghum (from 2.73% unpretreated to 60.42%) is noted. This therefore confirms that pretreatment is a fundamental step that has the potential of increasing glucose yield.

It can further be observed from Figure 4.13 that trihexyltetradecylphosphonium chloride was the least effective when used to pretreat both biomass substrates that were used in this study as it gives the least glucose yields in comparison to 1-butyl-4-methylpyridinium chloride and 1-butyl-3- methylimidazolium chloride. Its dismal performance in comparison to the other ionic liquids that were used in this study could be attributed to its large cation and hydrophobicity. Trihexyltetradecylphosphonium chloride has a bulky cation and a halide in its matrix. Essentially, the bulky cation decreases the concentration of active chloride ion. This reduces the effective chloride concentration within the liquids and hence reduces the effect of breaking down the hydrogen-bond network. In turn, the solvating capacity of the ionic liquid is reduced (Swatloski et. al., 2002). Similarly, 1-butyl-3- methylimidazolium chloride has a slightly smaller cation than 1-butyl-4-methylpyridinium chloride hence the likely reason behind its reported high yields with *prosopis juliflora*. Therefore, it had a slightly higher degree of interaction

with the cellulose chain at temperatures of 80℃ and 100℃. Nevertheless, at a temperature of 120℃, its efficiency decreased citing a decline in its stability.

The explorative studies and screening experiments carried out using various hydrophobic ILs suggest that hydrophobic ILs do not dissolve cellulose as effectively as hydrophilic ILs. (Swatloski et. al., 2002). 1-butyl-3- methylimidazolium chloride and 1-butyl-4-methylpyridinium chloride being hydrophilic are more effective in dissolving cellulose as compared to their hydrophobic counterpart- trihexyltetradecylphosphonium chloride.

.2.4 Effect of Biomass Type on Glucose Yield

The degree of biomass recalcitrance varies depending on whether the biomass is grass, softwood or hardwood. Other elements that can contribute towards the variation in recalcitrance include: age, storage conditions and extent of drying. That said, the physical and chemical characteristics vary broadly depending on the type of lignocellulosic biomass. Cumulatively, all these aspects impact the pretreatment efficiency. Therefore, due consideration should be given to type of biomass in the pretreatment with ionic liquids.

In this study, the biomass samples that were used are *gadam* sorghum and *prosopis julifora*. On average, the chemical composition of *prosopis julifora* is 49.4% cellulose, 18% hemicellulose, 4.3% ash, 28.3% lignin and extractives (Naseeruddin, et al., 2013). Similarly, the chemical composition of *gadam* sorghum is 35.87%, cellulose, 26.04% hemicelluloses, 7.52% ash and 30.57% lignin & Extractives (Cardoso et. al., 2013).

Table 4.14 Average Glucose Yield on Imidazolium Pretreatment

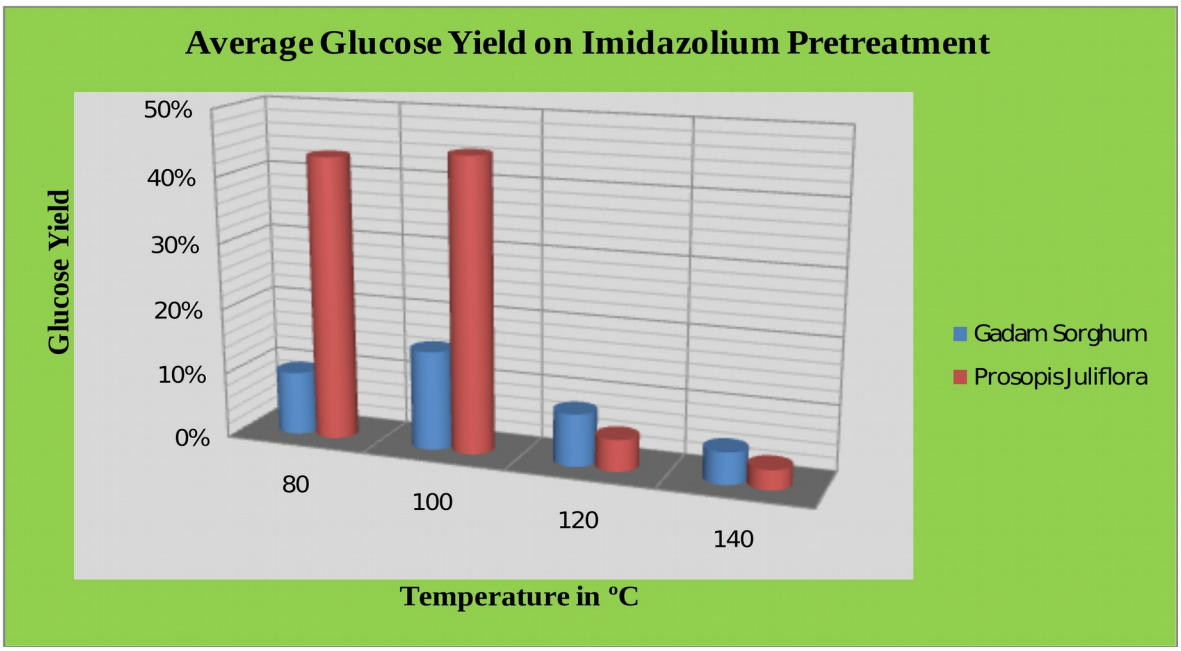
Pretreatment Temperature (◦C)	Average %age Glucose Yield on pretreatment with Imidazolium	
	Gadam Sorghum	*Prosopis Juliflora*
80	9.66%	43.07%
100	15.09%	44.54%
120	7.96%	4.83%
140	4.85%	3.02%

Table 4.15 Average Glucose Yield on Phosphonium Pretreatment

Pretreatment Temperature (◦C)	Average %age Glucose Yield on pretreatment with Phosphonium	
	Gadam Sorghum	*Prosopis Juliflora*
80	1.40%	1.36%
100	2.24%	2.11%
120	3.06%	6.25%
140	2.64%	2.83%

