International Research Journal of Engineering and Technology (IRJET) e-ISSN: 2395 -0056
Volume: 04 Issue: 03 | March -2017 www.irjet.net p-ISSN: 2395-0072

Towards Bioethanol Production in Kenya – Enhanced Pretreatment of Prosopis Juliflora Using Ionic Liquids

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Abstract - *Lignocellulosic biomass especially from nonfood crops is widely regarded as a sustainable second-generation bioethanol raw material. However, production of bioethanol from the abundant and inexpensive sources of lignocellulosic biomass such as Prosopis Juliflora 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 Prosopis Juliflora stem 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 Prosopis Juliflora 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 1.78 times with phosphonium IL, 18.1 times with imidazolium IL and 15.21 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 (7.19% in the P66614]Cl pretreated case, 73.27% in the [BMIM]Cl pretreated case and 61.63% in the [4MBP]Cl pretreated case. Prosopis Juliflora yielded a substantial amount of glucose hence qualifying as a potential non food-based biomass substitute suitable for bioethanol production.*

Key Words: Pretreatment, Ionic Liquids, Lignocellulosic Biomass, *Prosopis Juliflora*, bioethanol, Kenya.

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 (1) have led to a push towards decreased fossil fuel consumption. Given this reality, nations around the world are investing in alternative sources of energy, including bioethanol. The leading nations in bioethanol production (Table 1) are Brazil and the USA (2). Asian countries altogether account for about 14% of world's bioethanol production.

Table-1: Leading Bioethanol Producers In the World (3)

Country/Group of countries	Ethanol Production	
	Million litres	MTOE
USA	26,500	14.55
Brazil	19,000	10.44
European Union	2,250	1.24
China	1,840	1.01
Canada	1,000	0.55
India	400	0.22
Others	1,017	0.56
World (Total)	**52,007**	**28.57**

MTOE: million tones of oil equivalents

1.1 Lignocellulosic biomass and ethanol production

Bioethanol production from sugarcane and starch rich feedstock 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 (4). Estimates clearly point to the fact that first generation ethanol production process cannot sufficiently meet the

global energy needs. Therefore, second generation processes to produce bioethanol are gaining momentum. The second-generation processes use lignocellulosic materials for this purpose of which the biosphere has sufficient supplies (5). The production of ethanol from lignocellulosic biomass [sorghum straw, 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. 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. This was introduced into the Kenyan 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 (6). *Prosopis Juliflora* has survived vastly in these areas and become a major nuisance. On average, the chemical composition of *Prosopis Juliflora* stem is 49.4% cellulose, 18% hemicellulose, 4.3% ash, 28.3% lignin and extractives (7). This makes it a potential woody biomass that can be explored for bioethanol production.

1.2 Pretreatment of lignocellulosic biomass

Pretreatment is a crucial process step for the biochemical conversion of lignocellulosic biomass into bioethanol. 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 required to alter the structure of cellulosic biomass to make cellulose more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars (8). 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 over-view of the field (9-12). Pretreatment involves the alteration of biomass so that 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 1). The following criteria lead to an improvement in 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

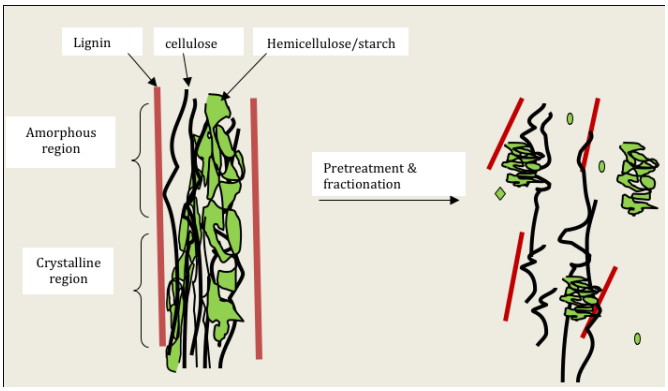

Figure-1: Effects of pretreatment on lignocellulosic biomass (12)

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.

Four main pretreatment methods are available namely, biological, physical, chemical and physico-chemical. Unfortunately, each of these methods has some disadvantages, such as:
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 (13). These encouraging results indicate the possible utilization of ILs as

alternative and non-volatile solvents in cellulose pretreatment processes (14).

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. *Prosopis Juliflora* stems were obtained from Marigat area in Baringo County in Kenya.

2.2 Experimental Set-up

Experimentation majorly consisted of the pretreatment of the *Prosopis Juliflora* stems 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.

2.3 Experimental Procedure

Prosopis Juliflora stems 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 *Prosopis Juliflora* 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 effects of reaction time and temperature. 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 (20). 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 (20) to give the glucose concentration. The overall scheme of the experimental process is illustrated in figure 2.

