

Use of Protic Ionic Liquids as Biomass Pretreatment for Lignocellulosic Ethanol Production

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Although sugar cane bagasse is considered as a promising material for the production of second-generation ethanol, obtained through enzymatic hydrolysis and subsequent fermentation, the strong recalcitrance of its internal structures increases the production costs of this biofuel, requiring the application of pre-treatment and technological routes for its processing, sometimes unfeasible technically and economically. More recently, ionic liquids have been studied as an environmentally friendly alternative. Although the majority of the published works consider aprotic ionic liquids, the interest in protic ionic liquids has grown in recent years, especially due to its capacity to establish strong hydrogen bonds. Moreover, the functionality of the mobile nature of the proton H^+ makes these materials potential candidates for conducting protons for use in fuel cells and several other applications. In this work, the protic ionic liquid bis(2-hidroxyethylammonium) acetate, 2-HE2AA, was produced and purified and then evaluated as a pre-treatment agent for sugarcane bagasse. The pre-treated material was enzymatically hydrolyzed for subsequent ethanol fermentation. The expectation is that a high yield be achieved in second-generation ethanol production, so that it can provide scientific and economic basis for a biomass conversion integrated and environmentally friendly process. Preliminary results indicated a yield of 84% in the pre-treatment step. The enzymatic saccharification showed a yield of 77% and a glucose conversion efficiency of 35% after 72 h.

1. Introduction

The growing need to expand in a sustainable manner using renewable energy sources (Qiu et al., 2012), providing greater security to energy supply and reducing the environmental impacts associated with fossil fuels (Wang et al., 2011), finds an alternative economically viable (Zhu et al., 2012) and with significant expansion potential in the production of bioethanol from the agricultural waste (Li et al., 2010).

The production of biofuels from lignocellulosic materials requires the transformation of cellulose into glucose monomers and subsequent conversion thereof, by micro-organisms, in ethanol. However, the native cellulose is very protected by lignin-carbohydrate matrix due to the strong existing connections (D'Almeida, 1988), and so the cellulose becomes highly recalcitrant (Santos et al., 2011). In order to improve the ethanol yield, a general trend has been to introduce a pre-treatment in the process to facilitate hydrolysis and achieve high rates of saccharification (Figure 1), low formation of undesirable by-products and high conversion rates of the polysaccharide portions (cellulose and hemicellulose) into their monomeric units (pentoses and hexoses) (Soares and Rossell, 2007).

In recent years, various types of pre-treatments were investigated, including dilute acid (Gomez et al., 2010), steam explosion (Ramos, 2003), hot water, organic solvent, alkaline hydrogen peroxide, but all had their own disadvantages for large-scale applications; some of these methods were not selective and often produce undesirable byproducts that inhibit fermentation and conversion of monosaccharides into ethanol and other products (Wang et al., 2011). More recently, ionic liquids have come to be considered as an environmentally friendly alternative (Bose et al., 2010). These ionic solvents with melting point below 373.15 K, are salts formed by organic or inorganic cations and anions, which allows adjustments of their physicochemical properties through the choice of these ions. These materials form biphasic systems that allow the product separation and recovery of the solvent with low power consumption. Such properties make these solvents

appropriate and with potential ability to replace most of the common organic solvents used by the chemical industries (Pinto, 2012).

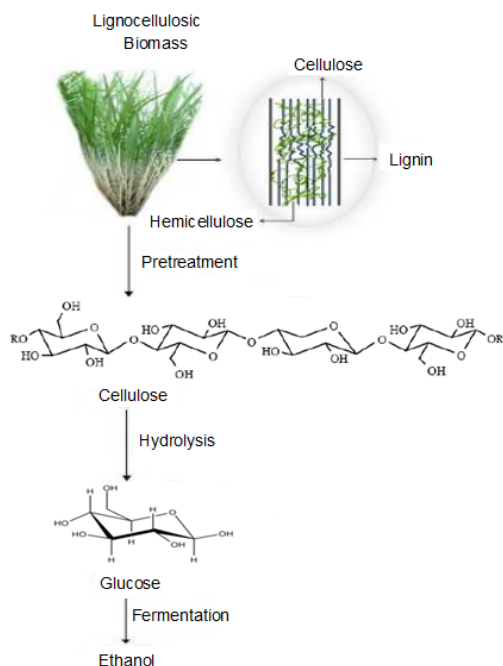


Figure 1. Simplified scheme of the production of ethanol from lignocellulosic biomass (Soares, 2013)