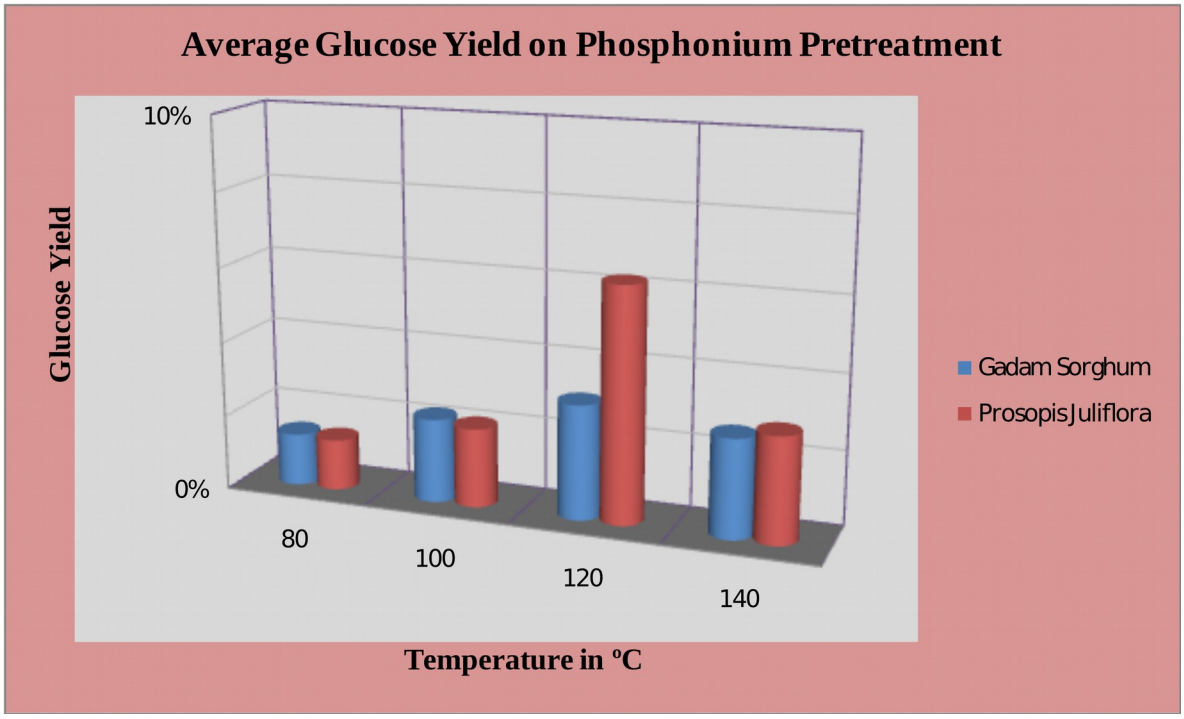
Table 4.16 Average Glucose Yield on Pyridinium Pretreatment

Pretreatment Temperature (◦C)	Average %age Glucose Yield on pretreatment with Pyridinium	
	Gadam Sorghum	*Prosopis Juliflora*
80	15.59%	22.99%
100	20.05%	27.84%
120	27.32%	23.70%
140	31.20%	41.40%



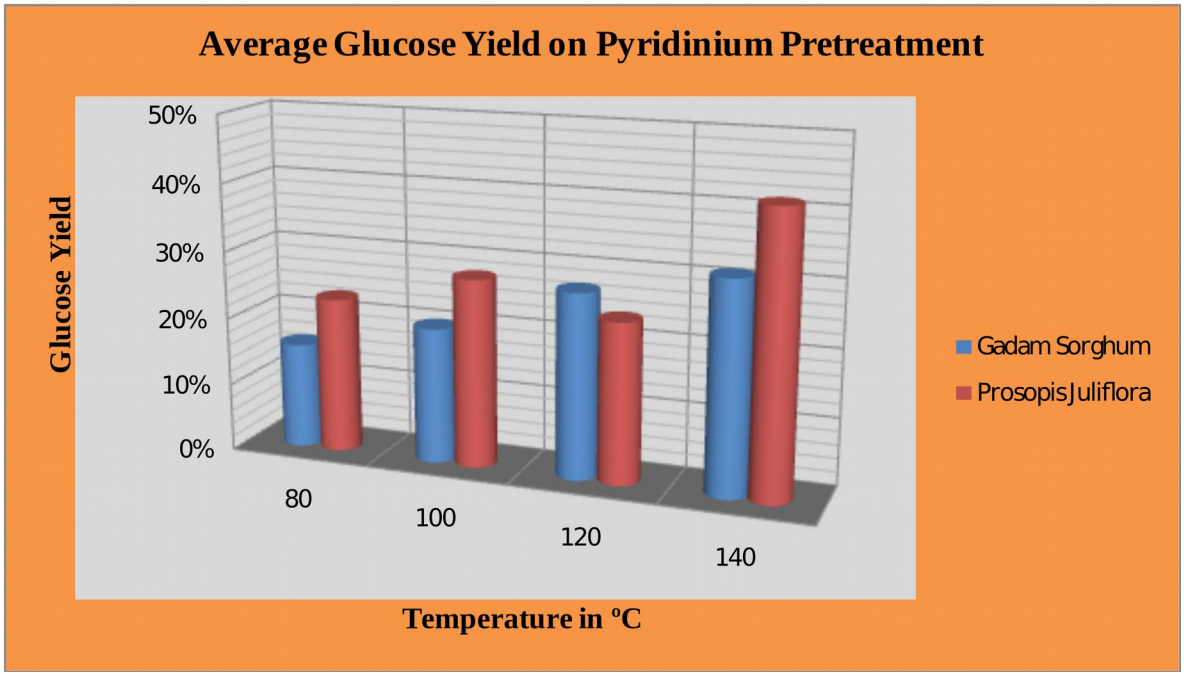
(Source: Table 4.7)

Figure 4.26 Average Glucose Yield on Imidazolium Pretreatment



(Source: Table 4.8)

Figure 4.27 Average Glucose Yield on Phosphonium Pretreatment



(Source: Table 4.9)

Figure 4.28 Average Glucose Yield on Pyridinium Pretreatment

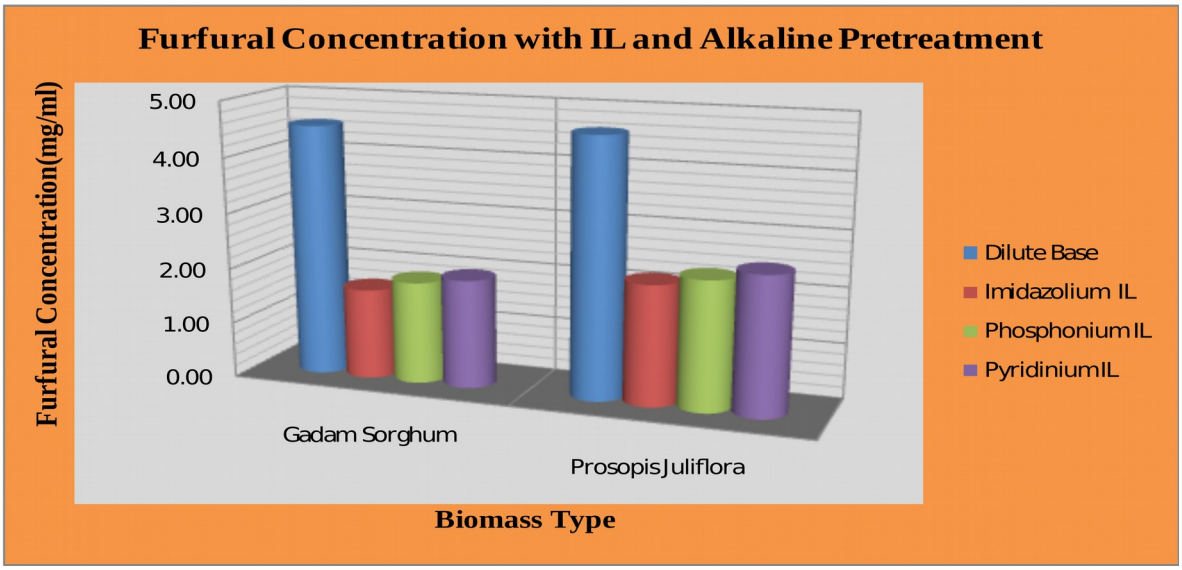
Generally, *gadam* sorghum had a more facile solubility in the various ionic liquids as opposed to *prosopis julifora*. This could be attributed to its lower density. Additionally, the absence of guaiacyl units in its lignin polymer makes it easier to deconstruct. The higher lignin content in *prosopis julifora* not only influences dissolution but it also impacts on the lignin-carbohydrate interactions. In spite of the foregoing, *prosopis julifora* produced a higher yield of glucose across the selected range of temperature as depicted in the Figures 4.14 – 4.16 above. This could be attributed to the higher cellulose content in its structure thereby giving it the potential to release more cellulose with every successful deconstruction.

