



Pretreatment of energy cane bagasse with recycled ionic liquid for enzymatic hydrolysis



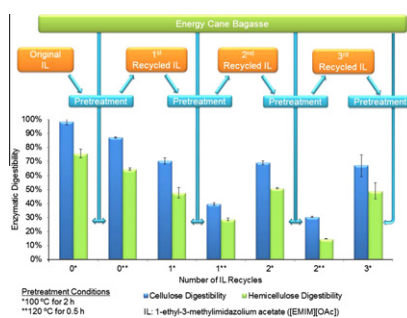
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HIGHLIGHTS

- ▶ Recyclable [EMIM][OAc] is effective on energy cane bagasse pretreatment.
- ▶ Enzymatic digestibility decreased as the number of IL recycles increased.
- ▶ Recycled IL pretreatment efficiency improved with adequate temperature and time.

GRAPHICAL ABSTRACT



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ABSTRACT

A previous study revealed that energy cane bagasse (ECB) pretreated with ionic liquid (IL), 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]), exhibited significantly higher enzymatic digestibility than untreated or water-treated ECB due to delignification and reduction of cellulose crystallinity. This study evaluated the effect of multiple recycled IL on the pretreatment of ECB for enzymatic hydrolysis. ECB was pretreated with [EMIM][OAc] (5% (w/w)) at 100 °C or 120 °C for 0.5 h upto 4 h followed by hydrolysis with commercially available enzymes. The post-pretreatment IL-containing liquid was evaporated at 100 °C for 12 h to remove water and then reused during pretreatment without any further purification. The enzymatic digestibility decreased as the number of pretreatment recycles increased. Decreasing pretreatment temperatures from 120 °C to 100 °C and extending the residence times from 0.5 h to 2 h brought significant improvement to the pretreatment efficiency of recycled [EMIM][OAc] on ECB.

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1. Introduction

Lignocellulosic biomass appears to be a prospective renewable energy resource that can be used for the generation of biofuels and bioproducts. A jointed study supported by the US Department of Energy (DOE) and the US Department of Agriculture (USDA) indicated that the land resources in the United States are sufficient to sustain production of over 1.3 billion dry tons of biomass annually, which could be available for large-scale bioenergy and biorefinery industries by mid-21st century while still meeting

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demand for forestry products, food and fiber (Perlack et al., 2005). Crop residues (sugarcane bagasse, corn stover, rice straw, wheat straw, sorghum bagasse), hardwood (black locust, poplar, eucalyptus), softwood (pine, spruce), herbaceous biomass (switchgrass, Bermuda grass), cellulose waste, and municipal solid wastes are some traditional potential lignocellulosic biomass resources for biofuels production (Aita and Kim, 2010). Energy cane, a hybrid of commercial and wild sugarcanes, is a relatively new and ideal lignocellulose resource. Compared to sugarcane, energy cane has higher fiber content, better cold tolerance, less fertilizer and water input requirements, and longer replanting time (Kim and Day, 2011; Sierra et al., 2008).

Lignocellulose is composed mainly of cellulose, hemicellulose and lignin. The cellulose chain is made up of glucose units joined together by β -1,4 glycosidic bonds. Individual cellulose chains are

held together by strong hydrogen bonds and van der Waals forces, which make cellulose a highly crystalline polymer (Dadi et al., 2007). Hemicellulose, a polymer of five carbon sugars, is relatively amorphous and it is readily degraded by glycosidases (Lee et al., 2009); however, the xylan layer with its covalent linkage to lignin and its non-covalent interaction with cellulose may play a role in preventing enzymatic degradation (Beg et al., 2001). Lignin is a highly branched and aromatic polymer, which consists mainly of ether linked phenylpropanoid units, and it serves as the “glue” that binds cellulose and hemicellulose, giving both rigidity and resistance to the lignocellulosic structure (Aita and Kim, 2010; Lee et al., 2009). The complex structure makes lignocellulose biomass highly resistant to enzymatic hydrolysis, which results in low sugar yields (Yang and Wyman, 2008).

Pretreatment is an essential step for overcoming the recalcitrance of lignocellulose, as it reduces the lignin content, breaks the carbohydrate-lignin complex and disrupts the crystalline structure of cellulose (Hendriks and Zeeman, 2009; Tan and Lee, 2012). Numerous methods have been developed to pretreat lignocellulosic biomass. They can be classified into several categories: (1) biological (e.g., fungi degradation), (2) mechanical (e.g., milling, grinding), (3) physicochemical (e.g., autohydrolysis, liquid hot water, steam, supercritical fluids, steam explosion), and (4) chemical (e.g., alkali, acid, oxidizing agents, organic solvent) (Aita and Kim, 2010; Liu et al., 2012; Zhao et al., 2009). However, several drawbacks are found with each of these methods. Biological methods have excessive residence times, mechanical methods suffer from intensive energy and capital costs, physicochemical methods require specialized equipment that can stand high pressures and high temperatures, and chemical methods have cost, safety and environmental issues (Aita and Kim, 2010; Hendriks and Zeeman, 2009; Shill et al., 2011; Zhao et al., 2009). Therefore, the development of alternative, cost-effective and energy efficient pretreatment processes are needed.