Some ionic liquids usually used in chemical processes are 1-butyl-3-methylimidazolium chloride ([bmim][Cl]) (Swatloski et al., 2002; Lee et al., 2009; Holm et al., 2012) and 1-ethyl-3-methylimidazolium acetate ([emim][Ac]) (Tan et al., 2009; Li et al., 2010; Fu and Mazza, 2011). Although ionic liquid applications are still dominated by aprotic ionic liquids, the interest in protic ionic liquids has grown in recent years, especially due to their capacity to establish strong hydrogen bonds. Furthermore, the functionality of the mobile nature of these H^+ of these ionic liquids makes them potential candidates to various applications (Clare et al., 2009; Mirjafari et al. 2012).

The main focus of this work is to investigate the efficiency of the use of protic ionic liquids as a pretreatment technology from sugarcane bagasse, in order to offer a technical and economic feasibility study for the production of second generation ethanol.

2. Materials and Methods

The sugarcane bagasse was ceded by Usina São João (USJ Group), located in Araras-SP (Brazil). The material was from mechanical harvesting and extraction by diffusion 2012/2013 harvest. The protic ionic liquid 2-HE2AA, highly viscous at room temperature, was produced by using equivalent molar ratio (7:7) of glacial acetic acid (60.05 g/mol, 99.8%) and diethanolamine (105.14 g/mol, 99%), and its synthesis was based on the procedures of Álvarez et al. (2012). Purification and characterization of the ionic liquids followed the methodology used by Pinto (2012).

The chemical characterization of the biomass followed the procedures used by Rabelo (2010), which was based on international norms from TAPPI (Technical Association of the Pulp and Paper Industry) and NREL (National Renewable Energy Laboratory) and the specific requirements for analysis of sugarcane bagasse proposed by Gouveia et al. (2009).

The bagasse was subjected to an initial milling process, followed by sieving, in order to obtain particles lesser than 5 mm, according to the methodology adopted by Machado et al. (2013) The pre-treatment was based on the methods described by Marabezi (2008), Li et al. (2010), Sun (2010), Wang et al. (2011), Zhu et al. (2012), Holm et al. (2012) and Vancov et al. (2012). The pre-treatment conditions were 4 hours of reaction at 373.15 K and 5% solids; a scheme is shown in Figure 2.

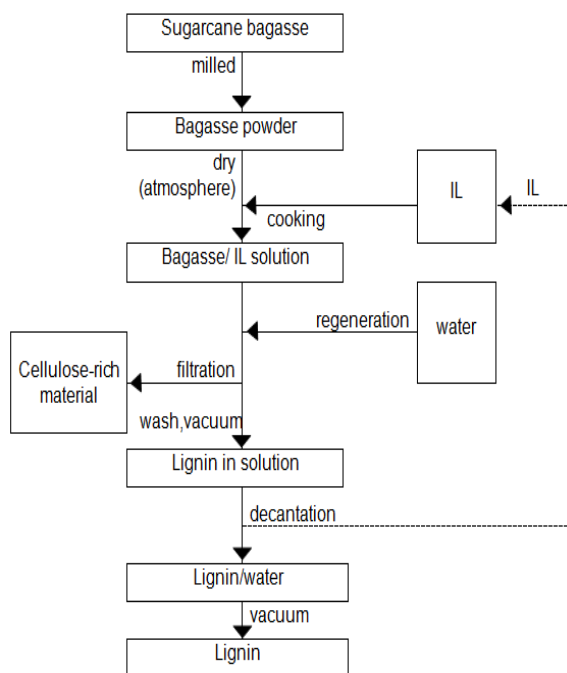


Figure 2. Flowchart for the process of dissolution and regeneration of cellulose of the sugarcane bagasse in ionic liquid

The enzymatic hydrolysis was based on the study by Rabelo (2010). The pretreated biomass (cellulose) had 12% moisture. Seven grams of this material (dry basis, particles lesser than 5 mm) were used and the enzymatic hydrolysis was carried out in a shaker at 100 rpm at 323.15 K (Marconi MA-832), with an enzyme load of 15 FPU/g dry biomass of cellulase and 25 CBU/g dry biomass of β -glucosidase. The activities of cellulase and β -glucosidase were determined as recommended by IUPAC (Ghose, 1987; Wood and Bhat, 1988; Adney and Baker, 1996). Samples of the reaction liquid hydrolysis (about 2 mL) were collected at 0, 1, 2, 6, 12, 24, 48, and 72 h and then boiled for 15 minutes in tubes identified to inactivate the enzymes. Analyses were performed in duplicate.