4.1 Comparison of Furfural Production in IL and Alkaline Pretreatment

During pretreatment of lignocellulosic biomass, hexose and pentose sugars as well as lignin are prone to degrade into undesirable by-products. A majority of these byproducts are toxic to bio-fuel producing microbes. In this study, the quantity of furfural in the hydrolysate was determined. The concentration of furfurals in various IL-Biomass mixtures after ionic liquid pretreatment and consecutive simple acid hydrolysis is as shown in the Table 4.10 below.

Table 4.17 Furfural Concentration Levels Upon Pretreatment

Pretreatment Method	Furfural Concentration (mg/ml)	
	Gadam Sorghum	*Prosopis Juliflora*
Dilute Base	4.53	4.63
Imidazolium IL	1.63	2.16
Phosphonium IL	1.84	2.32
Pyridinium IL	1.96	2.49



(Source: Table 4.10)

Figure 4.29 Furfural production from IL and dilute alkaline pretreatment

Generally, it was observed that the concentration of furfurals in the hydrolysate were lower when ILs were used as compared to when weak alkaline pretreatment was used for both biomasses. When the Imidazolium Based Ionic liquid was used, a 64% decrease was noted for *gadam* biomass and a 53.3% decrease noted for the *prosopis juliflora* biomass on the furfural concentration levels from those recorded when the weak alkaline method was used for pretreatment. Similarly, when the Phosphonium Based Ionic liquid was used, a 59.4% decrease was noted for *gadam* biomass and a 49.9% decrease noted for the *prosopis* biomass. Upholding the same trend, when the Pyridinium Based Ionic liquid was used, a 56.7% decrease was noted for gadam biomass and a 46.2% decrease noted for the prosopis biomass.

Based on the above data, pretreatment using dilute sodium hydroxide resulted into a higher concentration of furfurals in the hydrolysate as illustrated in the Figure 4.17 above.

4.2 Effect of Ionic Liquid Recycling on Pretreatment

Reuse of ILs is extremely important in reducing its cost and alleviating any potential environmental and/or ecological effects that may arise from its use in the pretreatment process. Therefore, it is in the interest of this that IL recycling was carried out to establish the number of times the IL could be reused without having any deleterious effect on the glucose yield. 1-butyl-3-methyl imidazolium chloride ionic liquid was used for this purpose at a pretreatment temperature of 100ºC and pretreatment time of 120 minutes

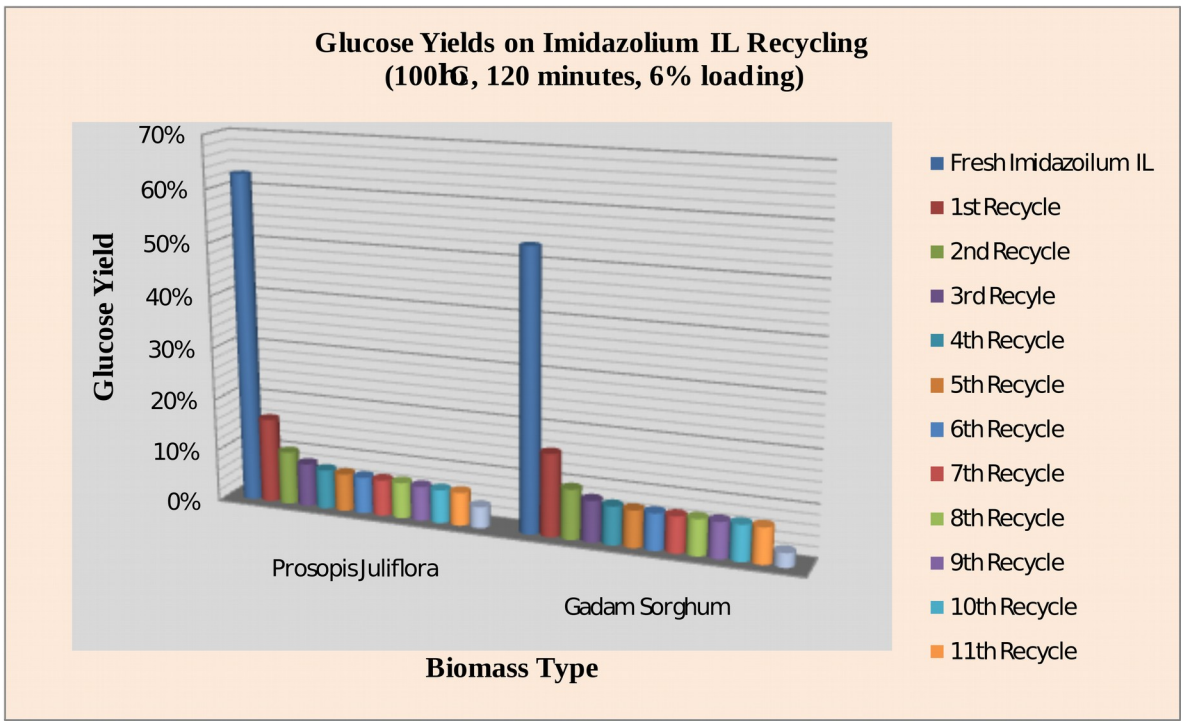
(temperature and time at which maximum glucose yields were observed during the study) at a biomass loading of 6% on both biomasses.

Due to the small IL quantities available for use, the recycling was done up to the second level experimentally and the values thereafter obtained by studying the decrease sequence / pattern of the glucose yield. The following values were thereafter extrapolated using the sequence upto a level which posted insignificant changes in the glucose yields.

The results were then tabulated as shown in Table 4.11 below.