Ionic liquids (ILs) are promising solvents for the pretreatment of lignocellulose as they exhibit excellent physical and chemical characteristics that include thermal stability, non-toxicity, good recyclability, low volatility, and are environmentally friendly (Gremos et al., 2011; Lee and Lee, 2005). In our previous work, [EMIM][OAc]-treated energy cane bagasse resulted in significant lignin removal (32.1%) with slight glucan and xylan losses (8.8% and 14.0%, respectively), and exhibited a much higher enzymatic digestibility (87.0%, 64.3%) than untreated (5.5%, 2.8%) or water-treated (4.0%, 2.1%) energy cane bagasse in terms of both cellulose and hemicellulose digestibilities, respectively (Qiu et al., 2012). The enhanced digestibilities of IL-treated energy cane bagasse were attributed to delignification and reduction of cellulose crystallinity as confirmed by FTIR and XRD analysis (Qiu et al., 2012). Although [EMIM][OAc] is highly effective on the pretreatment of energy cane bagasse, the relatively high cost of [EMIM][OAc] as well as other ILs is a major disadvantage. Therefore, recycling of ILs post pretreatment will aid in lowering processing costs for future commercial application.

This study aimed to assess the effect of recycled [EMIM][OAc] on the pretreatment of energy cane bagasse in terms of its chemical composition and enzymatic hydrolysis.

2. Methods

2.1. Biomass

Energy cane (L79-1002) was harvested at the Louisiana State University Agricultural Center Sugar Research Station located in St. Gabriel, LA. Leaves and roots were removed and the stalks were

crushed in a roller press (Farrel Company, Ansonia, CT) three times to extract the juice. The remaining crushed fibers (bagasse) were stored at -20°C .

2.2. Ionic liquid pretreatment and ionic liquid recycle

Ionic liquid 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]) (Sigma-Aldrich, Inc., St. Louis, MO) was mixed with biomass at a 20:1 ratio and heated to 120°C for 0.5 h or at 100°C for 0.5 h, 1 h, 2 h, and 4 h, among which 120°C was the pretreatment used in our previous study (Qiu et al., 2012). The other pretreatment conditions were selected based on results published by Lee et al. (2009), Li et al. (2010a) and Nguyen et al. (2010).

Post-pretreatment, deionized water was added to the IL solution at a 5:1 ratio to recover the biomass. The ionic liquid/water mixture and biomass were separated by vacuum filtration. The solids were washed repeatedly with deionized water to remove any remaining IL from the samples until the wash solution appeared colorless and solids were collected. The filtrate was evaporated at 100°C for 12 h in a drying oven to remove excess water, and then reused to pretreat energy cane bagasse without any further purification. Approximately, 85–90% of IL was recovered on each recycle.

Based on the yields obtained from the enzymatic hydrolysis studies, pretreatment conditions at 120°C for 0.5 h and 100°C for 2 h were selected for assessing the efficiency of recycled IL pretreatment. The ionic liquid/solid mixture separation and ionic liquid recovery were accomplished as described above. A total of two (120°C for 0.5 h) or three (100°C for 2 h) IL recycles post pretreatment were evaluated. Studies were carried out in duplicate.

2.3. Chemical composition of energy cane bagasse

All ionic liquid-treated energy cane bagasse samples were analyzed for glucan, xylan, arabinan, mannan, and lignin following Laboratory Analytical Procedures (LAP TP-510-42618, 42619, 42622) as documented by the National Renewable Energy Laboratory (NREL). NREL reference material (8491 sugarcane bagasse) was analyzed as an internal standard to ensure the accuracy of the procedures. Percent lignin removal, glucan recovery and xylan recovery were calculated as described below:

$$\text{Percent lignin removal} = \frac{1 - \% \text{ Lignin in treated biomass} \times \% \text{ Recovered solids}}{\% \text{ Lignin in untreated biomass}}$$

$$\text{Percent glucan recovery} = \frac{\% \text{ Glucan in treated biomass} \times \% \text{ Recovered solids}}{\% \text{ Glucan in untreated biomass}}$$

$$\text{Percent xylan recovery} = \frac{\% \text{ Xylan in treated biomass} \times \% \text{ Recovered solids}}{\% \text{ Xylan in untreated biomass}}$$

2.4. Enzymatic hydrolysis

A combination of two commercially available enzymes, Spezyme CP (Genencor, Danisco US Inc., Rochester, NY,) and Novozyme 188 (Sigma-Aldrich, Inc., St. Louis, MO), were used for the hydrolysis studies. Enzymatic hydrolysis was measured by following NREL's LAP TP-510-43629. Briefly, hydrolysis was carried out with 1% (w/v) substrate at 50°C , in 0.1 M sodium citrate buffer at pH 4.8 in a shaker incubator (Amerex Instruments Inc., Lafayette, CA) at 150 rpm. The substrates were hydrolyzed with Spezyme CP at 30 FPU/g glucan and Novozyme 188 at 30 CBU/g glucan. Samples were taken at 0 h (before the addition of enzymes), 24 h, 48 h and 72 h. Experiments were run in duplicate.

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