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The use of ionic liquid for the pretreatment of energy cane bagasse

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THE USE OF IONIC LIQUID FOR THE PRETREATMENT OF ENERGY CANE BAGASSE

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DEDICATION

♥

To my beloved parents

Ms. Lilin Zeng and Mr. Faqi Qiu

without whom

I would not have come so far...

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ABSTRACT

Lignocellulosic biomass appears to be a prospective renewable resource that can be used for the generation of biofuels and bioproducts. The major concern in lignocellulose conversion is overcoming biomass recalcitrance through pretreatment while still maintaining a green, energy efficient and cost-effective process. Energy cane is a promising energy crop with high fiber content, cold tolerance, and less fertilizer and water input requirements as compared to conventional sugarcane. Ionic liquids (ILs) are promising solvents for the pretreatment of lignocellulose as they are thermally stable, environmentally friendly, recyclable, and have low volatility. This study assessed the use of ionic liquid 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]) as a solvent during the pretreatment of energy cane bagasse (ECB) and its effect on the chemical composition, surface morphology, cellulose crystallinity, and enzymatic hydrolysis of the pretreated biomass.

IL-treated ECB resulted in significant lignin removal (32.1%) with slight glucan and xylan losses (8.8% and 14.0%, respectively), and exhibited much higher cellulose and hemicellulose enzymatic digestibilities (87.0%, 64.3%) than untreated (5.5%, 2.8%) or water-treated (4.0%, 2.1%) ECB, respectively. The enhanced digestibilities of IL-treated biomass can be attributed to delignification and reduction of cellulose crystallinity as confirmed by FTIR and XRD analysis. When pretreating ECB with recycled IL, enzymatic digestibility decreased as the number of pretreatment recycles increased. Decreasing the pretreatment temperature from 120 ℃ to 100 ℃ and extending the residence time from 30 min to 2 h brought significant improvement to the pretreatment efficiency of recycled [EMIM][OAc] on ECB. However, response surface methodology model indicated that a higher glucose yield of IL-treated biomass could be obtained at higher pretreatment temperatures with shorter residence times. The optimal

processing conditions were pretreatment of ECB at 131.9 ℃ for 28.1 min at 8.4% solids loading resulting in a final glucose yield of 35.96 g glucose per 100 g of native biomass.

The results presented in this thesis demonstrated that [EMIM][OAc] can be used as a potential solvent for the pretreatment of lignocellulosic biomass such as ECB. Furthermore, the sugar yields obtained post pretreatment have great potential as building blocks in the production of renewable fuels and chemicals.

CHAPTER 1

INTRODUCTION

1.1 Lignocellulosic Biomass

Renewable fuels and chemicals have gained increased interest worldwide due to growing concerns on energy consumption, depletion of fossil fuels, and increasing greenhouse gas emissions (Liu et al., 2012). Current production of bioethanol (first generation biofuels) relies on the use of sugars from food crops (Ajanovic, 2011). The major crops for biofuels are corn, wheat, barley, sugarcane, rapeseed, soybean, and sunflower which are all directly or indirectly used in food production (Sims et al., 2010). The sustainable and economic production of first generation fuels has, however, come under close scrutiny in the last decade attributed in most part to the competition for limited land and water used for food and fiber production (Alvira et al., 2010). Mueller et al. (2011) claimed that biofuel production contributed to 3-30% increase in commodity food prices from 2007 to 2008. Boddiger (2007) reported that the number of food-insecure people would rise by over 16 million for every percentage increase in the real prices of staple foods, raising the total number of chronically hungry to 1.2 billion by 2025. The recently identified limitations of first generation biofuels have caused great emphasis on second generation biofuels produced from lignocellulosic biomass or biomass not use for human consumption (Sims et al., 2010).

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 U. S. Department of Energy (DOE) and the U. S. 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 demand for forestry products, food and fiber (Perlack et al., 2005). A 30 percent replacement of the current U.S. petroleum consumption with biofuels by 2030 was also envisioned in this study. 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 lignocellulosic biomass sources with potential for biofuels production (Aita and Kim, 2010).

Energy cane, a hybrid of commercial and wild sugarcanes, is another ideal energy crop and lignocellulose resource, which is bred for high fiber content and low sucrose (Kim and Day, 2011). Unlike sugarcane, energy cane is more cold tolerant, requires less fertilizer and water input, and requires replanting only every ten years, as compared to every three years for sugarcane (Sierra et al., 2008). A non-commercial energy cane variety, L79-1002, developed in collaboration with the U. S. Department of Agriculture-Agricultural Research Service (USDA-ARS) in Houma, LA and the Louisiana State University Agricultural Center Sugar Research Station in St. Gabriel, LA has an average fiber content of 257 g/kg (dry basis) and cane yield of 83.3 Mg/ha (dry basis) (Bischoff et al., 2008) as compared to LCP 85-384, the predominant commercial variety of sugarcane grown in Louisiana, with a fiber content of 117 g/kg (dry basis) and cane yield of 59.2 Mg/ha (dry basis) (Gravois et al., 2009).

Lignocellulosic biomass including energy cane bagasse is not readily available for bioconversion into biofuels and bioproducts due to its recalcitrant structure. Lignocellulose is

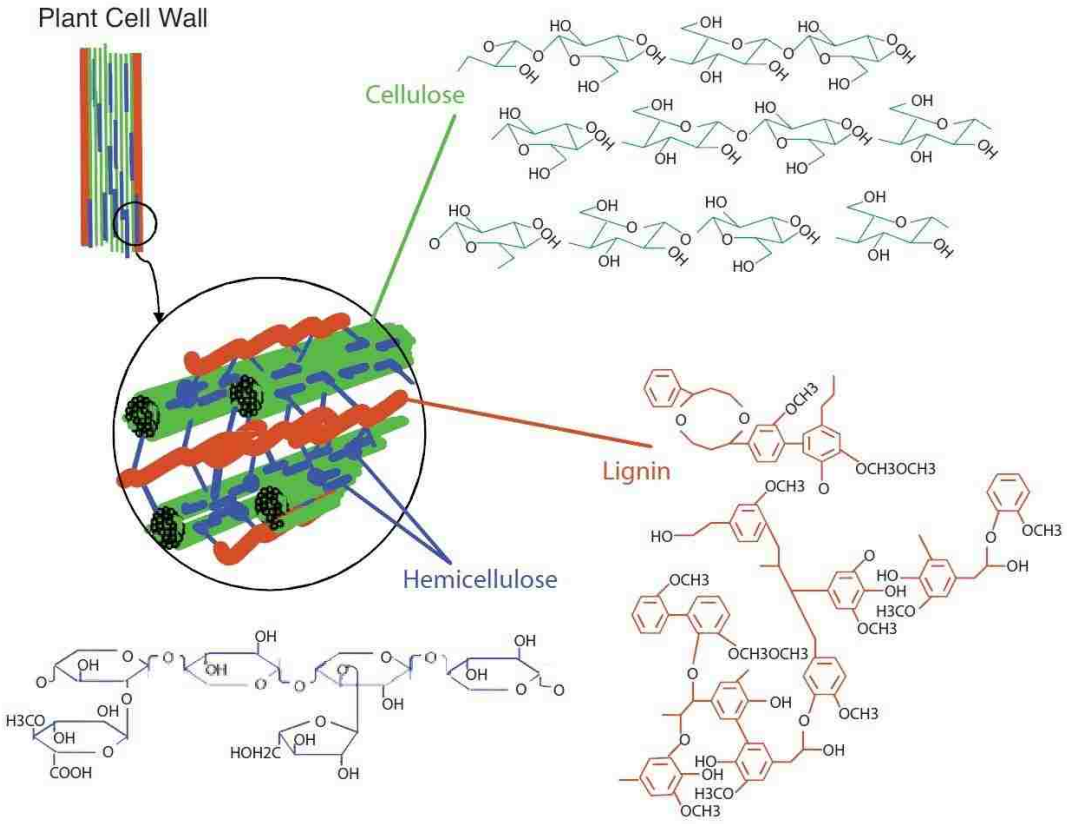

Fig. 1.1. Chemical Composition of Lignocellulosic Biomass (Macrae et al., 1993).

composed mainly of cellulose, hemicellulose and lignin as shown in Fig. 1.1. A cellulose chain is made up of glucose units joined together by β-1,4 glycosidic bonds (Aita and Kim, 2010). 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). The crystalline structure makes cellulose water insoluble and highly resistant to chemical and biological degradation (Mosier et al., 2005). 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 close association and complexity of the carbohydrates-lignin complex which results in low enzymatic accessibility is the main obstacle in lignocellulosics degradation (Lee et al., 2009; Zhu et al., 2008).

1.2 Pretreatment and Enzymatic Hydrolysis

Four major steps are needed to convert lignocellulosic biomass to bioethanol and can be summarized as follow: (1) pretreatment to breakdown the carbohydrates-lignin complex and enhance the access of enzymes to sugar polymers, (2) enzymatic hydrolysis to break down the cellulose and hemicellulose structures into their corresponding monosaccharides, (3) fermentation of monomeric sugars into ethanol by yeast or bacteria, and (4) distillation of ethanol (Hu et al., 2008). Pretreatment aims at breaking the lignin structure and disrupting the crystalline structure of cellulose to make cellulose and hemicellulose available for enzymatic hydrolysis (Fig. 1.2).

Cellulose is hydrolyzed by cellulases after pretreatment. The cellulases are a mixture of endo-1, 4-β-glucanase, exo-1, 4- β-glucanase, and β-glucosidase, among which endo-1, 4-β-glucanase randomly cleaves internal bonds in the amorphous structure of cellulose, exo-1, 4- β-glucanase removes tetrasaccharides or disaccharides from the non-reducing ends of cellulose chains, and β-glucosidase breaks down the tetrasaccharides and disaccharides into glucose (Aita and Kim, 2010; Lynd et al., 2002). Three steps take place during enzymatic hydrolysis of cellulose: (1) cellulases attach to the surface of cellulose, (2) cellulases break down cellulose to glucose, and (3) cellulases detach from the surface of cellulose (Sun and Cheng, 2002).