Table 4.18 Percentage Glucose Yield with IL Recycling (100◦C, 120 minutes, 6% loading)

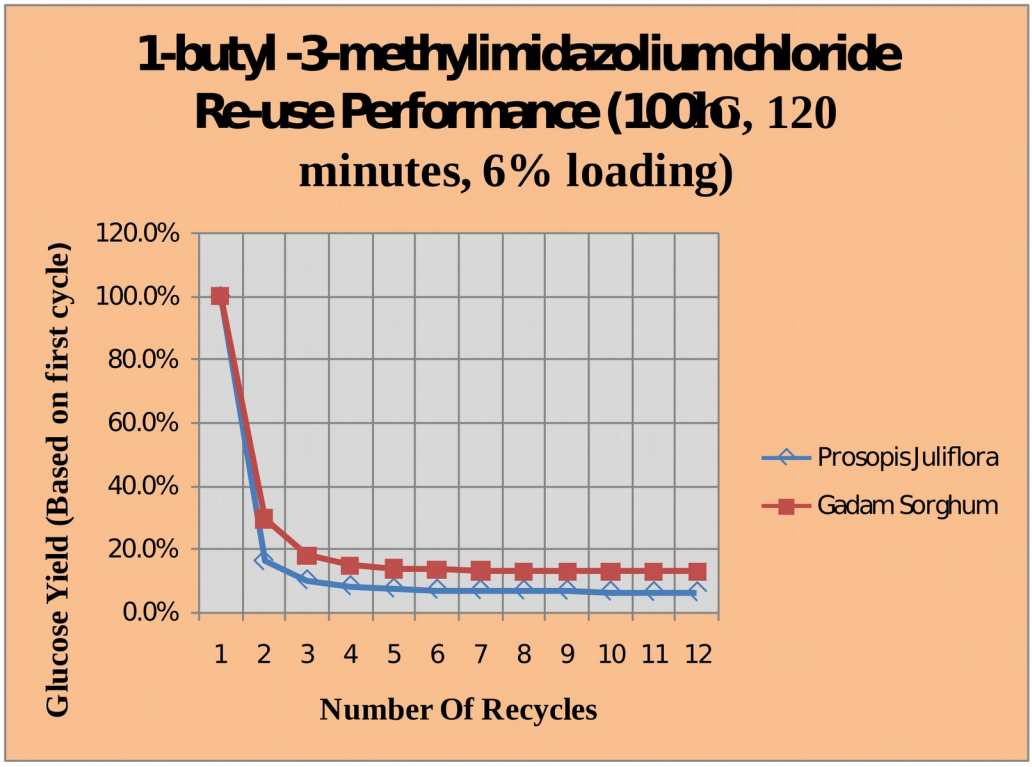
Sample	**%age Glucose Yield**	
	Prosopis Juliflora	***Gadam* Sorghum**
Fresh Imidazoilum IL	62.750%	53.260%
1st Recycle	16.190%	15.810%
2nd Recycle	10.120%	9.560%
3rd Recyle	8.250%	7.920%
4th Recycle	7.490%	7.330%
5th Recycle	7.140%	7.100%
6th Recycle	6.970%	7.000%
7th Recycle	6.890%	6.960%
8th Recycle	6.840%	6.940%
9th Recycle	6.540%	6.930%
10th Recycle	6.390%	6.927%
11th Recycle	6.315%	6.926%
Unpretreated	4.050%	2.730%



(Source: Table 4.11)

Figure 4.30 Effect of IL Recycling on Glucose Yield

From the figure 4.18 above, it is evident that the IL can be recycled upto about six times before an almost constant glucose yield is obtained even though it is still above the yield recorded with the unpretreated samples. As is evident from the Figure 4.18 above, the pretreatment efficiency of 1-butyl -3-methylimidazolium chloride decreases with an increase in the number of cycles.



(Source: Table 4.11)
Figure 4.31 1-butyl -3-methylimidazolium chloride Recycling Performance

Starting from the fourth recycle as displayed in Figure 4.19 above, both curves (for PJ and GS) tend to plateau off. This can be attributed to an increase in the amounts of phenolic and aliphatic hydroxyl groups in 1-butyl -3-methylimidazolium chloride which hinder the dissolution of more lignin into the ionic liquid. An increase in the number of recycles reports a decline in the extractive glucose yield. This can be attributed to the degradation of hemicelluloses and/or cellulose during IL pretreatment process. Moreover, organic acids such as acetic acid- liberated from hemicelluloses acetates could become enriched with the recycled IL and in turn catalyze the acidic fragmentation of the carbohydrates (Zhuang et. al., 2007).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

.1 Conclusions

The primary objective of this investigation was to study and rate the performance of ionic liquids (i.e. Imidazolium based, Phosphonium based and Pyridinium based) as an alternative method of pretreatment for non-food based lignocellulosic materials (*Prosopis Juliflora* and *Gadam* sorghum stalks). The main conclusions to be drawn from the experimental results reported in this research work can be summarized as follows:

1. Both biomass (*Prosopis Juliflora* and *Gadam* Sorghum) contain a substantial amount of cellulose in their composition (over 25%); 34% in PJ and 29.68% in GS hence can be used as potential substitutes for the food based biomass in bioethanol production as an alternative fuel. Depletion of non-renewable source of energy, such as fossils, demands the exploration of large-scale non-petroleum-based alternative fuels, such as bioethanol. Bioethanol made from inexpensive and abundant sources of lignocellulosic biomass is highly desirable. The development of non-petroleum based fuels using non-food based biomass subjected to environmentally friendly fuel production techniques will go a long way in supplementing the dwindling petroleum oil reserves while easing food versus fuel competition.

In addition, higher overall glucose yields were observed with *Prosopis Juliflora* (73.27%) as compared to *Gadam* Sorghum(60.42%). This can be attributed to the higher cellulose content in its structure (34% as opposed to 29.68% in gadam sorghum).

2. Efficiency of ionic liquids in the pretreatment of lignocellulosic biomass is evident as observed in the significant increase in glucose yield for samples which had been pretreated with ILs prior to hydrolysis than those that were unpretreated. Table 5.1 below provides an overview of these findings.

Table 5.19 Summary of IL Efficiency

Type of Ionic Liquid	Biomass and optimum parameters	Highest Glucose Yield	Improvement in Yield(number of times yield increases)
Unpretreated	PJ	4.05%	**control level**
	GS	2.73%	**control level**
1-butyl -4-methyimidazolium chloride	PJ, 100◦C,120mins	73.27%	**18.1**
	GS,100◦C,120mins	24.10%	**8.83**
1-butyl -4-methypyridinium chloride	PJ,140◦C,160mins	61.63%	**15.21**
	GS,140◦C,160mins	60.42%	**22.13**
trihexyltetradecylphosphonium chloride	PJ,120◦C,120mins	7.19%	**1.78**
	GS,120◦C,120mins	3.93%	**1.44**

An increase in glucose yield of between 1.44 times when trihexyltetradecylphosphonium chloride ionic liquid was used on *gadam* sorghum (from 2.73% unpretreated to 3.93%) and 22 times when 1-butyl-4-methypyridinium chloride ionic liquid was used on *gadam* sorghum (from 2.73% unpretreated to 60.42%) is noted.

