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Article *in* International Journal of Scientific and Engineering Research · February 2017

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International Journal of Scientific & Engineering Research, Volume 8, Issue 2, February-2017
ISSN 2229-5518

Enhancement of Simple Sugar Production: Pretreatment of Gadam Sorghum Stalks using Imidazolium, Pyridinium and Phosphonium Based Ionic Liquids

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ABSTRACT
Production of bioethanol from the abundant and inexpensive sources of lignocellulosic biomass requires that the latter undergo pretreatment processes. A new pretreatment method by use of Ionic Liquids has shown to be promising. This research work analyzed pretreatment of *Gadam* Sorghum stalks using 1-butyl-3-methylimidazolium chloride ([BMIM]Cl),1-butyl-4-methylpyridinium chloride ([4MBP]Cl) and trihexyltetradecylphosphonium chloride ([P66614]Cl). Regeneration of cellulose from the Ionic Liquids was carried out and the performance of the Ionic Liquids established through analysis of glucose concentrations upon hydrolysis. Pretreatment of Gadam Sorghum was carried out at varied temperatures (80°C–140°C) , pretreatment periods (40min–160min) and a biomass loading of 6%wt. Simple acid hydrolysis was then performed at a temperature of 130°C for a duration of 10 minutes. Finally glucose measurement was done using a Shimadzu UV–Vis Spectrophotometer at a wavelength of 520nm. Pretreatment of the biomass with the ionic liquids resulted in an increase in glucose yield of 1.43 times with phosphonium IL , 8.83 times with imidazolium IL and 22.13 times with pyridinium IL as compared to the unpretreated biomass. Ionic liquid pretreatment is therefore an effective, and viable process that can be applied towards unlocking lignocelluloses recalcitrance. However [BMIM]Cl and [4MBP]Cl showed better performance generally as compared to P66614]Cl with respect to maximum glucose yield obtained with each of the ILs (3.93% in the P66614]Cl pretreated case, 24.01% in the [BMIM]Cl pretreated case and 60.42% in the [4MBP]Cl pretreated case. A substantial amount of glucose is yielded from Gadam Sorghum hence qualifying it as a potential substitute for the food based biomass in bioethanol production as an alternative fuel.
Key Words: Pretreatment, Ionic Liquids, Lignocellulosic Biomass, Gadam Sorghum

1. INTRODUCTION

Energy accessibility and cost is a determining factor for the economical, political and social interrelations among nations. In most developing countries, the current sustainable source of energy is plant biomass which 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. First and second generation biofuels derived from plant biomass are currently the only sustainable class of liquid fuels (Lowe, 2008) such as 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. However, 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.,* 2008). 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.

Nevertheless, non-volatile solvents called ionic liquids (ILs) have since been discovered to be 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.,* 2005, 2006). These encouraging results indicate the possible utilization of ILs as alternative and non-volatile solvents in cellulose pretreatment processes (Zhao *et al.,* 2009). 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 that 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 (Remsing *et al*. , 2006). 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 in benzene, toluene and styrene but immiscible in higher alkyl benzenes (Vitz e*t al*., 2009).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 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. The goal of the present research work was to study pretreatment of Gadam Sorghum Stalks using Imidazolium, Pyridinium and Phosphonium Based Ionic Liquids. In addition, the regeneration of cellulose from the Ionic Liquids was carried out and the performance of the Ionic Liquids established through analysis of glucose concentrations upon hydrolysis.

2. MATERIALS AND METHODOLOGY

2.1 Experimental Materials

Commercial ionic liquids trihexyltetradecylphosphonium chloride (P66614Cl), 1-butyl -3-methylimidazolium chloride ([$BMIM^+$][Cl^-]), and 1-butyl -4-methypyridinium chloride (4MBPCl), were produced by Sigma Aldrich and supplied by Kobian Kenya Limited. Pure Analytical Standards; D(+) Glucose and furfural were also produced by Sigma Aldrich and supplied by Kobian Kenya Limited. Gadam sila sorghum stalks were obtained from Nang'eni in Bungoma County in Kenya.