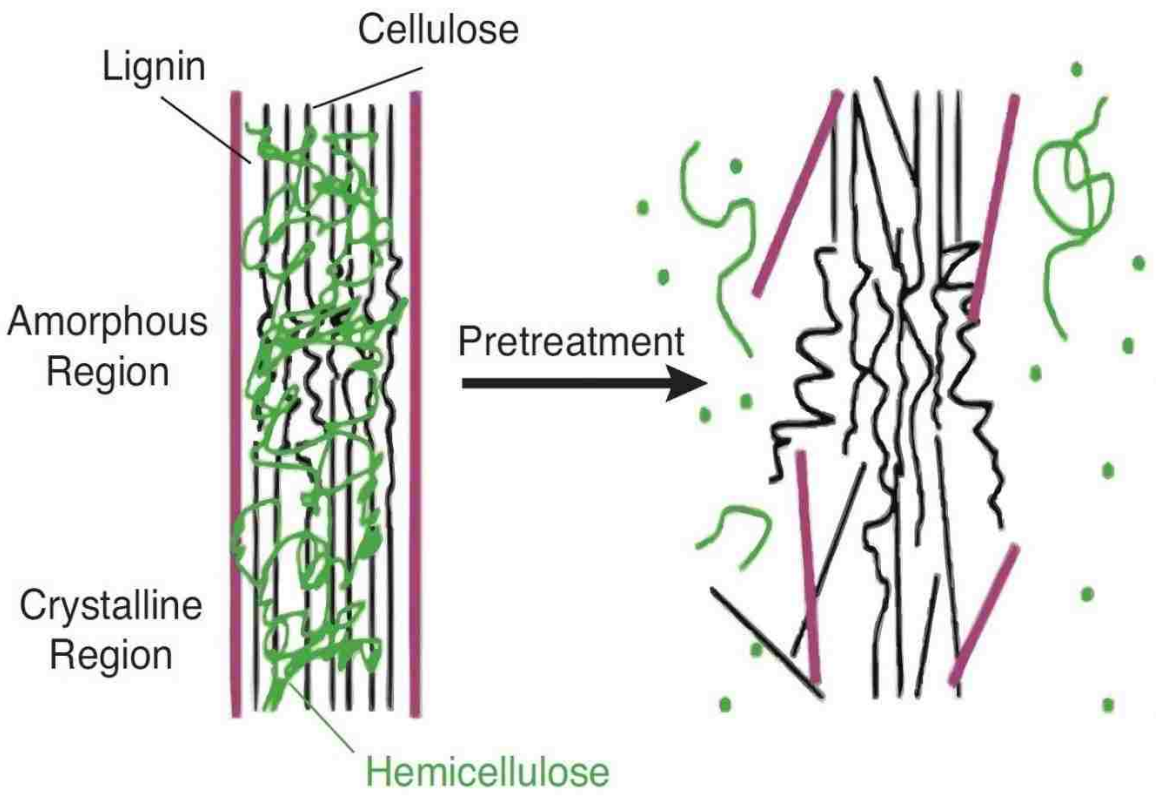

Fig. 1.2. Effect of Pretreatment on Lignocellulose (Mosier et al., 2005).

A promising pretreatment (1) minimizes the loss of cellulose and hemicellulose during pretreatment, (2) reduces the production of compounds that are inhibitory to both enzymes during enzymatic hydrolysis and microorganisms during fermentation, (3) increases sugar yields after enzymatic hydrolysis, (4) requires less energy input, and (5) minimizes capital and operating costs (Aita and Kim, 2010; Sierra et al., 2008). Pretreatment methods can be classified into biological, mechanical, physicochemical, and chemical processes (Aita and Kim, 2010; Brodeur et al., 2011; Hendriks and Zeeman, 2009; Liu et al., 2012; Shill et al., 2011; Sierra et al., 2008; Zhao et al., 2009). Specifically, biological pretreatments (e.g., fungi degradation) are carried out by aerobic fungi such as white rot, brown rot and soft rot. Aerobic fungi make

lignocellulose more digestible by attacking both cellulose and/or lignin, but this process generally requires excessive residence time of 10-14 days. Mechanical pretreatments (e.g., milling, grinding) break down lignocellulosic biomass into small particles by milling, grinding or chopping, which results in increase of specific surface area, decrease of the degree of polymerization and the crystallinity of cellulose, and, consequently, improvement of hydrolysis yields. However, intensive energy and capital cost is the main drawback. Physicochemical pretreatments (e.g., steam explosion, liquid hot water, supercritical fluids) are effective and promising methods to pretreat lignocellulosic biomass. Generally, they are cost-effective and result in high hydrolysis yields, but specialized equipment which can withstand high pressures and high temperature is needed. Moreover, steam explosion produces toxic compounds during pretreatment. Chemical pretreatments (e.g., alkali, acid, oxidizing agents, organic solvent, ionic liquids) have been shown to be highly effective in lignin removal, reducing cellulose crystallinity and improving hydrolysis yields. Some drawbacks include corrosion of equipment, release of toxic pollutants, and the high cost of catalysts and solvents. Therefore, the major concern in lignocellulose conversion is overcoming biomass recalcitrance through pretreatment while still maintaining a green, energy efficient and cost-effective process (Lee et al., 2009).

1.3 Ionic Liquid and Ionic Liquid Pretreatment

Ionic liquids (ILs) are a group of new organic salts that exist as liquids at relative low temperature (usually below 100 ℃). ILs exhibit excellent physical characteristics including the ability to dissolve polar and non-polar organic, inorganic and polymeric compounds (Lee and Lee, 2005). They are generally considered as green solvents which can be potential substitutes for traditional flammable and volatile solvents due to their desirable properties which include

low volatility, high thermal stability, non-flammability, and good recyclability (Quijano et al., 2010). Physical properties of ILs such as melting points, viscosity, hydrophobicity, and hydrolysis stability depend on both the type of cation/anion pair and the alkyl chain of the anion (Huddleston et al., 2001). Therefore, the physical properties of ILs can be adjusted by changing the structure of either the cation or the anion, or both to meet a special application. The main cations and anions commonly used for the synthesis of ILs are shown in Fig. 1.3. Mäki-Arvela et al. (2010) reported that physicochemical properties of ILs (i.e., viscosity, melting point, dipolarity, and hydrogen bond basicity) have significant effects on pretreatment of lignocellulosic biomass.

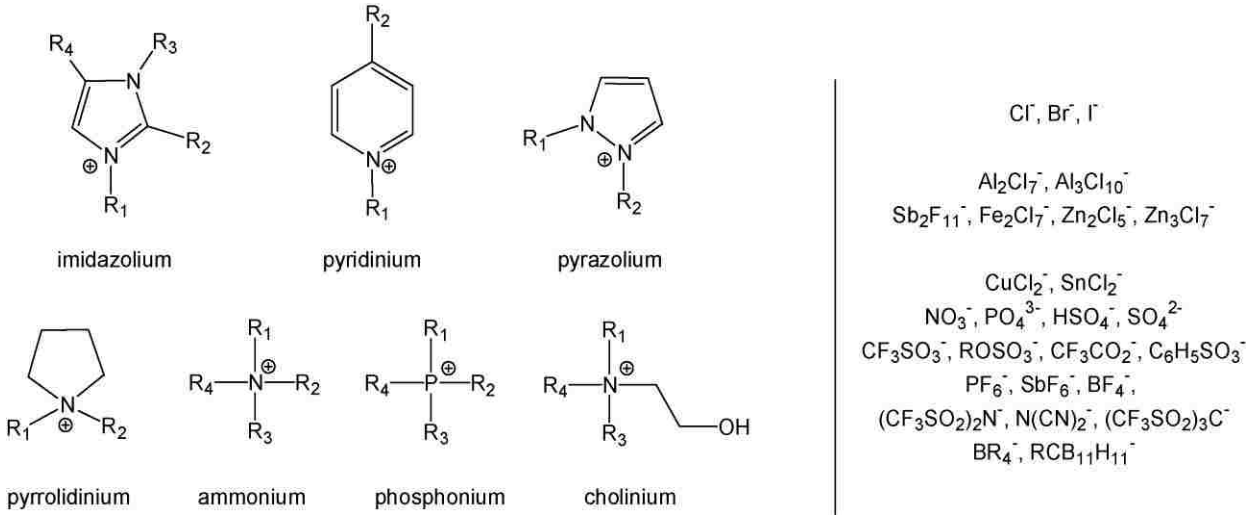

Fig. 1.3. Main Cations and Anions Present in Ionic Liquids (Olivier-Bourbigou et al., 2010).

Pretreatment with ILs can reduce the crystallinity of cellulose and partially remove hemicellulose and lignin while not generating degradation products which are inhibitory to enzymes or fermenting microorganisms (Dadi et al., 2007; Lee et al., 2009). Meanwhile, ILs

pretreatments are less energy demanding, easier to handle and more environmentally friendly than other pretreatment methods such as mechanical milling, steam explosion, acid, base, or organic solvent processes (Rogers and Seddon, 2003; Zhao et al., 2009). More than 20 ILs have been reported to be able to dissolve cellulose, which can be regenerated by the addition of anti-solvents, such as water, ethanol or acetone (Fukaya et al., 2008; Holm and Lassi, 2011; Shill et al., 2011). The polar characteristics and the ability to generate hydrogen bonds are the main properties of ILs, affecting the dissolution of cellulose and carbohydrates (Holm and Lassi, 2011). The mechanism for the dissolution of cellulose in ionic liquids is shown in Fig. 1.4. Feng and Chen (2008) indicated that interactions between the oxygen and hydrogen atoms of cellulose-OH and the cation and anion of ionic liquid results in the dissolution of cellulose. The cellulose atoms serve as electron pair donors and hydrogen atoms act as electron acceptors. Upon interaction, the oxygen and hydrogen atoms from hydroxyl groups are separated, which leads to the opening of hydrogen bonds between molecular chains of the cellulose and, as a result, the cellulose dissolves.

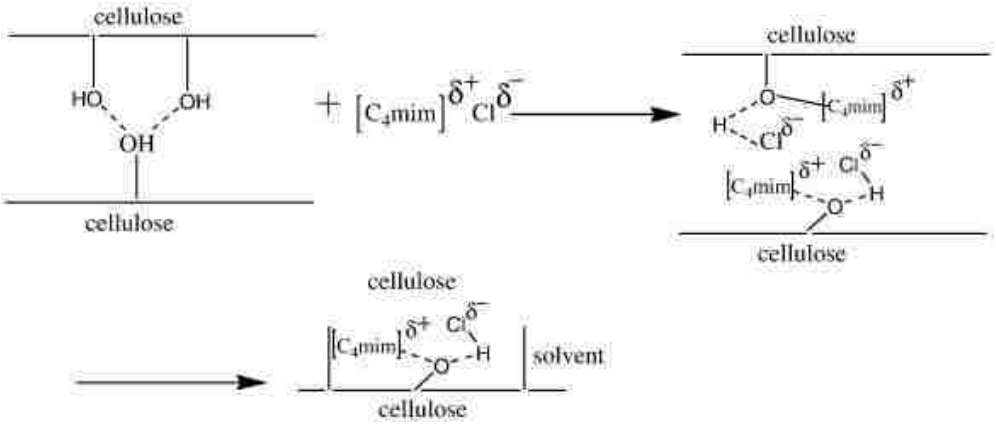

Fig. 1.4 Dissolution Mechanism of Cellulose by Ionic Liquids (Feng and Chen, 2008).

Typical ionic liquids used during biomass pretreatment contain an anion of chloride, formate, acetate or alkylphosphonate, which forms strong hydrogen bonds with cellulose and other carbohydrates (Zhao et al., 2009). In earlier studies, two chloride-based ILs, 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) and 1-allyl-3-methylimidazolium chloride ([AMIM]Cl), have been demonstrated to be effective ILs for pretreatment (Wu et al., 2004; Zhao et al., 2009; Zhu et al., 2006). However, [BMIM]Cl is corrosive and toxic, and [AMIM]Cl is viscous and has a reactive side chain (Zhao et al., 2009). In general, acetate-based ILs are less viscous than chloride-based ILs, and are more thermally stable than formate-based ILs (Fukaya et al., 2008; Zhao et al., 2008). Sun et al. (2011) pointed out that the anions affect the solubility of cellulose in ILs with the same cation in the following decrease order: $[OAc]^- \approx [(CH_3CH_2O)_2PO_2]^- > [SHCH_2COO]^- > [HCOO]^- > Cl^- > Br^- \approx [SCN]^-$. Therefore, the acetate-based IL 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]) (Fig. 1.5) was selected as the pretreatment solvent in this study because of its high lignocellulose solubility, low melting temperature (-20 ℃), low viscosity, non-toxicity, and non-corrosiveness (Samayam and Schall, 2010).