3. This work has evaluated the effect of cation type, pretreatment period and pretreatment temperature on the pretreatment process as summarized in Table 5.1 above.

From Table 5.1, it can be deduced as follows:

- The ionic liquids that were used in this study reported highest glucose yields at different pretreatment conditions. 1-butyl -3-methyimidazolium chloride recorded highest glucose yield under pretreatment temperature of 100℃ and a duration of 120 minutes for both *prosopis juliflora* and *gadam* sorghum. 1-butyl-4-methypyridinium chloride recorded highest glucose yield at 140℃ after pretreating both biomass for 160 minutes whereas trihexyltetradecylphosphonium chloride yielded highest glucose content at a temperature of 120℃ after 120minutes of pretreatment.
- Of the three ionic liquids used, trihexyltetradecylphosphonium chloride had the least glucose extraction efficiency. This is attributable to its hydrophobicity coupled with a relatively large cation and a relatively lower thermal stability.
- 1-butyl-4-methylpyridinium chloride demonstrates the highest thermal stability since its glucose extraction efficiency increased with the severity of the pretreatment temperature.
- 1-butyl-4-methylpyridinium chloride Ionic liquid showed an equally good glucose extraction efficiency (61.63% for *prosopis juliflora* and 60.42% for *gadam* sorghum) as 1-butyl -3-methyimidazolium chloride ionic liquid (73.27% for *prosopis juliflora* and 24.10% for *gadam* sorghum). It can therefore be used as a suitable substitute of the commonly studied Imidazolium ionic liquid.

4. Low fermentation inhibitor concentrations are reported when ionic liquids are used as compared to when dilute alkaline solution is used for pretreatment of lignocellulosic biomass. Table 5.2 below reports the furfural concentration levels when the different pretreatment agents are used and tabulates the percentage decrease in the furfural concentration levels with the dilute base agent as the control level.

Table 5.2 Furfural Concentration Levels

Pretreatment Agent	Biomass and optimum parameters	Furfural Concentration (mg/ml)	Percentage Decrease In Furfural Concentration
Dilute Base	PJ, 140◦C,30mins	4.63	**control level**
	PJ, 140◦C,30mins	4.53	**control level**
1-butyl -3-methyimidazolium chloride	PJ,140◦C,160mins	2.16	**53.3%**
	GS,140◦C,160mins	1.63	**64.0%**
1-butyl -4-methypyridinium chloride	PJ,140◦C,120mins	2.49	**46.2%**
	GS,140◦C,120mins	1.96	**56.7%**
trihexyltetradecylphosphonium chloride	PJ,120◦C,120mins	2.32	**49.9%**
	GS,120◦C,120mins	1.84	**59.4%**

The investigation on furfural inhibitor production showed a decrease of between 46.2% and 64% in furfural concentration when ionic liquids were used for pretreatment as compared to dilute alkaline pretreatment. This goes a long way in promoting use of ionic liquids as pretreatment agents for enhanced hydrolysis.

5. It was also established using 1-butyl -3-methyimidazolium chloride ionic liquid that it is possible to recycle the ionic liquid about six times without any deleterious effect on generation of fermentable sugars.

The overall conclusion is that, ionic liquid pretreatment is an effective, environmentally friendly and viable process that can be applied towards unlocking lignocelluloses recalcitrance. In addition, *prosopis juliflora* and *gadam* sorghum stalks are non-food based biomasses that can be embraced for bioethanol production.

.2 Recommendations

- From the results obtained in this study, the following are the recommendations:
- Both *prosopis juliflora* and *gadam* sorghum stalks can be embraced for bioethanol production since substantial glucose yields were obtained from each. In addition, prosopis juliflora and gadam sorghum stalks are non-food based biomass hence passing as potential alternatives for food based biomass in bioethanol production as an alternative fuel.
- 1-butyl -4-methypyridinium chloride Ionic liquid showed equally good glucose extraction efficiency as 1-butyl -3-methylimidazolium chloride ionic liquid. It can therefore be used in place of the commonly studied Imidazolium ionic liquid as it is cheaper hence saving on cost. Sugar yields can be increased by optimizing process conditions.
- Low fermentation inhibitor (furfural) concentrations are reported when ionic liquids are used as compared to when dilute alkaline solutions are used for pretreatment hence ionic liquids can be embraced as a pretreatment method in order to enhance high bioethanol production levels.
- With the high glucose yields reported with the use of 1-butyl -3-methylimidazolium chloride and a possibility of reuse established of upto six times, this ionic liquid can be embraced as a pretreatment method. However, a more suitable regeneration technique that can effectively remove the impurities in

ionic liquids in order to enhance further effective reuse of ILs thus reducing cost and alleviating any potential environmental and/or ecological effects that may arise from its use in the pretreatment process should be developed. Sugar yields can be increased by optimizing process conditions and using more advanced methods of phase separation and sugar recovery.

Further research can also be carried out to on the following:

- Study of the morphological structure of the biomass before pretreatment and the regenerated cellulose after pretreatment.

- Optimization of process conditions to maximize sugar yields and use of more advanced methods of phase separation and sugar recovery.

- Chemical kinetics study to be conducted using the ionic liquids used in this work and the two biomass to process pure cellulose.

- Mechanism of dissolution in ionic liquid to ascertain whether preferential cellulose dissolution is a property of the Imidazolium and Pyridinium cations whereas the Phosphonium cation had preference for another component of the lignocellulosic biomass.

- Relationship of the ionic liquid antisolvent ratio on the effectiveness of cellulose regeneration.

- Possibility of manufacturing low cost ionic liquids from renewable sources of carbon in order to make the process of ionic liquid manufacture more sustainable.

More exploration needs to be done in synthetic chemistry that would lead to the development of new low-cost ionic liquids to meet the process demand.

- Treatment and possible reuse of the waste water that results from the regeneration of dissolved cellulose in the ionic liquid.