2.2 Experimental Set-up

Experimentation majorly consisted of the pretreatment of the gadam sorghum stalks with the different ionic liquids at varied conditions (temperature and time) for cellulose dissolution. The chemical composition analysis of the biomass material before pretreatment was carried out Analysis of the pretreated biomass after hydrolysis for glucose concentrations was also done. 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. The following variables had a direct impact on the results that were attained from this study: particle size, biomass loading, 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. Pretreatment temperature and duration - were adjusted with the progression of the experiments. The overall scheme of the experimental process is illustrated in figure 1.

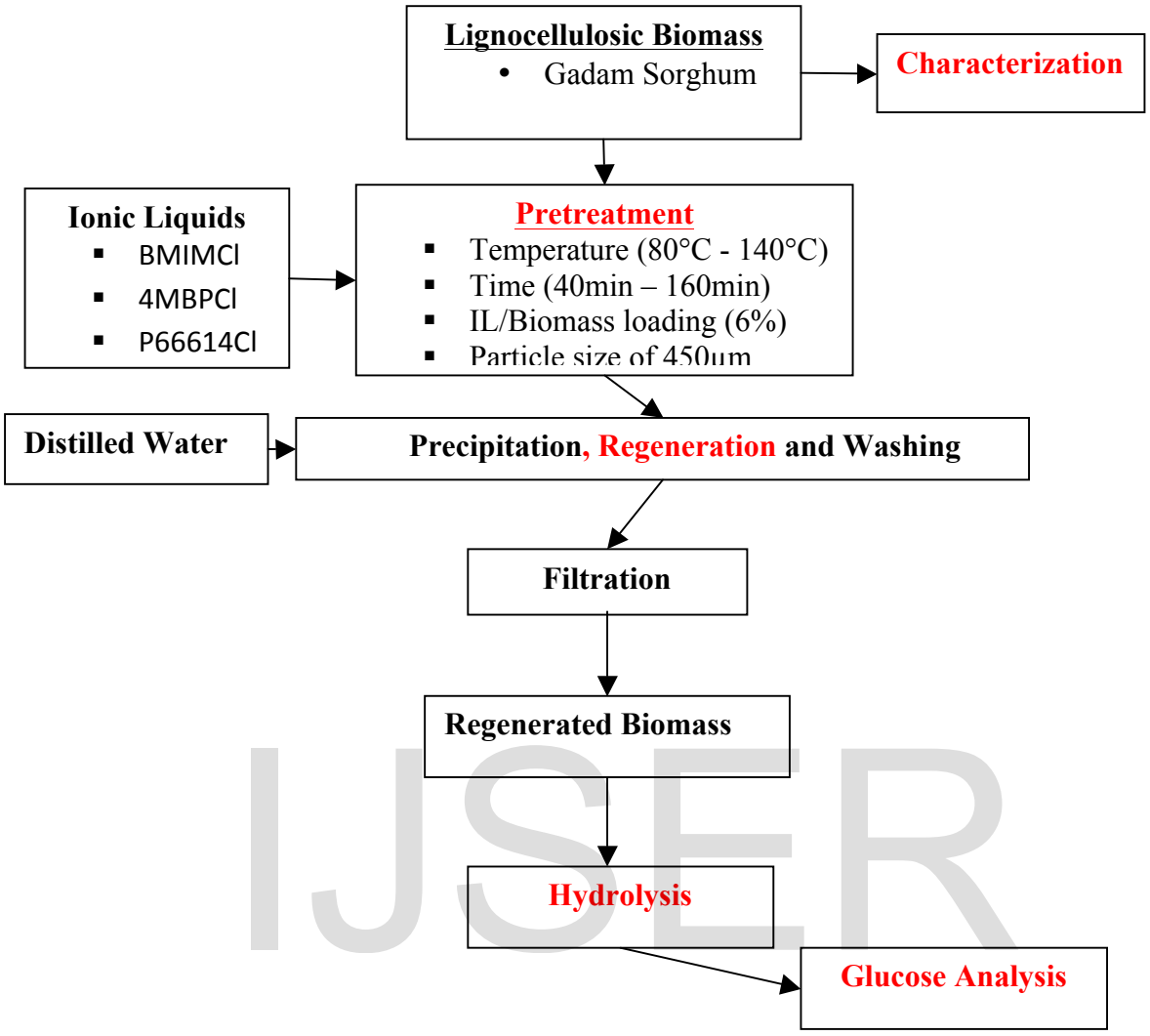


Figure 1: Overall Scheme of the Experimental Process

2.3 Experimental Procedure

Gadam Sorghum stalks were 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 105^{o}C to eliminate/reduce the moisture content of the biomass in an oven to 9.12%. The characterization of the biomass 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. In each case three replications were employed and averages obtained. The analysis of the results was done using Microsoft 2007 excel program.

2.3.1 Compositional Analysis of Biomass

The chemical composition of biomass under investigation was analyzed as described by National Renewable Energy Laboratory (NREL) - Laboratory analytical procedures (LAP) procedures, with minor modifications to suit the prevailing conditions. The procedures applied were for: Ash Determination (ASTM E1755-01), Determination of water soluble extractives (NREL/TP-510-42618), Determination of Ethanol soluble Extractives (NREL/TP-510-42618), and Determination of lignin and structural carbohydrates. ASTME 1757 - 01 Standard practice for preparation of biomass for compositional analysis was also applied.

2.3.2 Dissolution with Ionic Liquid and Regeneration of Cellulosic Materials

About 0.03g of milled gadam sorghum stalks 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). 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,* and *Temperature effect*. 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. 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. Simple acid hydrolysis was then carried out and the hydrolysates analyzed for glucose using a Shimadzu UV Vis Spectrophotometer at a wavelength of 520nm [10]. The concentration of glucose in the samples was then calculated based on a standard curve obtained using a standard glucose solution.