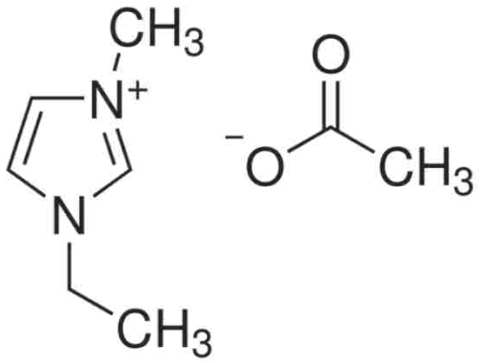

Fig. 1.5. Molecular Structure of 1-Ethyl-3-Methylimidazolium Acetate [EMIM][OAc].

1.4 Goal of This Study

This study aimed to assess the use of 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]) as a solvent during the pretreatment of energy cane bagasse and its effect on the chemical composition and enzymatic hydrolysis of pretreated biomass. This study is divided into three sections: (1) Chapter 2, assessment of the effect of [EMIM][OAc] during the pretreatment of energy cane bagasse by monitoring changes in biomass chemical composition (cellulose, hemicellulose and lignin), surface morphology, cellulose crystallinity, and enzymatic digestibility; (2) Chapter 3, investigation of the pretreatment efficiency of recycled [EMIM][OAc] on energy cane bagasse in terms of its chemical composition and enzymatic hydrolysis; (3) Chapter 4, optimization of the pretreatment processing parameters for [EMIM][OAc] on energy cane bagasse by response surface methodology.

CHAPTER 2

EFFECT OF IONIC LIQUID PRETREATMENT ON THE CHEMICAL COMPOSITION, STRUCTURE AND ENZYMATIC HYDROLYSIS OF ENERGY CANE BAGASSE

2.1 Introduction

Lignocellulose is a suitable and renewable energy resource that can be used for the generation of bio-based transportation fuels and chemicals. The polysaccharides (hemicellulose and cellulose) present in native or untreated lignocellulosic biomass are not readily available for bioconversion into fuels and chemicals. The close association and complexity of the carbohydrates-lignin complex is the main obstacle in lignocellulosics degradation (Lee et al., 2009; Zhu et al., 2008). Pretreatment aims at breaking the lignin structure and disrupting the crystalline structure of cellulose to make cellulose and hemicellulose available for enzymatic hydrolysis. Processing shortages such as long residence time, high energy demand, high cost, and environmental pollution exist in current biological, mechanical, chemical, and physicochemical pretreatment methods (Shill et al., 2011; Zhao et al., 2009). Therefore, the major concern in lignocellulose conversion is overcoming biomass recalcitrance through pretreatment while still maintaining a green and energy efficient process (Lee et al., 2009).

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Ionic liquids (ILs) are thermally stable organic salts with potential application as ‘‘green solvents’’ (Sheldon et al., 2002). ILs exhibit excellent physical characteristics including the ability to dissolve polar and non-polar, organic, inorganic, and polymeric compounds (Lee and Lee, 2005). Additionally, ILs have the advantages of having low volatility, being non-flammable and recyclable (Gremos et al., 2011). Pretreatment with ILs can reduce the crystallinity of cellulose and partially remove hemicellulose and lignin while not generating degradation products which are inhibitory to enzymes or fermenting microorganisms (Dadi et al., 2007; Lee et al., 2009). Pretreatment with ILs are less energy demanding, easier to handle and more environmentally friendly than other pretreatment methods such as mechanical milling, steam explosion, acid, base, or organic solvent processes (Rogers and Seddon, 2003; Zhao et al., 2009). Typical ionic liquids used during biomass pretreatment contain an anion of chloride, formate, acetate or alkylphosphonate which form strong hydrogen-bonds with cellulose and other carbohydrates (Zhao et al., 2009). In general, acetate-based ILs are less viscous than chloride-based ILs and are more thermally stable than formate-based ILs (Fukaya et al., 2008; Zhao et al., 2008). The acetate-based IL 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]) was selected in this study for the pretreatment of energy cane due to its low melting temperature (-20 ℃), low viscosity, non-toxicity, and non-corrosiveness (Samayam and Schall, 2010).

Energy cane, a hybrid of commercial and wild sugarcanes, is bred for high fiber content and low sucrose (Kim and Day, 2011). Unlike sugar cane, energy cane is more cold tolerant, requires less fertilizer and water input, and requires replanting only every ten years, compared to every three years for sugar cane (Sierra et al., 2008). A non-commercial energy cane variety, L79-1002, developed in collaboration by the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) in Houma, LA and the Louisiana State University

Agricultural Center Sugar Research Station in St. Gabriel, LA has an average fiber content of 257 g/kg (dry basis) and cane yield of 83.3 Ma/ha (dry basis) (Bischoff et al., 2008) as compared to LCP 85-384, the predominant commercial variety of sugarcane grown in Louisiana, with a fiber content of 117 g/kg (dry basis) and cane yield of 59.2 Mg/ha (dry basis) (Gravois et al., 2009).

This study aimed to assess the effect of an acetate-based ionic liquid 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]) during the pretreatment of energy cane bagasse by monitoring changes in biomass chemical composition (cellulose, hemicellulose and lignin), lignocellulose structure and enzymatic digestibility.

2.2 Materials and Methods

2.2.1 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 ℃.

2.2.2 Ionic liquid pretreatment

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 ℃ for 30 min. Post pretreatment, deionized water was added into 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. Untreated and

water-treated energy cane bagasse were used as controls. Water-treated bagasse (control) was prepared by combining water and bagasse at a 20:1 ratio and by heating the mixture to 120 ℃ for 30 min. Experiments were run in triplicates with three separate batches.

2.2.3 Chemical composition of energy cane bagasse

Untreated, water-treated and ionic liquid-treated energy cane bagasse were analyzed for glucan, xylan, arabinan, mannan, lignin, ethanol extractives, and ash content 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 sample to ensure the accuracy of the procedures. The percent lignin removal, glucan loss and xlyan loss were calculated as described below:

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

$$\%\text{Glucan loss} = 1 - \frac{\%\text{Glucan in treated biomass} \times \%\text{Recovered solids}}{\%\text{Glucan in untreated biomass}}$$

$$\%\text{Xylan loss} = 1 - \frac{\%\text{Xylan in treated biomass} \times \%\text{Recovered solids}}{\%\text{Xylan in untreated biomass}}$$

2.2.4 FTIR analysis

Fourier transform infrared spectroscopy (FTIR) was performed using a Thermo Scientific Nicolet Nexus 670 FT-IR Spectrometer and Smart iTR with a diamond window (Thermo Fisher

Scientific Inc., Waltham, MA). About 5 mg of sample material was placed on the diamond window of Smart iTR. The background spectrum of diamond window without sample was subtracted from that of each sample spectrum. Scans were conducted at 700-4000 cm^{-1} with a resolution of 4 cm^{-1} and at 64 scans per sample.

2.2.5 XRD analysis

X-ray diffraction (XRD) measurements were made at the synchrotron ring of J. Bennett Johnston, Sr., Center for Advanced Microstructures and Devices (CAMD), Louisiana State University, Baton Rouge, LA. The CAMD electron storage ring operates at 1.3 GeV with ring current varying between 100 to 200 mA. The measurements were performed at the double crystal monochromator 7.5 Tesla wavelength shifter beam line. The wavelength was set to that of the absorption edge of nickel foil (8333.0 eV, 1.4878 A) with the double crystal monochromator with Ge 220 crystals. The wavelength was refined to 1.4810 Angstrom with GSAS by running NIST LaB6 standard 660a. A Huber four-circle goniometer in Bragg-Brentano geometry was used for the measurements. The diffracted X-rays were detected with a Canberra germanium solid state detector. The source and receiving slits were 30 μm and 10 μm, respectively. The biomass samples were mounted on zero-background plates (50 μm depth) coated with a very thin layer of vacuum grease. Patterns were collected from 5° to 40° (2θ), with 0.05° step size and 3 seconds counting. Patterns were normalized by the ring current. Data reduction was accomplished with JADE 9.3.4. The crystallinity index (CrI) was calculated using the formula as described by Cheng et al. (2011):

$$CrI = (I_{002}-I_{am})/ I_{002}$$

Where, I_{002} is the scattered intensity at the main peak for cellulose Type I; I_{am} is the scattered intensity due to the amorphous portion evaluated as the minimum intensity between the main and secondary peaks.

2.2.6 SEM analysis

Scanning electron microscopy (SEM) was used to monitor the changes in morphology before and after IL pretreatment. A JEOL JSM-6610LV scanning electron microscope (JEOL USA, Inc., Peabody, MA) operated at 10 keV was used to image the samples. Prior to imaging, the samples were sputter-coated with platinum to make the fibers conductive, avoiding degradation and buildup of charge on the specimen.

2.2.7 Enzymatic hydrolysis

A combination of two commercially available enzymes, Spezyme CP (cellulases) (Genencor, Danisco US Inc., Rochester, NY) and Novozyme 188 (cellobiases) (Sigma-Aldrich, Inc., St. Louis, MO) were used for the hydrolysis of untreated, water-treated and ionic liquid-treated energy cane bagasse. Enzymatic hydrolysis was measured by following NREL's LAP TP-510-43629. Briefly, hydrolysis was carried out with 1% (w/v) substrate at 50 ℃, 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 15 FPU/g glucan and Novozyme 188 at 15 CBU/g glucan. A second test using a higher enzyme loading of Spezyme CP at 30 FPU/g glucan and Novozyme 188 at 30 CBU/g glucan was also conducted. Samples were taken at 0 h (before the addition of enzymes), 24 h, 48 h, and 72 h. Experiments were run in triplicates.

2.2.8 Chemical analysis of hydrolyzed samples

Collected samples (0 h, 24 h, 48 h, and 72 h) were centrifuged (8000 rpm) with a Spectrafuge 24D (Labnet International Inc., Woodbridge, NJ), filtered (0.2 μm Syringe Filters, Environmental Express, Inc., Mt. Pleasant, SC) and diluted accordingly. Sugars (glucose, cellobiose, arabinose, and xylose) from all collected samples were analyzed by high performance liquid chromatography (HPLC) (Agilent 1200 Series) with a BioRad Aminex HPX-87P, lead form, 3000 mm × 7.8 mm (ID), 9 μm column and a differential refractive index detector (G1362A Agilent). Percent theoretical cellulose and hemicellulose digestibilities were calculated using the equations provided by NREL's LAP TP-510-43630 as described below:

$$\%\text{Theoretical Cellulose Digestibility} = \frac{[\text{Glucose}] + 1.053\,[\text{Cellobiose}]}{1.111\,f\,[\text{Biomass}]} \times 100\%$$

$$\%\text{Theoretical Hemicellulose Digestibility} = \frac{0.9\,[\text{Xylose}] + 0.9\,[\text{Arabinose}]}{1.136\,f\,[\text{Biomass}]} \times 100\%$$

Where, [Glucose] is the residual glucose concentration (g/L), [Cellobiose] is the residual cellobiose concentration (g/L), [Xylose] is the residual xylose concentration (g/L), [Arabinose] is the residual arabinose concentration (g/L), 1.053 is the multiplication factor that converts cellobiose to equivalent glucose, [Biomass] is the dry biomass concentration at the beginning of the enzymatic hydrolysis (g/L), f is the cellulose or hemicellulose fraction in dry biomass (g/g), 1.111 is the factor that converts cellulose to equivalent glucose, 1.136 is the factor that converts hemicellulose to equivalent xylose.