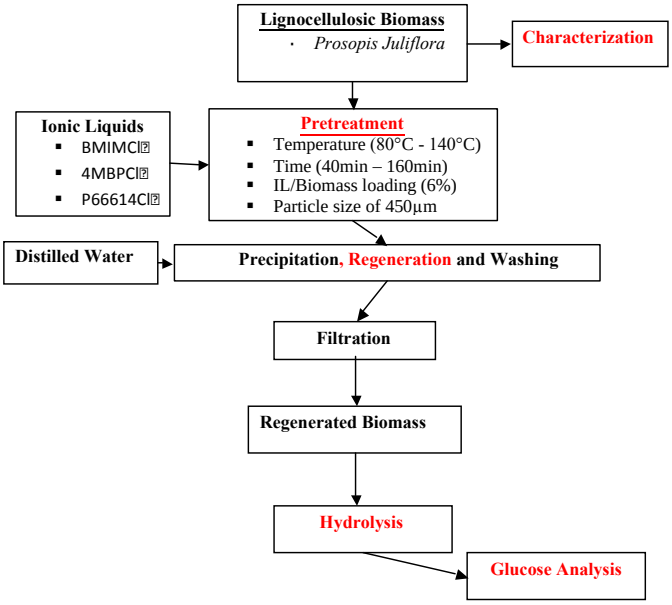

Figure-2: Overall Scheme of the Experimental Process

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 (table 2) were found to be quite comparable to those from established literature.

The chemical composition of *Prosopis Juliflora* stem compares favorably with that reported by (15) which indicates a composition of 35.87%, cellulose, 26.04% hemicelluloses, 7.52% ash and 30.57% lignin & extractives in *Prosopis Juliflora* stem. They are also in agreement with results from Sun and Cheng (16) who found a composition of 45-50% cellulose, 25-35% hemicellulose and 25-35% lignin for softwoods stems. The presence of high cellulose content in *Prosopis Juliflora* stem (34%) indicates its potential for use as a feedstock for the production of glucose.

Table-2: 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**

3.2 Effect of Pretreatment on Glucose Yield

The glucose yield was obtained after the regeneration process of each biomass followed by acid hydrolysis. Results for the effect of pretreatment of *Prosopis Juliflora* 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. The control experiment was carried out and it involved the unpretreated *Prosopis Juliflora* stem being hydrolysed and glucose yields determined in order to compare with those obtained with pretreated biomass. From the control experiment, unptretreated *Prosopis Juliflora* produced extractive glucose yield of 4.050%, which was rather low as compared to the yields from the pretreated *Prosopis Juliflora* (Table 3). Ionic liquid pretreatment thus appears to have great influence on the glucose yield after acid hydrolysis.

Table-3: 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%

3.3 Effect of the various IL Cations on Pretreatment

In reference to Figure 3, a higher glucose yield was reported for samples that had been pretreated with ionic liquids prior to hydrolysis as compared to the unpretreated samples. An increase in glucose yields of 1.78 times when

phosphonium IL was used (from4.05% unpretreated to 7.19%), 15.21 times when pyridinium IL was used (from 4.05% unpretreated to 61.63%), and 18.1 times when imidazolium IL is used (from 4.05% unpretreated to 73.27%) is noted.

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 (17). 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 (18).

The foregoing discussion 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 (19).

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. Similar IL behavior on cellulose has been reported by other researchers (19). 1-butyl-3- methylimidazolium chloride and 1-butyl-4-methylpyridinium chloride being hydrophilic are more effective in dissolving *Prosopis Juliflora* cellulose as compared to their hydrophobic counterpart-trihexyltetradecylphosphonium chloride.

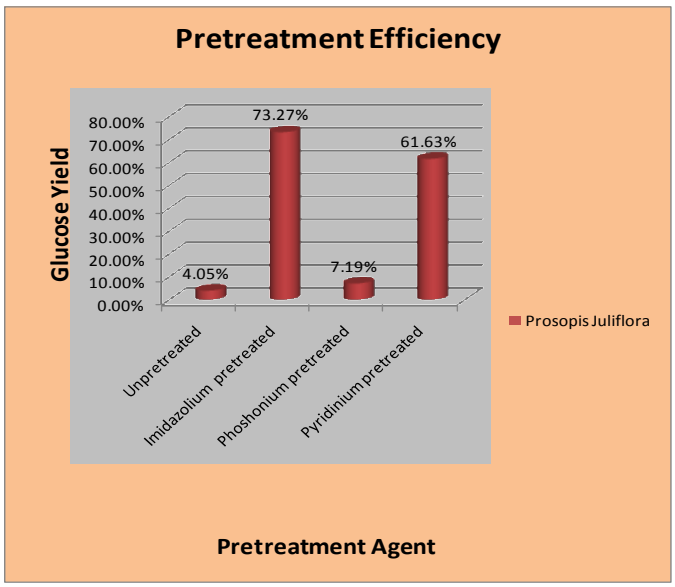

Figure-3: Comparison of Ionic Liquids Cations in terms of Glucose Extraction Efficiency

4 CONCLUSION

Prosopis Juliflora stem contains a substantial amount of cellulose in its composition (over 25%); 34% 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 that had been pretreated with ILs prior to hydrolysis than those that were unpretreated. An increase in glucose yields of 1.78 times when phosphonium IL was used (from 4.05% unpretreated to 7.19%), 15.21 times when pyridinium IL was used (from 4.05% unpretreated to 61.63%), and 18.1 times when imidazolium IL is used (from 4.05% unpretreated to 73.27%) 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°C and a duration of 120 minutes. 4MBPCL recorded highest glucose yield at 140°Cafter pretreating the biomass for 160 minutes whereas [P66614]Cl yielded highest glucose content at a temperature of 120°C 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 an equally good glucose extraction efficiency 61.63% as Imidazolium ionic liquid 73.27%. It can therefore be used as a suitable substitute of the commonly studied Imidazolium ionic liquid.

ACKNOWLEDGEMENT

This work was supported by research grants from the National Commission for Science, Technology and Innovation (NACOSTI), and Moi University, Kenya.

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