2.3.3 Acid Hydrolysis for Control Experiment

To the unpretreated biomass (0.03g of sample) 2ml of 2 w/v% sulphuric acid was added. The reaction mixture was heated in an oil bath at a temperature of 130°C for 10 minutes. The cellulosic hydrolysate was then separated from the solids by filtration. The hydrolysate was finally analyzed by the UV-visible spectrophotometer to give the glucose concentration

2.3.4 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. For each of the regenerated biomass samples, 2ml of 2 w/v% sulphuric acid was added. The reaction mixture was heated in an oil bath at a temperature of 130°C for 30 minutes. The cellulosic hydrolysate was then separated from the pretreated solids by filtration. The hydrolysate was finally analyzed by the UV-visible spectrophotometer to give the glucose concentration.

3. RESULTS, ANALYSIS AND DISCUSSION

3.1 Characterization of the Biomass

The biomass used in the present study was collected, processed mechanically and the chemical composition determined according to the standard methods was obtained. These results were tabulated (Table 1) and compared to those obtained from established references.

Table 1: 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**

The chemical composition of gadam sorghum stalks compares favorably with that reported by the work of [11] who reported 15 - 25% cellulose, 35 – 50% hemicellulose and 20 – 30% lignin content in sweet sorghum bagasse. The results are also in agreement with the report from [12] which indicates a composition of 35.87%, cellulose, 26.04% hemicelluloses, 7.52% ash and 30.57% lignin & extractives in gadam sorghum stalks.The presence of high cellulose content in gadam sorghum stalks (29.68%) indicates its potential for use as a feedstock for the production of glucose.

3.2 Effect of Pretreatment 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 glucose yield under the different experimental conditions are presented in tables 2 and 3 as well as in figure 2. Generally 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 of gadam sorghum samples with the various ionic liquids on glucose yield by heating at various pretreatment temperatures and times at 6%wt biomass loading were tabulated as shown in Tables 3 below.