2.3 Results and Discussion

2.3.1 Effect of IL pretreatment on biomass composition

The chemical composition of untreated, water-treated and IL-treated energy cane bagasse were analyzed and reported in Table 2.1 as dry weight basis. The chemical composition of energy cane bagasse before pretreatment was 40.9% glucan, 20.8% xylan and 24.8% lignin which are comparable to those reported by Aita et al. (2011) and Kim and Day (2011). It was observed that 15.1% of the total mass was lost during pretreatment with IL and that 52.6% of the loss was attributed to lignin removal. Only 4.0% of mass loss was observed in water-treated energy cane bagasse. Recent studies have indicated that [EMIM][OAc] is effective in removing lignin (Fu et al., 2010; Lee et al., 2009; Samayam and Schall, 2010). Composition analysis revealed that 32.1% of the initial lignin was removed in IL-treated energy cane samples, whereas only 2.3% of the initial lignin was removed in water-treated samples. Shill et al. (2011) indicated that the π-π interactions of the IL cation with lignin assisted in lignin solubilization. However, complete delignification of biomass is difficult due to the location of lignin within the lignin-carbohydrate complex, strong poly-ring bonds of C-O-C, and hydrophobicity (Kim et al., 2003). IL pretreatment exhibited a lesser effect on delignification as compared to other pretreatment technologies such as dilute ammonia in which 55% of the initial lignin was removed from energy cane bagasse (Aita et al., 2011). A more effective delignification was observed with acid insoluble lignin. Specifically, 41.7 % of the initial acid insoluble lignin was removed from energy cane bagasse by [EMIM][OAc] at 120 ℃ for 30 min. Fu et al. (2010) reported that 52.7% initial acid insoluble lignin was extracted from triticale straw by [EMIM][OAc] at 150 ℃ for 2 h. Another study with switchgrasss reported a 69.2% total lignin removal using [EMIM][OAc] at 160 ℃ for 3 h (Li et al., 2010b). The higher acid insoluble lignin loss and total lignin loss

reported in previous studies can be attributed to the different lignocellulosic materials, higher processing temperatures (150 ℃, 160 ℃) and longer retention times (1.5 h, 3h) used.

Table 2.1. Chemical composition of untreated, water-treated and [EMIM][OAc]-treated energy cane bagasse.

Biomass component (%, dry weight basis)	Untreated	Water-treated	IL-treated
Ash	1.44 ± 0.01	1.10 ± 0.01	1.39 ± 0.01
Extractives	1.49 ± 0.04	1.44 ± 0.14	1.52 ± 0.09
Acid soluble lignin	3.98 ± 0.08	4.15 ± 0.14	5.54 ± 0.36
Acid insoluble lignin	20.83 ± 0.22	21.10 ± 0.14	14.31 ± 1.06
Total lignin	24.81 ± 0.14	25.25 ± 0.01	19.85 ± 1.45
Glucan	40.87 ± 0.22	43.41 ± 0.27	43.89 ± 0.21
Xylan	20.82 ± 0.10	21.85 ± 0.18	21.10 ± 0.33
Arabinan	1.53 ± 0.05	1.59 ± 0.06	2.05 ± 0.27
Mannan	ND	ND	ND
Recovered solids	100	95.99 ± 1.67	84.89 ± 2.32

ND: none detected

The loss of glucan in IL-treated and water treated samples were less than 9%. Similar observations were reported by Aita et al. (2011) in which 91.4% of the initial glucan was retained in dilute ammonia-treated energy cane bagasse. The loss of initial xylan in [EMIM][OAc]-treated sample was 14.0% as compared to dilute ammonia pretreatment which removed 30.1% of the initial xylan (Aita et al., 2011). Compared with other [EMIM][OAc] pretreated lignocellulosic materials, Samayam and Schall (2010) reported 15.0% and 32.0% of initial xylan losses in pretreated poplar and switchgrass, respectively, at 120 ℃ for 30 min. Li et al. (2010b) reported that the loss of xylan in pretreated switchgrass was 62.6% at 160 ℃ for 3 h. Xylan losses of 6% and 26% had been reported in Maple wood flour pretreated at 110 ℃ and 130 ℃ for 1.5 h, respectively (Lee et al., 2009).

2.3.2 Effect of IL pretreatment on lignocellulose structure

2.3.2.1 FTIR analysis

FTIR analysis was conducted to examine the cellulose structure of untreated, water-treated and IL-treated samples (Fig. 2.1). Two infrared ratios related to cellulose structure were calculated: (1) α 1426 cm^{-1}/ α 896 cm^{-1}, the ratio of peak areas at 1426 and 896 cm^{-1} ,which is referred to as the crystallinity index (O'Connor et al., 1958) or lateral order index (LOI) (Hurtubise and Krassig, 1960); (2) α 1373 cm^{-1}/ α 2917 cm^{-1}, the ratio of peak areas at 1373 and 2917cm^{-1}, which is known as the total crystallinity index (TCI) (Nelson and O'Connor, 1964a). The ratios of peak areas were determined following the method of Nelson and O'Connor (1964a). The 1426 cm^{-1} band represents CH_2 scissoring motion (Nelson and O'Connor, 1964b); the 896 cm^{-1} band indicates the vibrational mode involving C_1 and four atoms attached to it, which is characteristic of β-anomers or β-linked glucose polymers (Nelson and O'Connor, 1964b); the 1373 cm^{-1} band is for C-H bending mode (Nelson and O'Connor, 1964a); and the 2917 cm^{-1} band represents C-H and CH_2 stretching, which is unaffected by changes in crystallinity (Nelson and O'Connor, 1964a). Therefore, higher values of LOI and TCI are indicative of biomass with a higher crystallinity and more ordered structure of cellulose. Both LOI and TCI decreased significantly post IL pretreatment as shown in Table 2.2. LOI decreased from 0.9593 to 0.3718 and TCI decreased from 0.4057 to 0.1937 in IL-treated bagasse; whereas, water-treated samples just had a slight decrease from 0.9593 to 0.8174 and 0.4057 to 0.3747 in terms of LOI and TCI, respectively. The results indicated that the highly crystalline cellulose in energy cane bagasse was transformed to amorphous form after pretreatment with [EMIM][OAc]. Decrease in crystallinity of Avicel (Zhao et al., 2009), switchgrass (Li et al., 2010b), straw (Fu and Mazza,

2011a), sugarcane (Yoon et al., 2011) and kenaf powder (Ninomiya et al., 2012) have also been reported after pretreatment with ILs.

2.3.2.2 XRD analysis

XRD analysis was conducted to further examine the crystallinity of cellulose since determination of crystallinity index (CrI) by FTIR spectroscopy gives only relative values from both crystalline and amorphous regions. Therefore, the CrI calculated from an FTIR spectrum is often compared with those from XRD and/or NMR measurements (Park et al., 2010). In this study, two typical diffraction peaks were observed at $2\theta=15°$ and 21°, which correspond to (101) and (002) lattice planes of crystalline cellulose type I (Fig. 2.2). After IL pretreatment, the peak (101) disappeared and the peak (002) became broader and weaker (Fig. 2.2C). The XRD pattern of [EMIM][OAc]-treated energy cane bagasse was similar to the XRD pattern of amorphous cellulose as reported by Nelson and O'Connor (1964b).

The CrI of various energy cane bagasse samples was determined based on the XRD patterns for quantitative comparison and are depicted in Table 2.2. The CrI of untreated, water-treated and IL-treated samples were 0.5628, 0.5338 and 0.2452, respectively. The CrI of IL-treated sample was significantly lower than those reported for water-treated and untreated samples. No significant difference was observed between the CrI for water-treated and untreated samples. A lower CrI is indicative of a material with lower crystallinity. The CrI obtained through XRD was also in accordance with the LOI and TCI values obtained through FTIR as reported earlier. Results from both FTIR and XRD suggest that pretreatment with [EMIM][OAc] can reduce the cellulose crystallinity in energy cane bagasse. Reports suggested that anions and

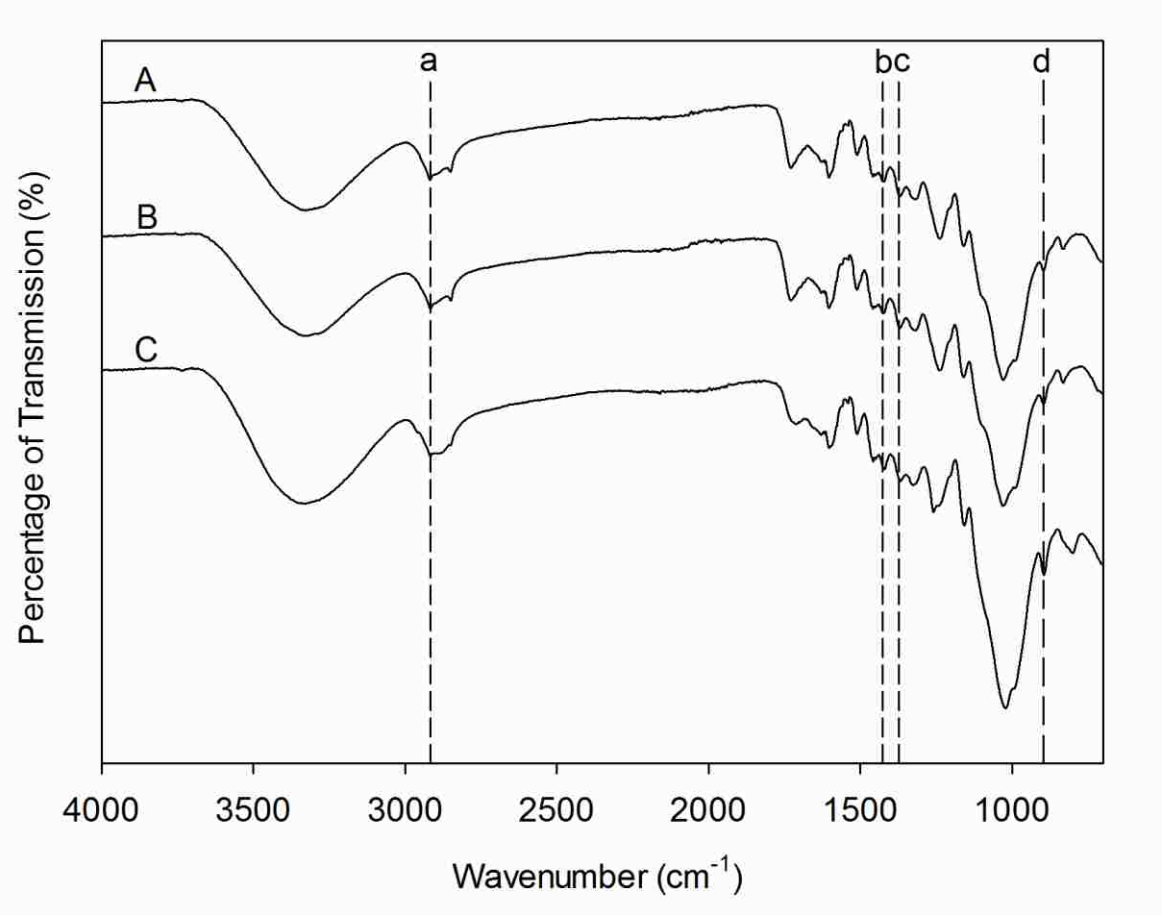

Fig. 2.1. FTIR Spectra of (A) Untreated, (B) Water-Treated and (C) [EMIM][OAc]-Treated Energy Cane Bagasse. (a) 2917 cm^{-1}, (b) 1426 cm^{-1}, (c) 1373 cm^{-1}, (d) 896 cm^{-1}.

Table 2.2. Crystallinity index of untreated, water-treated and [EMIM][OAc]-treated energy cane bagasse.