The methodologies used in the analysis of the main components of the lignocellulosic residues and those resulting from steps of synthesis and pretreatment consisted in the determination of the extractives by Soxhlet (Marconi MA-188), ash (furnace Quimis Q-318), protein, carbohydrate, bagasse and inhibitor concentration by HPLC (Agilent 1260 Infinity). For ionic liquids, purity and water content were analyzed; the analytical techniques included density (Anton Paar DMA 5000, $\pm 0,005 \text{ kg.m}^{-3}$), viscosity (Anton Paar AMVn, $0,04 \text{ mm}^2/\text{s}$ at 293.2 K), water content (Karl Fischer titrator Mettler Toledo DL31, ± 0.005 mass fraction), UV/VIS (Mettler Toledo RE40D, $\pm 0,0001$), IR (Thermo Scientific FTIR 670, 2 nm) and NMR (Varian Inova 500). In the analysis of the enzymatic activity of β -glucosidase, glucose concentration was determined according to the GOD-PAP glucose enzymatic method described by Henry et al. (1974).

In the future stages of this work, changes will be made in the process, which aims to assess the influence of variables such as the pre-treatment temperature, the type of cation and the chain size of the anion of the protic ionic liquids on the solubilization of lignin. Tests will be performed in the range of 353.15 to 433.15 K and other protic ionic liquids will be also analyzed.

3. Results

The analysis of the sugarcane bagasse is presented in Table 1. The characterization of the ionic liquid is presented in Table 2 and Figures 3 and 4. The pre-treatment yield was 84%. Figure 5 shows the cellulose digestibility profile for ionic liquid 2-HE2AA pre-treated sugarcane bagasse. The enzymatic hydrolysis showed a yield of 77% with a glucose conversion efficiency of 35%.

Table 1. Chemical composition of untreated bagasse

sugarcane bagasse composition <i>in natura</i>	exp. (g)	lit. (g)*
ash	0,82 ± 0,02	0,60 ± 0,30
extractives	9,51 ± 0,21	1,60 ± 0,10
hemicellulose	17,58 ± 0,38	24,50 ± 0,40
lignin	28,95 ± 0,63	26,40 ± 0,50
cellulose	43,13 ± 0,94	40,50 ± 0,60
total	100 ± 2,19	93,60 ± 0,50

*Rabelo (2010)

Table 2. Characterization of ionic liquid 2-HE2AA at 323.15 K

property	units	value
specific gravity	g/cm ³	1,16165
dynamic viscosity	Poise	46,451
viscosity	cSt	39,987
water content	%	0,3945
refractive Index	-	1,4754