Table 2: 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%

3.3 Effect of the Various IL Cations 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

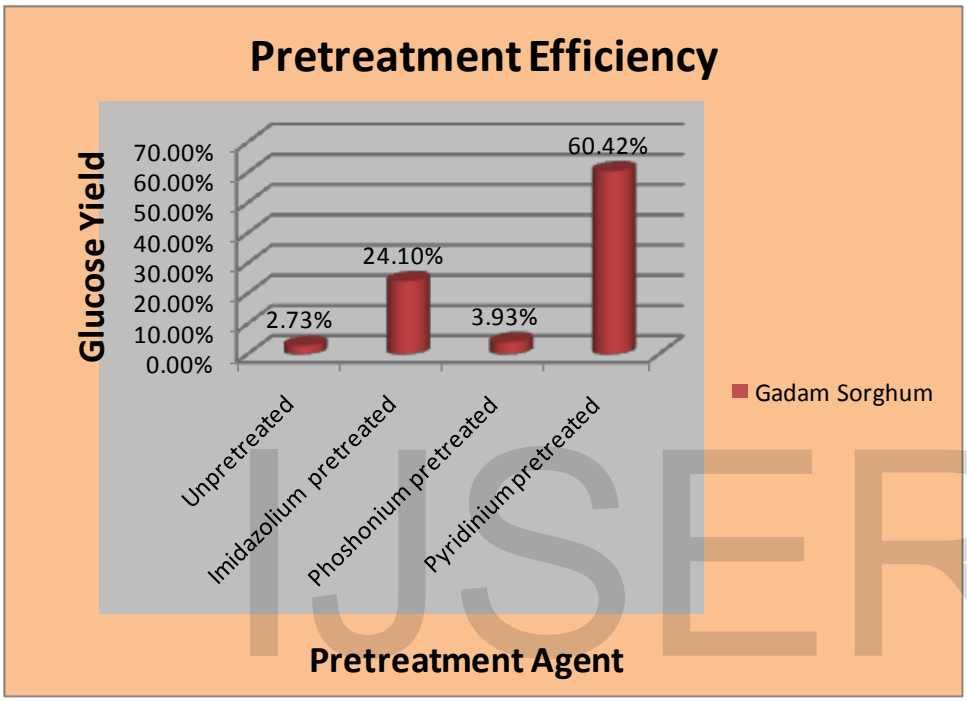
Ionic liquids have the ability to dissolve carbohydrates and lignin since they can effectively disrupt the intricate network of non-covalent interactions between these polymers. The fundamental interaction between the anion of the ionic liquid with the substrate carbohydrate is more prevalent in comparison to the interaction between the cation and the carbohydrate [13]. 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 [14].

Table 3 Glucose Yield Achieved for each pretreatment method

Sample Condition	Glucose Yield
Unpretreated	2.73%
Imidazolium pretreated	24.10%
Phoshonium pretreated	3.93%
Pyridinium pretreated	60.42%

In reference to Figure 2, 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 3 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 [15]. 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 [15]. 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.



(Source Table 5)

Figure 2: Glucose Extraction Efficiency Comparison of Ionic Liquids Cations

4. CONCLUSION

Gadam Sorghum contains a substantial amount of cellulose in its composition (over 25%); 29.68% hence can be used as a potential substitute for the food based biomass in simple sugar production for 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.

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. An increase in glucose yield of between 1.43 times when phosphonium IL was used on gadam sorghum (from 2.73% unpretreated to 3.93%) and 22 times when pyridinium IL was used on gadam sorghum (from 2.73% unpretreated to 60.42%) is noted.

The ionic liquids that were used in this study reported highest glucose yields at different pretreatment conditions. BMIMCl recorded highest glucose yield under pretreatment temperature of 100℃ and a duration of 120 minutes. 4MBPCL recorded highest glucose yield at 140℃ after pretreating the biomass for 160 minutes whereas [P66614]Cl yielded highest glucose content at a temperature of 120℃ after 120minutes of pretreatment. Of the three ionic liquids used, [P66614]Cl 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. Pyridinium Ionic liquid showed better glucose extraction efficiency 60.42% than Imidazolium ionic liquid 24.10%. It can therefore be used as a suitable substitute of the commonly studied Imidazolium ionic liquid.

ACKNOWLEDGEMENT

The authors gratefully acknowledge support by grants from the National Commission for Science, Technology and Innovation, Kenya and Moi University, Kenya Research Department.

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