Sample	LOI (1426/896 cm^{-1})	TCI (1373/2917 cm^{-1})	CrI (XRD)
Untreated	0.9593	0.4057	0.5628
Water-treated	0.8174	0.3747	0.5338
IL-treated	0.3718	0.1937	0.2452

LOI: lateral order index or crystallinity index based on FTIR

TCI: total crystallinity index based on FTIR

CrI: crystallinity index based on XRD

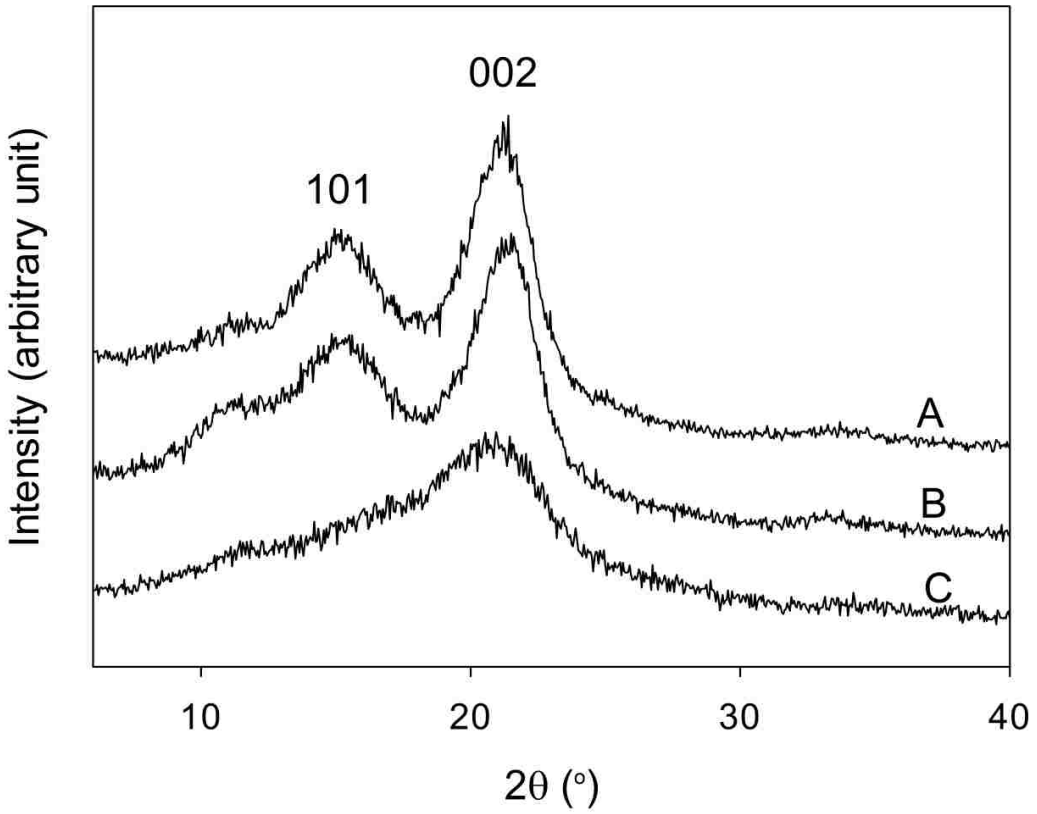

Fig. 2.2. XRD Patterns for (A) Untreated, (B) Water-Treated and (C) [EMIM][OAc]-Treated Energy Cane Bagasse.

cations in ILs are responsible for the dissolution and disruption of cellulose (Dadi et al., 2007; Feng and Chen, 2008; Shill et al., 2011; Yoon et al., 2011). It was indicated that the anion in ILs attacked the free hydroxyl group on cellulose and deprotonated it, while the cation interacted with the hydroxyl oxygen atoms. The hydrogen bonds in cellulose were disrupted and replaced by hydrogen bonding between the anion of ILs and cellulose hydroxyls. Consequently, cellulose dissolution occurred and the crystalline structure was disrupted. Li et al. (2009) also suggested that the decrease of CrI, probably due to the rapid precipitation with water, prevented the dissolved lignocellulosic material from restructuring into its original crystalline structure, which

resulted in a fragmented and porous biomass with amorphous structure and greater surface area for enzymes to attach.

2.3.2.3 SEM analysis

SEM images of untreated, water-treated and IL-treated energy cane bagasse are shown in Fig. 2.3. Both untreated and water-treated biomass showed compact, ordered and rigid fibril structures (Fig. 2.3A and 2.3B). After pretreatment of energy cane bagasse with ionic liquid, the structure became loose, disordered and curly (Fig. 2.3C). This was probably due to the removal of lignin and decrease of cellulose crystallinity, which have already been confirmed by FTIR and XRD analysis.

2.3.3 Enzymatic hydrolysis of energy cane bagasse

Enzymatic hydrolysis of untreated, water-treated and [EMIM][OAc]-treated energy cane bagasse are summarized in Fig. 2.4. Detailed data on enzymatic hydrolysis (Tables A1, A2) is listed in Appendix A and B. Significantly higher cellulose digestibilities (64.6%, 68.4% and 68.9%) were observed in IL treated energy cane bagasse samples at an enzyme loading of 15 FPU Spezyme CP and 15 CBU Novozyme 188/g glucan as compared to untreated samples (2.6%, 3.2% and 4.1%) and water-treated samples (2.9%, 3.3% and 3.4%) at 24 h, 48 h and 72 h post hydrolysis, respectively. Cellulose digestibility of IL-treated sample at 24 h was approximately 25 and 22 times higher than untreated and water-treated samples, respectively. The limited enzymatic hydrolysis of untreated and water-treated energy cane bagasse can be explained by the unmodified crystalline structure of cellulose and hindrance of lignin (Chandra et al., 2007; Yang and Wyman, 2008). Higher cellulose digestibilities (75.4%, 81.2% and 87.0%) were observed with enzyme loadings of 30 FPU Spezyme CP /g glucan and 30 CBU Novozyme

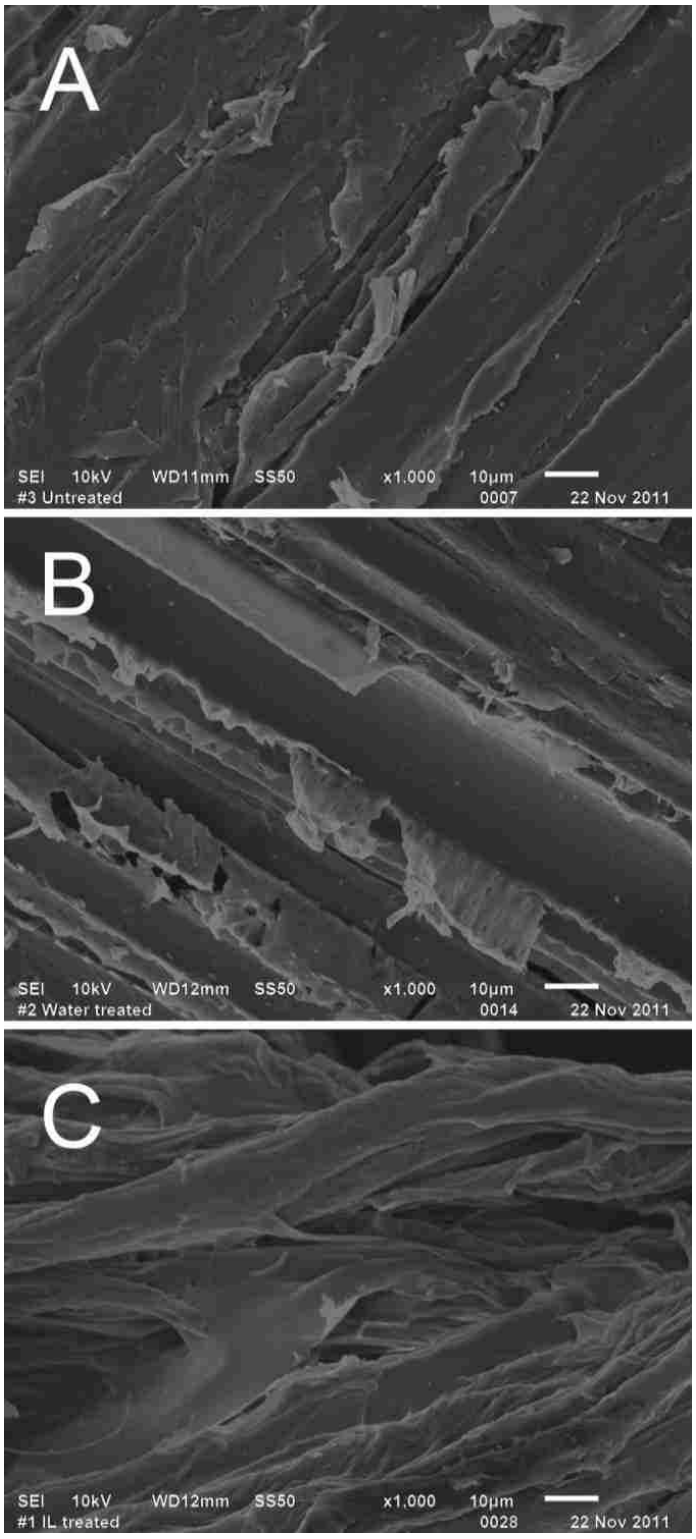

Fig. 2.3. SEM Images of (A) Untreated (B) Water-Treated and (C) [EMIM][OAc]-Treated Energy Cane Bagasse at 1000X Magnification.