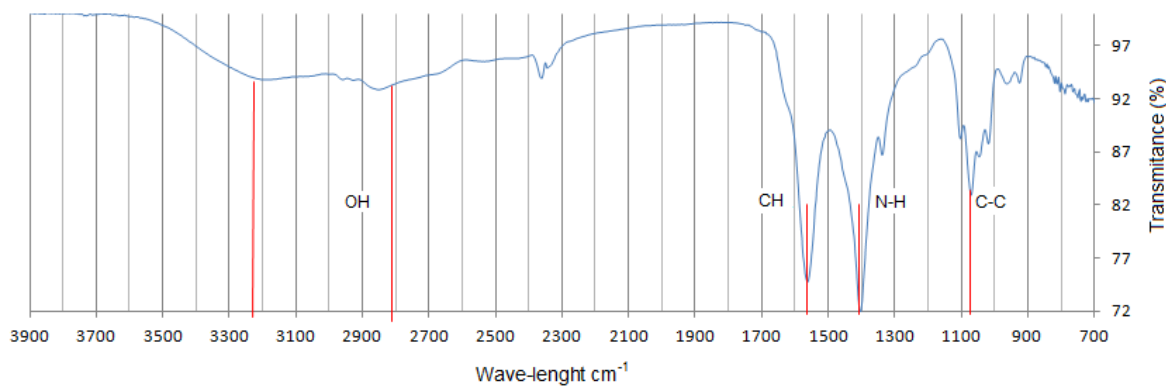
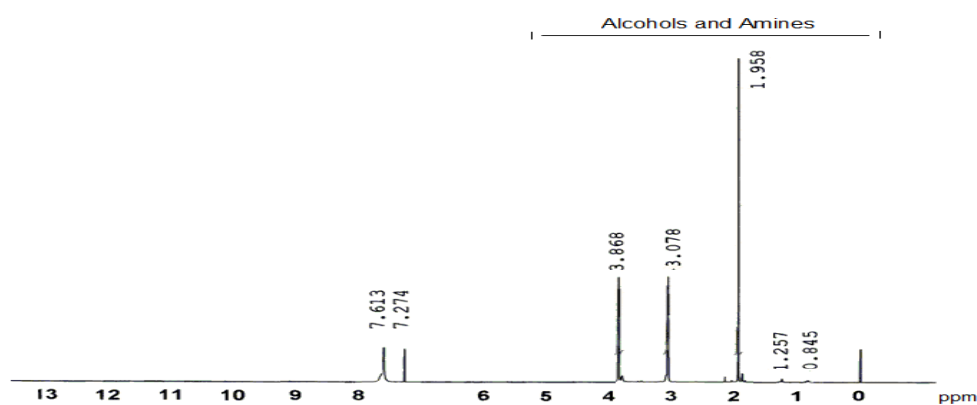


Figure 3. FTIR of ionic liquid 2-HE2AA

Figure 4. ¹H NMR of ionic liquid 2- HE2AA

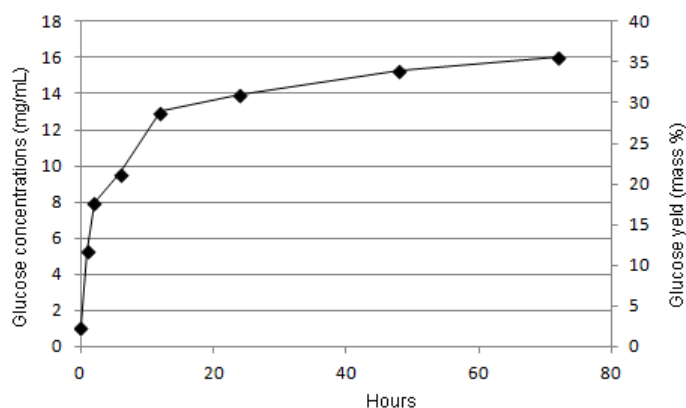


Figure 5. Efficiency of enzymatic hydrolysis of the pretreated bagasse with 2-HE2AA

4. Discussion and Conclusion

The formation of the salt in the synthesis of the ionic liquid can be confirmed by FTIR analysis, based on studies of Iglesias et al. (2010) and Oliveira (2012). The broad absorption band in the spectrum of the ionic liquid, in the range of $3500\text{--}2500\text{ cm}^{-1}$, is characteristic of the ammonia structure for all the products of the neutralization; the stretching vibration of OH is incorporated in this band and the compound can be an alcohol (confirming the results obtained by NMR), phenol or carboxylic acid. The band in the range $1580\text{--}1495\text{ cm}^{-1}$ can be assigned to the carbonyl stretching and the vibration of the bending in NH structure, confirming the presence of the amine group (NH_3). The range of $1700\text{--}1200\text{ cm}^{-1}$ corresponds to symmetric vibration of the stretching of the C-H bond and the band $1260\text{--}700\text{ cm}^{-1}$ shows vibrations of C-C structure. The region between $2300\text{--}2400\text{ cm}^{-1}$ can represent the interference of carbon dioxide during the analysis.

The preliminary results show that the pretreatment with the protic ionic liquid 2-HE2AA enabled the conversion of cellulose into glucose by enzymatic hydrolysis. Although the conversion of 35% is considered low, further studies varying pre-treatment time, temperature and solids concentration will be performed; and other ionic liquids will also be evaluated.

In the future stages of this work, an economical assessment of the use of protic ionic liquids as a pretreatment of sugarcane bagasse will be made. The recycling of the ionic liquid will reduce operating costs, maintaining the efficiency of the treatment. The recovery and characterization of the lignine fraction (energy-sustainability balance) will also reduce costs, since this residue will become feedstock for power supply.

Acknowledgements

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