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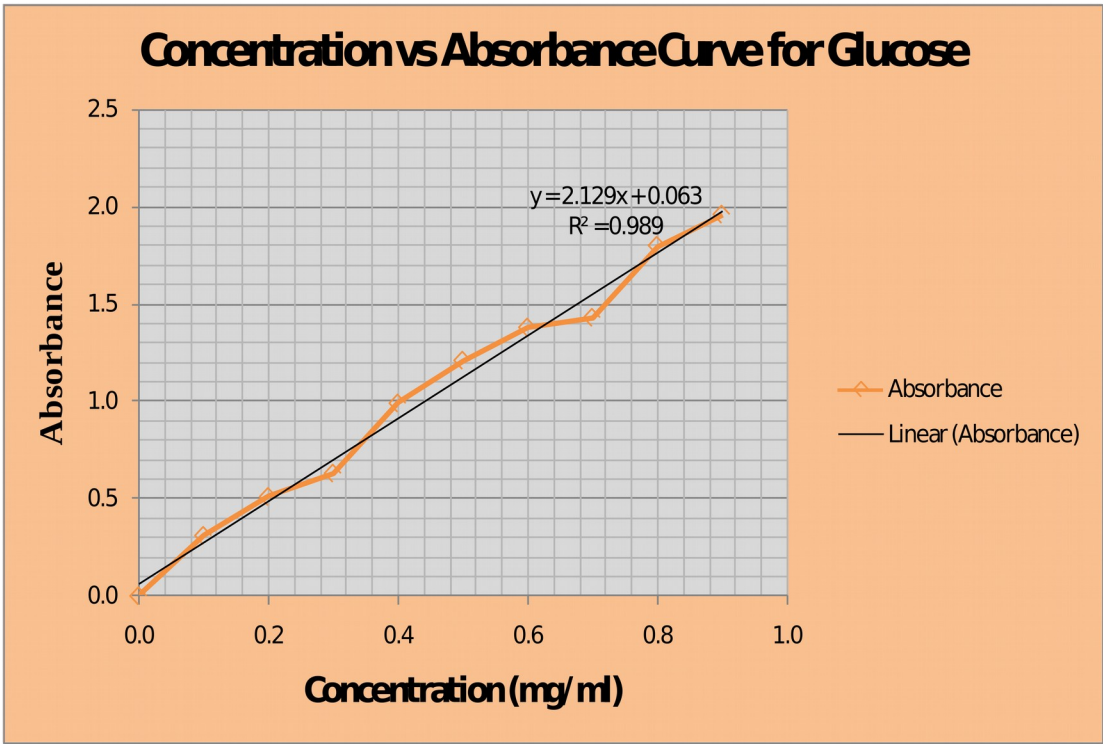
APPENDICES

APPENDIX I

STANDARDIZATION CURVE DATA GENERATED BY MEASURING THE ABSORBANCE OF DIFFERENT CONCENTRATIONS OF PURE GLUCOSE AT 520 NM

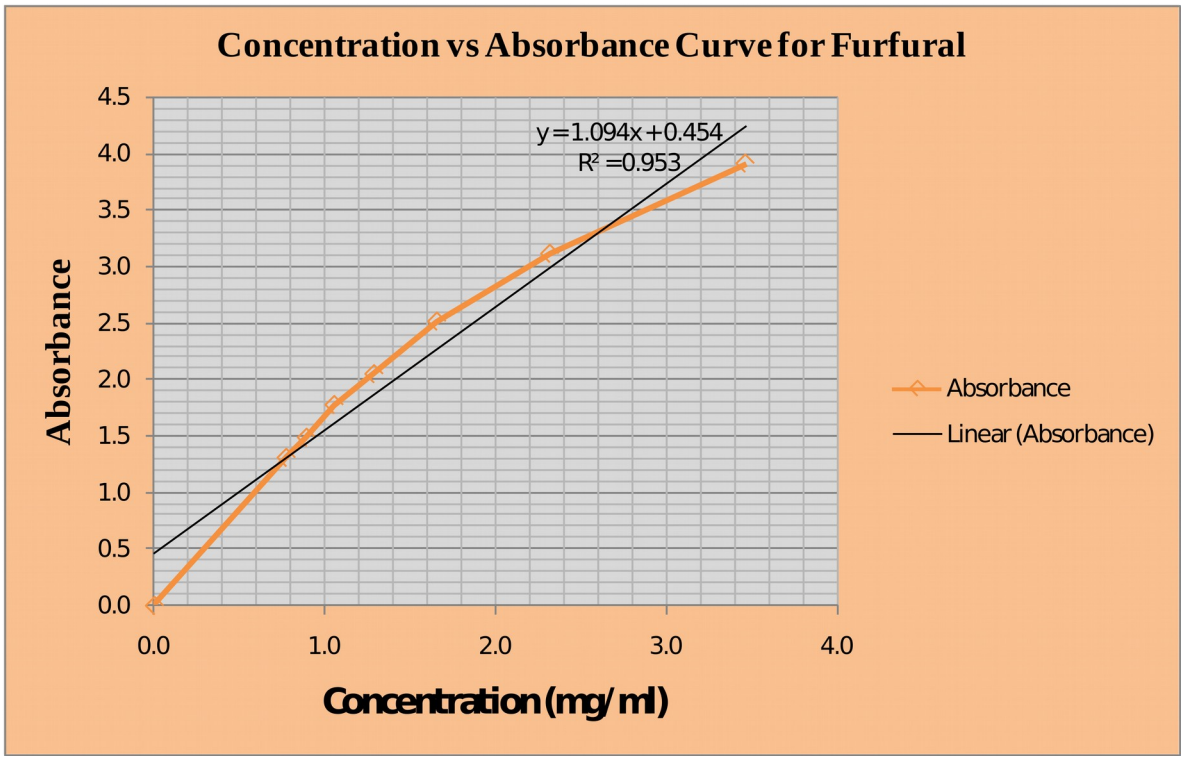
Concentration (g/ml)	Concentration (w/v%)	Absorbance
0	0	0
0.1	10	0.31
0.2	20	0.51
0.3	30	0.63
0.4	40	0.99
0.5	50	1.21
0.6	60	1.38
0.7	70	1.43
0.8	80	1.8
0.9	90	1.96



APPENDIX II

STANDARDIZATION CURVE DATA GENERATED BY MEASURING THE ABSORBANCE OF DIFFERENT CONCENTRATIONS OF PURE FURFURAL AT 284NM

Concentration (mg/ml)	Absorbance
0	0
0.773	1.31
0.892	1.488
1.0545	1.777
1.288	2.05
1.657	2.515
2.32	3.114
3.467	3.912



APPENDIX III

CHEMICAL AND PHYSICAL PROPERTIES OF GLYCEROL

Physical Description	Hygroscopic viscous liquid
Molecular Weight	92.09382 g/mol
Exact mass	92.047344 g/mol
Monoisotropic mass	92.047344 g/mol
Boiling Point	290 ºC
Melting Point	18.1 ºC
Flash Point	171 ºC
Colour	Clear, colourless syrupy liquid
Odor	Mild odor
Taste	Sweet
Viscosity	954 cP at 25 ºC
pH	Neutral to litmus test

(Source: National Centre for Biotechnology Information, 2016)

APPENDIX IV

CALCULATIONS

1) Quantity of glucose expected from Gadam Sorghum, assuming 100% cellulose conversion calculated as follows:

Quantity of biomass used $= 0.03$g

% cellulose in Gadam Sorghum = 35.87%

Quantity of glucose expected

$$¿ 0.03 \times \frac{35.87}{100} = 10.761\, mg$$

2) Quantity of glucose expected from Prosopis Juliflora, assuming 100% cellulose conversion calculated as follows:

Quantity of biomass used $= 0.03$g

% cellulose in Gadam Sorghum = 49.4%

Quantity of glucose expected

$$¿ 0.03 \times \frac{49.4}{100} = 14.82\, mg$$

3) The glucose yield is calculated using:

$$glucose\ yield = 100 \times \frac{m_{glucose}}{m_{initial}}$$

4) The cost of ionic liquid used per gram of glucose produced:

Ionic Liquid	**[BMIM]Cl**	**4MBPCl**	**P66614Cl**
Purchase Cost	Ksh 23,200 for 5gms	Ksh 17,400 for 5gms	Ksh 26,100 for 50gms
Ionic Liquid Cost per Gram	23,200/5 =Ksh 4,650	17,400/5 =Ksh 3,480	26,100/50 =Ksh 522
Quantity of Ionic Liquid Used was	0.47g	0.47g	0.47g
Cost of Ionic Liquid Used	Ksh. 2,186	Ksh 1,636	Ksh. 245
Maximum Glucose Yield with *Prosopis Juliflora*	0.7327 * 14.82 = 10.85mg =0.011g	0.6163 * 14.82 = 9.13mg =0.00913g	0.0719 * 14.82 = 1.0656mg =0.001066g
Cost of Ionic Liquid per gram of Glucose Produced with Prosopis Juliflora	2186/0.011 = **Ksh 198,727**	1636/0.00913 = **Ksh 179,189**	522/0.001066 = **Ksh 489,681**
Maximum Glucose Yield with *Gadam* Sorghum	0.2410 * 10.761= 2.59mg =0.0026g	0.6042 * 10.761 = 6.5mg =0.0065g	0.0393 * 10.761 = 0.423mg =0.000423g
Cost of Ionic Liquid per gram of Glucose Produced with *Gadam* Sorghum	2186/0.0026 = **Ksh 840,769**	1636/0.0065 = **Ksh 251,692**	522/0.000423 = **Ksh 1,234,042**

5) w/w% to w/v% conversion

w/w% is the number of grams of solute per 100g solution

w/v% is the number of grams of solute per 100ml solution

Concentration of sulphuric acid 98%w/w

Density of sulphuric acid 1.84g/ml

Converting %w/w to %w/v:

$$98\ \frac{w}{w} \times \frac{1.84\ g}{ml} = 180.32\ w/v$$

We want 250ml of 2%w/v solution of sulphuric acid. To find the volume of sulphuric acid required we use the equation:

$$Volume\ of\ acid\ required = \frac{mass\ of\ solute}{concentration\ of\ solution}$$

$$Mass\ of\ sulphuric\ acid \in 2\ \frac{w}{v} = \frac{2\ g}{100\ ml} \times 250\ ml = 5\ g$$

$$Volume\ of\ acid\ required = 5\ g \div \frac{180.32\ g}{100\ ml} = 2.77\ ml$$

APPENDIX IV

EXPERIMENTAL DATA COLLECTED ON PRETREATMENT WITH THE VARIOUS ILS AT VARIED TEMPERATURES AND TIME.

Feedstock	Ionic liquid	Sample	Temp (°C)	Time(mins)	LED Abs	Dilution factor	Actual Abs	Concentration(mg/ml)	Extractive Yield
Gadam Sorghum	Pyridinium	1AGPy	80	40	0.132	2.222	0.293	0.683	6.35%
		1BGPy		80	0.147	2.365	0.348	0.803	7.46%
		1CGPy		120	0.158	2.395	0.377	0.865	8.04%
		1DGPy		160	0.957	2.089	2.000	4.359	40.50%
									15.59%
Gadam Sorghum	Pyridinium	2AGPy	100	40	0.157	2.088	0.327	0.758	7.04%
		2BGPy		80	0.208	2.222	0.462	1.047	9.73%
		2CGPy		120	0.405	2.207	0.894	1.979	18.39%
		2DGPy		160	2.017	1.103	2.224	4.843	45.00%
									20.04%
Gadam Sorghum	Pyridinium	3AGPy	120	40	0.170	2.062	0.350	0.807	7.50%
		3BGPy		80	0.402	2.238	0.900	1.992	18.51%
		3CGPy		120	0.707	2.222	1.572	3.438	31.95%
		3DGPy		160	0.578	4.389	2.537	5.515	51.25%
									27.30%
Gadam Sorghum	Pyridinium	4AGPy	140	40	0.675	1.081	0.730	1.624	15.10%
		4BGPy		80	1.001	1.127	1.128	2.482	23.07%
		4CGPy		120	1.142	1.125	1.284	2.819	26.20%
		4DGPy		160	1.347	2.222	2.993	6.497	60.38%
									31.18%

Feedstock	Ionic liquid	Sample	Temp (°C)	Time(mins)	LED Abs	Dilution factor	Actual Abs	Concentration(mg/ml)	Extractive Yield
Prosopis Julifora	Pyridinium	1APPy	80	40	0.192	2.222	0.426	0.970	6.54%
		1BPPy		80	0.264	2.222	0.586	1.316	8.88%
		1CPPy		120	1.037	2.222	2.305	5.015	33.84%
		1DPPy		160	1.620	2.222	3.599	7.803	52.65%
									25.48%
Prosopis Julifora	Pyridinium	2APPy	100	40	0.094	2.222	0.209	0.502	3.39%
		2BPPy		80	0.536	2.222	1.191	2.618	17.66%
		2CPPy		120	0.949	2.821	2.677	5.817	39.25%
		2DPPy		160	1.205	1.750	2.109	4.594	31.00%
									22.83%
Prosopis Julifora	Pyridinium	3APPy	120	40	0.278	2.222	0.619	1.386	9.35%
		3BPPy		80	0.358	2.222	0.796	1.768	11.93%
		3CPPy		120	0.596	2.222	1.325	2.907	19.61%
		3DPPy		160	1.657	2.222	3.683	7.983	53.86%
									23.69%
Prosopis Julifora	Pyridinium	4APPy	140	40	0.801	2.222	1.780	3.887	26.23%
		4BPPy		80	1.008	2.222	2.240	4.876	32.90%
		4CPPy		120	1.977	1.546	3.056	6.634	44.76%
		4DPPy		160	1.370	3.076	4.214	9.127	61.59%
									41.37%

Feedstock	Ionic liquid	Sample	Temp (°C)	Time(mins)	LED Abs	Dilution factor	Actual Abs	Concentration(mg/ml)	Extractive Yield
Gadam Sorghum	Phosphonium	1AGPh	80	40	0.012	1.022	0.012	0.079	0.73%
		1BGPh		80	0.040	1.075	0.043	0.146	1.36%
		1CGPh		120	0.066	1.090	0.072	0.207	1.92%
		1DGPh		160	0.020	2.932	0.058	0.177	1.65%
									1.42%
Gadam Sorghum	Phosphonium	2AGPh	100	40	0.077	1.658	0.128	0.329	3.05%
		2BGPh		80	0.028	2.222	0.062	0.186	1.73%
		2CGPh		120	0.049	2.106	0.103	0.274	2.55%
		2DGPh		160	0.054	1.083	0.058	0.179	1.66%
									2.25%
Gadam Sorghum	Phosphonium	3AGPh	120	40	0.032	3.384	0.109	0.288	2.67%
		3BGPh		80	0.046	2.734	0.126	0.325	3.02%
		3CGPh		120	0.055	3.135	0.172	0.423	3.93%
		3DGPh		160	0.100	1.047	0.105	0.280	2.60%
									3.05%
Gadam Sorghum	Phosphonium	4AGPh	140	40	0.026	1.684	0.044	0.147	1.37%
		4BGPh		80	0.026	4.559	0.120	0.311	2.89%
		4CGPh		120	0.047	4.322	0.205	0.494	4.59%
		4DGPh		160	0.051	2.222	0.113	0.297	2.76%
									2.90%