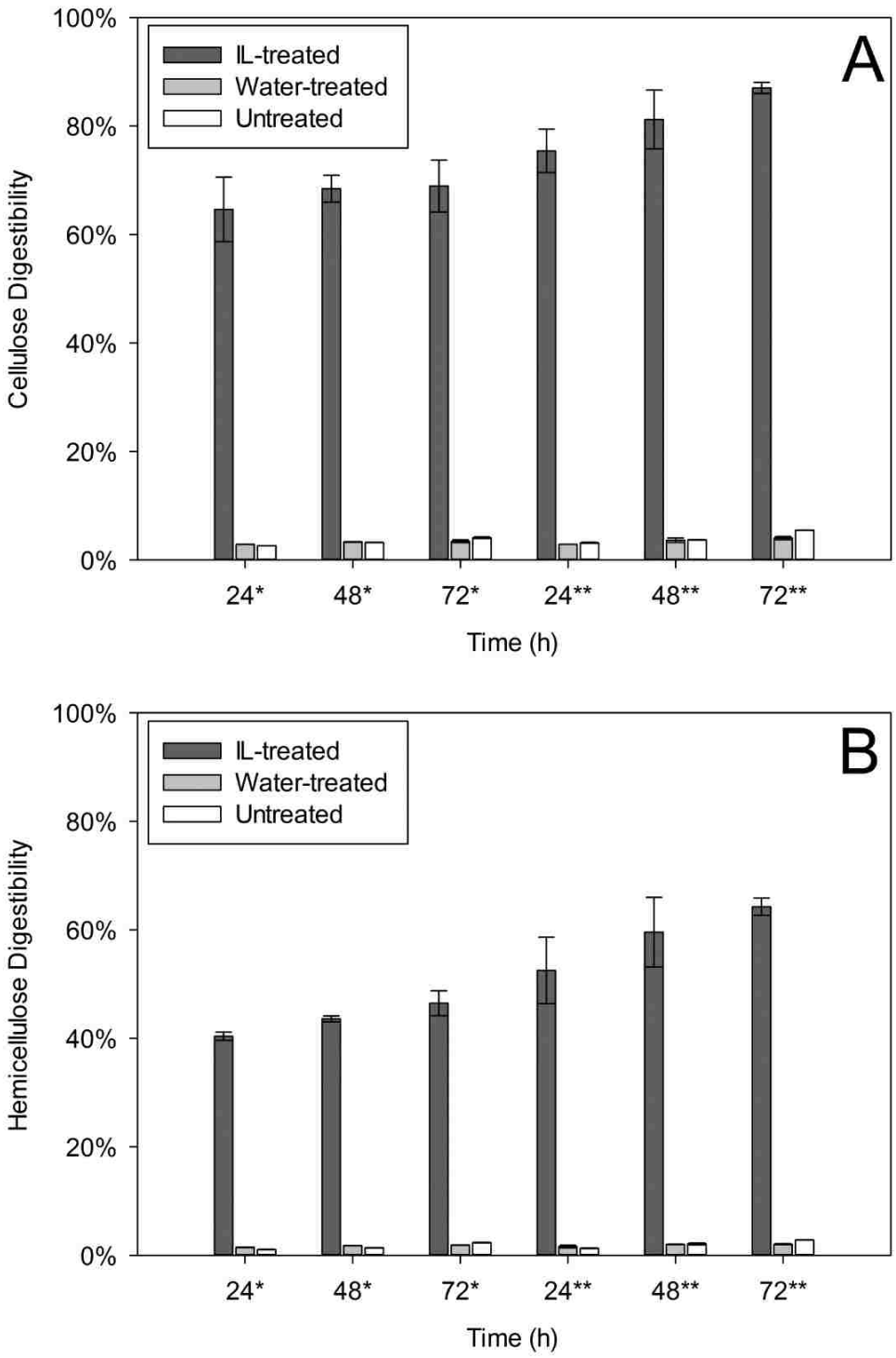

Fig. 2.4. (A) Cellulose and (B) Hemicellulose Digestibility of [EMIM][OAc]-Treated, Water-Treated and Untreated Energy Cane Bagasse at Two Enzyme Loadings.
* 15 FPU Spezyme CP/g glucan and 15 CBU Novozyme 188/g glucan.
** 30 FPU Spezyme CP/g glucan and 30 CBU Novozyme 188/g glucan.

188 /g glucan at 24, 48 and 72 h, respectively, than lower enzyme loadings. No significant increases in digestibilities were observed for untreated and water-treated samples. A slightly higher cellulose digestibility (77.0%) has been reported with dilute ammonia-treated energy cane bagasse at an enzyme loading of 30 FPU Spezyme CP and 32 CBU Novozyme 188/ g glucan at 24 h post hydrolysis (Aita et al., 2011). This result suggested that a higher delignification (55.0% versus 32.1%) did not directly result in a higher cellulose digestibility (77.0% versus 75.4%). Similarly, other studies have indicated high cellulose digestibilities (85% - 95%) post ILs pretreatment with lignocellulosic materials containing 60–70% of initial lignin, such as wood flour (Lee et al., 2009), straw (Fu et al., 2010), switchgrass, and poplar (Samayam and Schall, 2010). Ninomiya et al. (2012) reported 95% cellulose digestibility with almost 100% initial lignin content in the kenaf powder post ILs pretreatment.

Hemicellulose digestibilities were lower than those observed for cellulose since the enzyme mixture used in this study contained mostly cellulase-degrading enzymes. Hemicellulose digestibilities of [EMIM][OAc]-treated samples (40.4%, 43.6% and 46.5%) at an enzyme loading of 15 FPU Spezyme CP and 15 CBU Novozyme 188/g glucan were significantly higher than both untreated (1.1%, 1.4% and 2.4%) and water-treated samples (1.5%, 1.8% and 1.9%) at 24 h, 48 h and 72 h post hydrolysis, respectively. An increase in enzyme loading to 30 FPU Spezyme CP and 30 CBU Novozyme 188/g glucan resulted in higher hemicellulose digestibilities (52.5%, 59.6% and 64.3%, respectively) in all IL-treated samples. As observed with cellulose digestibilities, no significant increases in digestibilities were detected in untreated and water-treated samples. IL-treated sample exhibited a significantly higher hemicellulose digestibility than those reported with dilute ammonia in which only a 39% hemicellulose digestibility was observed after 24 h hydrolysis at an enzyme loading of 30 FPU Spezyme CP

and 32 CBU Novozyme 188/ g glucan (Aita et al., 2011). The higher hemicellulose digestibility observed in [EMIM][OAc]-treated bagasse may be attributed to minimal loss of initial xylan (14.0% versus 30.1%). The enhanced cellulose and hemicellulose digestibilities observed in energy cane bagasse treated with [EMIM][OAc] could be attributed to delignification, lignin structure disruption and the reduction in cellulose crystallinity.

2.4 Conclusions

[EMIM][OAc]-treated energy cane bagasse resulted in significant lignin removal (32.0%) with slight glucan and xylan losses (8.8% and 14.0%, respectively), and exhibited significant higher enzymatic digestibilities (87.0%, 64.3%) than untreated (5.5%, 2.8%) or water (4.0%, 2.1%) treated energy cane bagasse in terms of both cellulose and hemicellulose yields, respectively. SEM images revealed a loose and disordered structure of biomass post pretreatment. FTIR analysis indicated that IL-treated biomass exhibited a significant loss of native cellulose crystalline structure. XRD analysis also confirmed that IL pretreatment resulted in a decrease of crystallinity index from 0.5628 to 0.2452.

CHAPTER 3

PRETREATMENT OF ENERGY CANE BAGASSE WITH RECYCLED IONIC LIQUID FOR ENZYMATIC HYDROLYSIS

3.1 Introduction

Lignocellulosic biomass appears to be a prospective renewable energy resource that can be used for the generation of biofuels and bioproducts. Energy cane, a hybrid of commercial and wild sugarcanes, is an ideal energy crop and 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, which together forms a complex structure. The recalcitrance of this complex structure makes lignocellulose biomass highly resistant to enzymatic hydrolysis, which results in low reducing sugar yield (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, which 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 (32.0%) 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 multiple recycled [EMIM][OAc] on the pretreatment of energy cane bagasse in terms of its chemical composition and enzymatic hydrolysis.

3.2 Materials and Methods

3.2.1 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 ℃.

3.2.2 Ionic liquid pretreatment and recycling

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 ℃ for 0.5 h or at 100 ℃ for 0.5 h, 1 h, 2 h, and 4 h. 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 ℃ for 12 h by air drying oven to remove water and then reused to pretreat energy cane bagasse without any further purification. Approximately, 85% to 90% of IL was recovered on each recycle.

Based on the yields obtained from enzymatic hydrolysis studies, pretreatment conditions at 120 ℃ for 0.5 h and 100 ℃ 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 ℃ for 0.5 h) or three (100 ℃ for 2 h) IL recycles post pretreatment were evaluated. Studies were carried out in duplicate with two separate batches.

3.2.3 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. The percent lignin removal, glucan recovery and xlyan recovery were calculated as described below:

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

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

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

3.2.4 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 ℃, 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.

3.2.5 Chemical analysis of hydrolyzed samples

Collected samples (0 h, 24 h, 48 h, and 72 h) were centrifuged (8000 rpm) with a Spectrafuge 24D (Labnet International Inc., Woodbridge, NJ), filtered (0.2 μm Syringe Filters, Environmental Express, Inc., Mt. Pleasant, SC) and diluted accordingly. Sugars (glucose, cellobiose, arabinose, and xylose) from all collected samples were analyzed by high performance liquid chromatography (HPLC) (Agilent 1200 Series) with a BioRad Aminex HPX-87P, lead form, 3000 mm × 7.8 mm (ID), 9 μm column and a differential refractive index detector (G1362A Agilent). Percent theoretical cellulose and hemicellulose digestibilities were calculated using the equations provided by NREL's LAP TP-510-43630 as described below:

$$\%\text{Theoretical Cellulose Digestibility} = \frac{[\text{Glucose}] + 1.053\,[\text{Cellobiose}]}{1.111\,f\,[\text{Biomass}]} \times 100\%$$

$$\%\text{Theoretical Hemicellulose Digestibility} = \frac{0.9[\text{Xylose}] + 0.9\,[\text{Arabinose}]}{1.136\,f\,[\text{Biomass}]} \times 100\%$$

Where, [Glucose] is the residual glucose concentration (g/L), [Cellobiose] is the residual cellobiose concentration (g/L), [Xylose] is the residual xylose concentration (g/L), [Arabinose] is the residual arabinose concentration (g/L), 1.053 is the multiplication factor that converts cellobiose to equivalent glucose, [Biomass] is the dry biomass concentration at the beginning of the enzymatic hydrolysis (g/L), f is the cellulose or hemicellulose fraction in dry biomass (g/g),

1.111 is the factor that converts cellulose to equivalent glucose, 1.136 is the factor that converts hemicellulose to equivalent xylose.

3.3 Results and Discussion

3.3.1 Effect of IL and recycled IL pretreatment on biomass composition

The chemical composition of IL and recycled IL-treated energy cane bagasse is summarized in Table 3.1, and the percent lignin removal, glucan recovery and xylan recovery are presented in Table 3.2. Previous studies have indicated that [EMIM][OAc] is effective in removing lignin, because the π-π interactions of the IL cation with lignin assist in lignin solubilization (Fu et al., 2010; Lee et al., 2009; Samayam and Schall, 2010; Shill et al., 2011). IL-treated energy cane bagasse with different pretreatment temperatures, residence times and IL recycles exhibited lignin removal in the range of 15.05% to 32.08% when compared to the initial lignin percentage in untreated bagasse as shown in Table 3.2. Specifically, lignin removal of the 1st and 2nd recycled IL-treated biomass at 120 ℃ for 0.5 h gradually decreased to 23.53% and 21.74% from 32.08% of the original IL-treated biomass, respectively. A temperature decrease in pretreatment also resulted in the decrease of lignin removal. The lignin removal of energy cane bagasse treated at 100 ℃ for 0.5 h, 1 h, 2 h, and 4 h were 15.05%, 18.66%, 17.45%, and 25.19%, respectively, among which lignin removal from biomass treated for 4 h was 6.89% less than the result observed at 120 ℃ for 0.5 h. The use of 1st, 2nd and 3rd recycled IL at 100 ℃ for 2 h did not cause any significant decrease in lignin removal (16.80%, 17.47% and 18.93%, respectively) compared to the original IL pretreatment (17.45%) under the same pretreatment conditions. Li et al. (2010a) reported that lignin removal of regenerated wood decreased with the increasing number of IL recycles at 120 ℃ for 5 h. In our study, a similar trend was observed with biomass

Table 3.1. Chemical composition analysis of biomass.

Pretreatment conditions				Biomass component (%, dry weight basis)						
Solvent	Number of recycles	Temp. (℃)	Time (h)	Acid soluble lignin	Acid insoluble lignin	Total lignin	Glucan	Xylan	Arabinan	Recovered solids
None	N/A	N/A	N/A	4.70 ± 0.93	19.17 ± 0.29	23.86 ± 0.64	43.51 ± 0.18	21.29 ± 0.90	1.59 ± 0.30	100.00
Water*	N/A	120	0.5	4.15 ± 0.14	21.10 ± 0.14	25.25 ± 0.01	43.41 ± 0.27	21.85 ± 0.18	1.59 ± 0.06	95.99 ± 1.67
IL*	0	120	0.5	5.54 ± 0.36	14.31 ± 1.06	19.85 ± 1.45	43.89 ± 0.21	21.10 ± 0.33	2.05 ± 0.27	84.89 ± 2.32
IL	1	120	0.5	4.91 ± 0.52	16.48 ± 0.33	21.39 ± 0.76	45.20 ± 2.45	20.87 ± 0.29	1.21 ± 0.14	85.30 ± 1.02
IL	2	120	0.5	3.70 ± 0.89	17.65 ± 0.51	21.35 ± 1.37	40.95 ± 1.72	21.27 ± 0.56	2.09 ± 0.33	87.46 ± 4.32
IL	0	100	0.5	4.56 ± 0.27	18.52 ± 0.23	23.07 ± 0.96	42.81 ± 1.68	22.27 ± 1.23	1.91 ± 0.21	87.86 ± 4.33
IL	0	100	1	4.39 ± 0.15	17.60 ± 1.11	21.99 ± 1.33	42.49 ± 2.53	22.04 ± 0.19	2.13 ± 0.12	88.26 ± 3.03
IL	0	100	2	4.86 ± 0.28	17.42 ± 0.40	22.27 ± 0.68	45.35 ± 0.56	22.42 ± 0.74	1.43 ± 0.26	88.44 ± 2.54
IL	0	100	4	6.01 ± 0.94	15.59 ± 1.08	21.60 ± 2.02	48.02 ± 2.30	20.89 ± 2.30	1.51 ± 0.28	82.64 ± 2.72
IL	1	100	2	3.99 ± 0.25	18.04 ± 0.53	22.04 ± 0.78	44.44 ± 2.61	22.85 ± 1.86	1.38 ± 0.11	90.07 ± 2.92
IL	2	100	2	4.12 ± 0.39	18.40 ± 0.51	22.53 ± 0.13	47.90 ± 0.06	22.96 ± 0.78	1.62 ± 0.13	87.40 ± 3.19
IL	3	100	2	4.82 ± 0.12	17.04 ± 0.80	21.85 ± 0.68	47.59 ± 2.63	22.71 ± 0.46	1.43 ± 0.04	88.53 ± 3.13

*Data obtained from previous study by Qiu et al. (2012).

Table 3.2. Lignin removal, glucan recovery and xylan recovery from IL-treated biomass.

Pretreatment conditions				Lignin removal	Glucan recovery	Xylan recovery
Solvent	Number of recycles	Temp. (℃)	Time (h)	(%, dry weight basis)		
None	N/A	N/A	N/A	0	100	100
Water*	N/A	120	0.5	2.31	101.96**	100.74**
IL*	0	120	0.5	32.08	91.16	86.03
IL	1	120	0.5	23.53	88.61	83.62
IL	2	120	0.5	21.74	82.31	87.38
IL	0	100	0.5	15.05	86.45	91.90
IL	0	100	1	18.66	86.19	91.37
IL	0	100	2	17.45	92.18	93.13
IL	0	100	4	25.19	91.21	81.09
IL	1	100	2	16.80	92.00	96.67
IL	2	100	2	17.47	96.22	94.26
IL	3	100	2	18.93	96.83	94.43

*Data obtained from previous study by Qiu et al. (2012).
**Considering systematic and random errors, these values were approximate to 100.

pretreated at 120 ℃ for 0.5 h; whereas, the lignin removal of biomass pretreated at 100 ℃ for 2 h did not differ significantly with the increasing number of IL recycles. This discrepancy between the two pretreatment condition groups is probably due to the disparity in lignin extraction ability under different temperatures and residence times. Lee et al. (2009) reported that although lignin continuously accumulated in recycled [EMIM][OAc] by repeating the pretreatment cycle at 90 ℃ for 24 h, the lignin extraction efficiencies remained largely unaffected. This result is in accordance with our observations of biomass pretreated at 100 ℃ for 2 h. A correlation between

pretreatment temperature, residence time and lignin removal efficiency can be seen as higher pretreatment temperature (120 ℃) and shorter residence time (0.5 h) resulted in a decrease in lignin removal efficiency with the increasing number of IL recycles; whereas, a relative lower pretreatment temperature (100 ℃) and longer residence time (2 h) maintained similar lignin removal efficiencies.

The glucan recovery of all IL-treated biomass ranged from 82.31% to 96.83% as shown in Table 3.2. The glucan recovery for pretreatment at 120 ℃ for 0.5 h decreased with the increasing number of IL recycles; whereas, the glucan recovery of pretreatment at 100 ℃ for 2 h increased with the increasing number of IL recycles. The decrease of glucan recovery at higher temperature (120 ℃) was probably due to cellulose degradation taking place during the pretreatment process. Nguyen et al. (2010) reported that the cellulose recovery of rice straw consistently increased with the increasing number of [EMIM][OAc] recycles. It was also pointed out that the constant increase of glucan recovery was due to the accumulation of solubilized cellulose from previous pretreatments (Nguyen et al., 2010), a possible explanation for the increase of glucan recovery observed in our study at the lowest pretreatment temperature (100 ℃).

The xylan recovery of biomass pretreated at 100 ℃, with the exception of 4 h residence time, was higher than biomass pretreated at 120 ℃, an indication that higher pretreatment temperature and longer residence times may lead to degradation of xylan. Li et al. (2010b) reported a 62.6% xylan loss in switchgrass pretreated at 160 ℃ for 3 h with [EMIM][OAc]. Lee et al. (2009) also reported that when the [EMIM][OAc] pretreatment temperature increased from 110 ℃ to 130 ℃ with 1.5 h residence time, the xylan loss in maple wood flour increased from 6% to 26%. The glucan and xylan recoveries of [EMIM][OAc]-treated biomass at 100 ℃ for 2 h

including 0, 1st, 2nd and 3rd recycles (glucan recovery: 92.18%, 92.00%, 96.22%, 96.83%, and xylan recovery: 93.13%, 96.67%, 94.26%, 94.43%, respectively) were higher than both [EMIM][OAc]-pretreated at 120 ℃ for 0.5 h (glucan recovery: 91.16%, xylan recovery: 86.03%) and dilute ammonia-treated (glucan recovery: 91.4%, xylan recovery: 69.9%) energy cane bagasse (Aita et al., 2011; Qiu et al., 2012).

3.3.2 Enzymatic hydrolysis

Cellulose digestibility and hemicellulose digestibility of IL and recycled IL-treated energy cane bagasse are summarized in Fig. 3.1 and Fig. 3.2, respectively. Detailed data on cellulose and hemicellulose digestibilities (Tables A3, A4) is listed in Appendix C and D. A similar pattern was observed in the data obtained for both percent enzymatic digestibilities of cellulose and hemicellulose. However, hemicellulose digestibilities were lower than those obtained for cellulose due to the enzyme mixture used in this study which contained mostly cellulase-degrading enzymes. Higher hemicellulose digestibility could be expected if xylanases were added. A decrease in the enzymatic hydrolysis efficiency was observed for the 1st and 2nd recycled IL-treated biomass at 120 ℃ for 0.5 h as shown in Fig. 3.1 (B) and Fig. 3.2 (B), respectively. Both cellulose and hemicellulose percent digestibilities decreased significantly with the increasing number of IL recycles. A 39.55% cellulose digestibility and a 28.73% hemicellulose digestibility were observed for the 1st recycle followed by 30.34% and 14.66% for the 2nd recycle as compared to 87.01% and 64.25% cellulose and hemicellulose digestibilities observed with the original IL-treated sample, respectively. No further recycles were evaluated due to the low cellulose and hemicellulose digestibilities observed after the 2nd recycle. Nguyen et al. (2010) reported that the glucose conversion of rice straw pretreated at 130 ℃ for 24 h

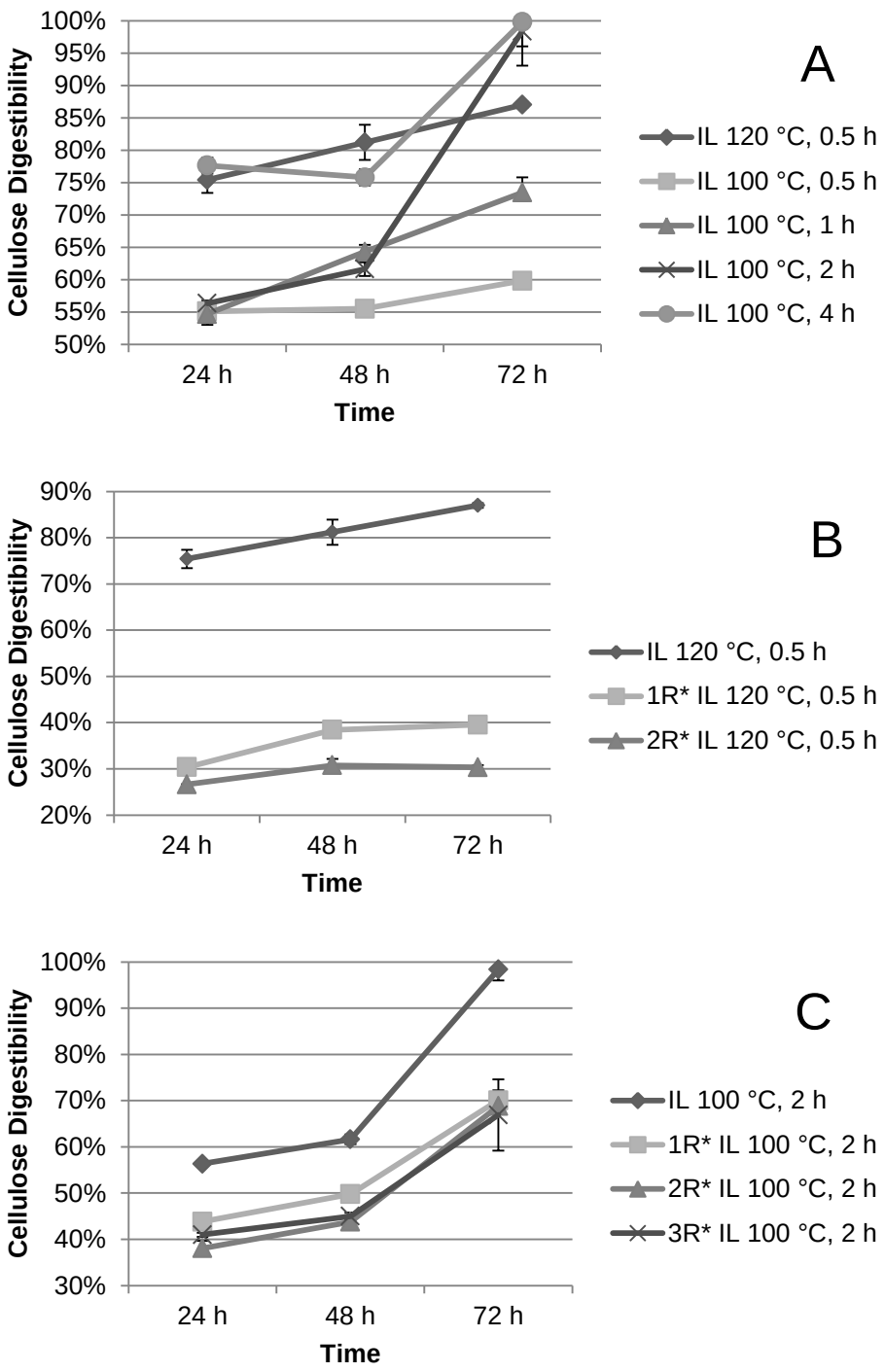

Fig. 3.1. Cellulose Digestibility of (A) Original [EMIM][OAc]-Treated Energy Cane Bagasse at 120 ℃ or 100 ℃ for Different Residence Times; (B) IL, 1st and 2nd Recycled IL-Treated Energy Cane Bagasse at 120 ℃ for 0.5 h; and (C) IL, 1st, 2nd and 3rd Recycled IL-Treated Energy Cane Bagasse at 100 ℃ for 2 h.
*1R, 2R, 3R IL: 1st, 2nd, 3rd recycled ionic liquid (IL).