Feedstock	Ionic liquid	Sample	Temp (°C)	Time(mins)	LED Abs	Dilution factor	Actual Abs	Concentration(mg/ml)	Extractive Yield
Prosopis Julifora	Phosphonium	1APPh	80	40	0.021	2.222	0.046	0.152	1.02%
		1BPPh		80	0.040	2.222	0.089	0.244	1.65%
		1CPPh		120	0.043	2.222	0.096	0.260	1.75%
		1DPPh		160	0.022	2.222	0.048	0.157	1.06%
									1.37%
Prosopis Julifora	Phosphonium	2APPh	100	40	0.037	2.394	0.088	0.243	1.64%
		2BPPh		80	0.040	2.253	0.089	0.245	1.65%
		2CPPh		120	0.059	2.222	0.132	0.337	2.27%
		2DPPh		160	0.078	2.222	0.173	0.425	2.87%
									2.11%
Prosopis Julifora	Phosphonium	3APPh	120	40	0.139	2.222	0.308	0.716	4.83%
		3BPPh		80	0.189	2.222	0.420	0.957	6.46%
		3CPPh		120	0.212	2.222	0.470	1.065	7.19%
		3DPPh		160	0.386	1.102	0.425	0.968	6.53%
									6.25%
Prosopis Julifora	Phosphonium	4APPh	140	40	0.070	2.222	0.156	0.388	2.62%
		4BPPh		80	0.072	2.222	0.159	0.396	2.67%
		4CPPh		120	0.109	2.222	0.243	0.576	3.88%
		4DPPh		160	0.092	1.350	0.124	0.319	2.15%
									2.83%

Feedstock	Ionic liquid	Sample	Temp (°C)	Time(mins)	LED Abs	Dilution factor	Actual Abs	Concentration(mg/ml)	Extractive Yield
Gadam Sorghum	Imidazolium	1AGIm	80	40	0.145	2.617	0.379	0.869	8.08%
		1BGIm		80	0.172	2.891	0.497	1.124	10.44%
		1CGIm		120	0.207	2.840	0.588	1.320	12.27%
		1DGIm		160	0.167	2.200	0.368	0.845	7.85%
									9.66%
Gadam Sorghum	Imidazolium	2AGIm	100	40	0.206	2.200	0.454	1.031	9.58%
		2BGIm		80	0.229	2.593	0.593	1.331	12.37%
		2CGIm		120	0.309	3.819	1.179	2.593	24.09%
		2DGIm		160	0.269	2.572	0.691	1.542	14.33%
									15.09%
Gadam Sorghum	Imidazolium	3AGIm	120	40	0.264	1.129	0.298	0.695	6.46%
		3BGIm		80	0.456	1.097	0.500	1.130	10.50%
		3CGIm		120	0.096	5.325	0.511	1.154	10.72%
		3DGIm		160	0.038	4.804	0.184	0.450	4.18%
									7.97%
Gadam Sorghum	Imidazolium	4AGIm	140	40	0.252	1.756	0.443	1.007	9.36%
		4BGIm		80	0.086	2.200	0.188	0.458	4.26%
		4CGIm		120	0.102	1.216	0.124	0.319	2.96%
		4DGIm		160	0.058	1.993	0.117	0.304	2.82%
									4.85%

Feedstock	Ionic liquid	Sample	Temp (°C)	Time(mins)	LED Abs	Dilution factor	Actual Abs	Concentration(mg/ml)	Extractive Yield
Prosopis Julifora	Imidazolium	1APIm	80	40	1.059	2.200	2.330	5.071	34.22%
		1BPIm		80	1.454	2.200	3.200	6.943	46.85%
		1CPIm		120	1.854	2.200	4.078	8.834	59.61%
		1DPIm		160	0.974	2.200	2.142	4.665	31.48%
									43.04%
Prosopis Julifora	Imidazolium	2APIm	100	40	0.493	2.200	1.084	2.388	16.11%
		2BPIm		80	0.514	2.200	1.132	2.490	16.80%
		2CPIm		120	2.279	2.200	5.014	10.850	73.21%
		2DPIm		160	2.238	2.200	4.923	10.653	71.88%
									44.50%
Prosopis Julifora	Imidazolium	3APIm	120	40	0.074	2.200	0.162	0.402	2.71%
		3BPIm		80	0.191	1.486	0.284	0.664	4.48%
		3CPIm		120	0.190	2.285	0.433	0.986	6.65%
		3DPIm		160	0.205	1.725	0.354	0.815	5.50%
									4.84%
Prosopis Julifora	Imidazolium	4APIm	140	40	0.083	1.275	0.106	0.280	1.89%
		4BPIm		80	0.177	1.028	0.182	0.444	2.99%
		4CPIm		120	0.194	1.354	0.262	0.618	4.17%
		4DPIm		160	0.095	1.943	0.185	0.452	3.05%
									3.03%

APPENDIX VI

EXPERIMENTAL DATA COLLECTED FOR GLUCOSE YIELDS ON HYDROLYSIS OF THE BIOMASSES WITHOUT PRETREATMENT

Feedstock	Actual Abs	Concentration(mg/ml)	Extractive Yield
Gadam Sorghum	0.1120	0.2941	2.733%
Prosopis Juliflora	0.1780	0.4363	4.054%

APPENDIXVII

EXPERIMENTAL DATA COLLECTED FOR FURFURAL PRODUCTION ON PRETREATMENT WITH THE VARIOUS ILS AND DILUTE ALKALINE SOLUTION

sample	IL/ dilute Base	Pretreatment Conditions	Abs	Inh. Concentration(mg/ml)
Gadam sorghum	Pyridinium	T=140 ◦C, t=160min	1.670	1.956
Prosopis Juliflora	Pyridinium	T=140 ◦C, t=160min	2.136	2.491
Gadam sorghum	Imidazolium	T=100◦C, t=120min	1.387	1.630
Prosopis Juliflora	Imidazolium	T=100 ◦C, t=120min	1.852	2.164
Gadam sorghum	Phosphonium	T=140 ◦C, t=120min	1.567	1.837
Prosopis Juliflora	Phosphonium	T=120◦C, t=120min	1.987	2.319
Gadam sorghum	Weak Base	T=140 ◦C, t=30min	3.913	4.531
Prosopis Juliflora	Weak Base	T=140 ◦C, t=30min	3.999	4.629