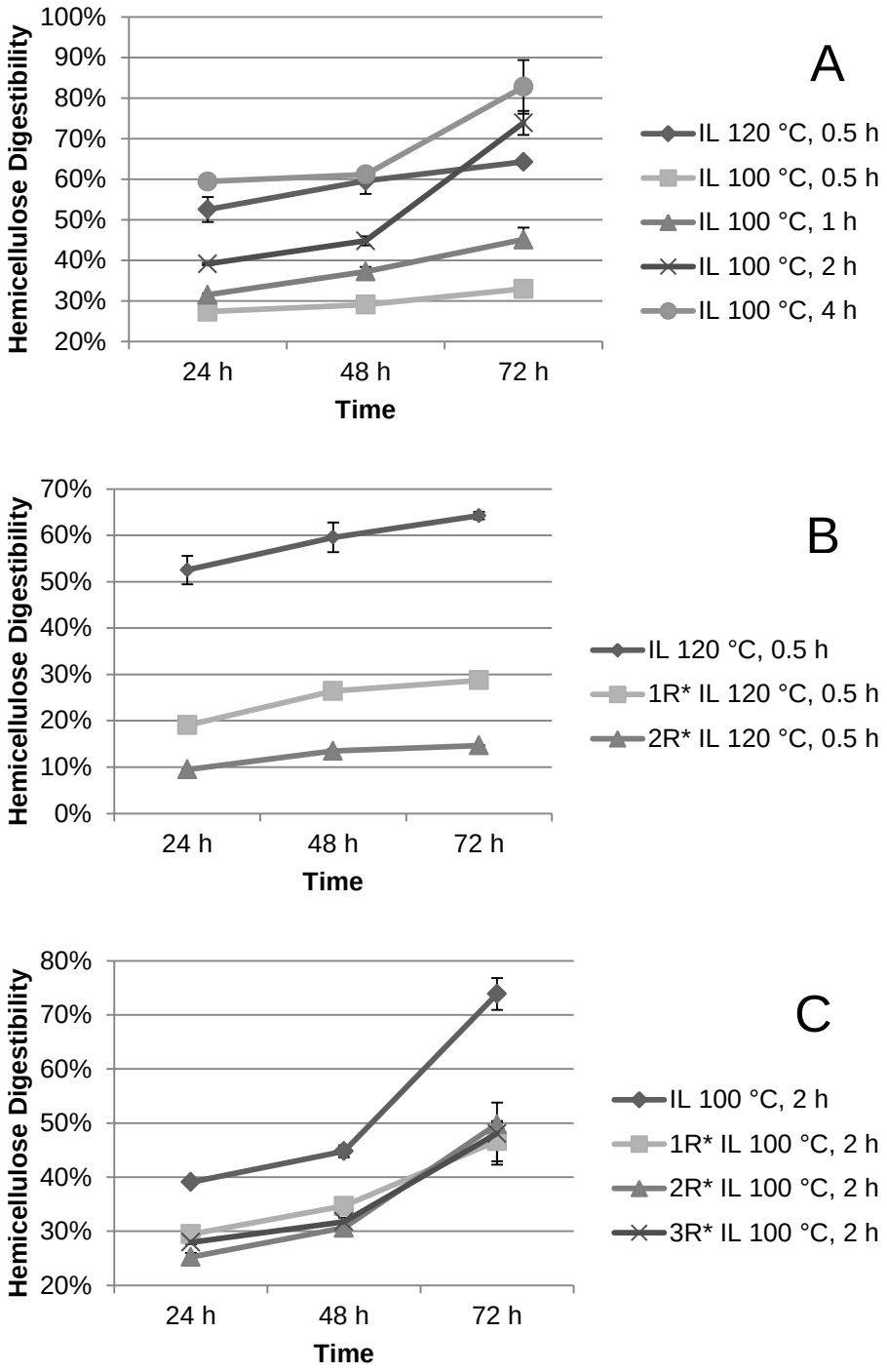

Fig. 3.2. Hemicellulose Digestibility of (A) Original [EMIM][OAc] -Treated Energy Cane Bagasse at 120 ℃ or 100 ℃ for Different Residence Times; (B) IL, 1st and 2nd recycled IL-Treated Energy Cane Bagasse at 120 ℃ for 0.5 h; and (C) IL, 1st, 2nd and 3rd Recycled IL-Treated Energy Cane Bagasse at 100 ℃ for 2 h.
*1R, 2R, 3R IL: 1st, 2nd, 3rd recycled ionic liquid (IL).

declined with increasing numbers of [EMIM][OAc] recycles. Li et al. (2010a) also reported that the amount of glucose released by the enzymatic hydrolysis from *Eucalyptus grandis* decreased by 56% after three recycles with [AMIM][Cl] at 120 ℃ for 5 h as compared to the original IL-treated sample. The decrease in IL pretreatment efficiency could be attributed to the increase of impurities in IL after each recycle. The recycled ILs were observed to be darker than the original IL. Li et al. (2010a) observed the presence of carboxylic acids and aliphatic hydroxyl groups which originated from the decomposition of cellulose and/or hemicellulose post-pretreatment by ^{31}P NMR analysis. After three recycles, the concentration of aliphatic hydroxyl groups increased by 2.3 fold, and phenolic hydroxyl groups which come from lignin degradation were also detected. Lee et al. (2009) also pointed out that the extracted lignin content increased from 6.9 g/kg after original IL pretreatment to 35.6 g/kg after the 4th recycled IL pretreatment, which clearly indicated the accumulation of lignin content with increasing numbers of IL recycles. Although the accumulation of impurities was observed in recycled [EMIM][OAc], similar cellulose digestibilities (1st, 2nd, 3rd, and 4th: 92.1%, 92.7%, 92.7%, and 90.2%, respectively) to that of original IL (95.7%) were observed for wood flour after four recycles pretreatment at 90 ℃ for 24 h.

Percent cellulose and hemicellulose digestibilities of [EMIM][OAc]-treated energy cane at 100 ℃ at various residence times are shown in Fig. 3.1 (A) and Fig. 3.2 (A), respectively. Both percent cellulose digestibility and hemicellulose digestibility gradually increased with the increase in residence time. Although the percent cellulose digestibility at 2 h residence time was lower at both 24 h and 48 h as compared to that of 4 h residence time, the cellulose digestibility at 2 h residence time after 72 h increased to 98.39%, which was comparable to the cellulose digestibility observed at 4 h residence time (99.80%). A similar trend was also observed with

percent hemicellulose digestibility. Therefore, the 2 h residence time was used to further evaluate the effect of recycled IL on energy cane bagasse pretreatment at 100 ℃.

Percent cellulose and hemicellulose digestibilities of energy cane bagasse pretreated by recycled IL at 100 ℃ for 2 h are shown in Fig. 3.1 (C) and Fig. 3.2 (C), respectively. The recycled IL-treated (100 ℃ for 2 h) energy cane bagasse also exhibited a significant decrease of both cellulose and hemicellulose digestibilities as compared to the original IL-treated biomass. Unlike the biomass pretreated at 120 ℃ for 0.5 h as shown in Fig. 3.1 (B) and Fig. 3.2 (B),enzymatic digestibility of biomass pretreated at 100 ℃ for 2 h did not decrease significantly with the increasing number of IL recycles. The enzymatic digestibilities of 1^{st}, 2^{nd} and 3^{rd} recycled IL-treated samples were comparable to each other. Especially after hydrolysis for 72 h, cellulose digestibilities were 70.09%, 68.81% and 66.91% for 1^{st}, 2^{nd} and 3^{rd} recycles, respectively, and the hemicellulose digestibilities were 46.66%, 49.78% and 48.05%, respectively. Compared to cellulose digestibility (39.55% and 30.34%) and hemicellulose digestibility (28.73% and 14.66%) of biomass pretreated by 1^{st} and 2^{nd} recycled IL at 120 ℃ for 0.5 h after hydrolysis for 72 h, the biomass pretreated with recycled IL at 100 ℃ for 2 h exhibited significantly higher digestibilities in terms of both cellulose and hemicellulose. Perhaps, this observation is due to faster decomposition of the ionic liquid at high temperatures. Li et al. (2011b) indicated that the decomposition of [EMIM][OAc] occurs upon heating, and higher temperatures result in more decomposition even for short residence times. Comparing these results with those reported by Lee et al. (2009), Li et al. (2010a) and Nguyen et al. (2010), it can be concluded that high pretreatment temperatures (≥ 120 ℃) are detrimental to the efficiency of recycled ILs pretreatment; whereas, relatively low temperatures (≤100 ℃) could improve the pretreatment efficiency of recycled ILs. However, this trend does not apply to all

biomass and ionic liquids. Li et al. (2009) reported that by the 5th recycle of [EMIM][DEP] for the pretreatment of wheat straw at 130 ℃ for 0.5 h, the yield of reducing sugars was still similar to that of the original IL pretreated sample. Shill et al. (2011) also reported that *Miscanthus* pretreated with 1st and 2nd recycled [EMIM][OAc] at 140 ℃ for 1 h still maintained a glucose conversion of 90%. Therefore, the pretreatment condition that leads to high pretreatment efficiency of recycled IL varies with different biomass and ionic liquids, and properly adjusting pretreatment temperature and residence time could be beneficial in improving the efficiency of recycled IL pretreatment and enzymatic digestibility of pretreated biomass. In this case, decreasing pretreatment temperature from 120 ℃ to 100 ℃ and extending the residence time from 0.5 h to 2 h resulted in significant improvements of the pretreatment efficiency of recycled [EMIM][OAc] on energy cane bagasse. However, further research is still needed to determine optimal pretreatment conditions for both [EMIM][OAc] and recycled [EMIM][OAc] on energy cane bagasse.

3.4 Conclusions

The energy cane bagasse pretreated with recycled [EMIM][OAc] resulted in less lignin removal as compared to the original ionic liquid pretreatment. Pretreatment with recycled IL at 120 ℃ for 0.5 h removed more lignin than pretreatment with recycled IL at 100 ℃ for 2 h. However, the lignin removal efficiency at 120 ℃ for 0.5 h decreased with increasing number of IL recycles; whereas, a relatively low pretreatment temperature (100 ℃) and a longer residence time (2 h) resulted in fixed lignin removal efficiency. The energy cane bagasse pretreated with recycled IL at 100 ℃ for 2 h retained more than 90% percent of both glucan and xylan in each recycle. The enzymatic digestibility decreased with increasing numbers of IL recycles for

pretreatments at 120 ℃ for 0.5 h and at 100 ℃ for 2 h. However, higher digestibilities in terms of both cellulose (70.09%, 68.81% and 66.91%) and hemicellulose (46.66%, 49.78% and 48.05%) were observed with 1st, 2nd and 3rd recycled IL at 100 ℃ for 2 h, respectively, as compared to cellulose digestibilities (39.55% and 30.34%) and hemicellulose digestibilities (28.73% and 14.66%) of 1^{st} and 2^{nd} recycled IL-treated biomass at 120 ℃ for 0.5 h. Decreasing pretreatment temperature from 120 ℃ to 100 ℃ and extending the residence time from 0.5 h to 2 h resulted in significant improvements to the pretreatment efficiency of recycled [EMIM][OAc] on energy cane. This study demonstrated that the recycle of [EMIM][OAc] for energy cane bagasse pretreatment has great potential for further industry application.

CHAPTER 4

OPTIMIZATION OF PROCESSING CONDITIONS FOR THE IONIC LIQUID PRETREATMENT OF ENERGY CANE BAGASSE BY RESPONSE SURFACE METHODOLOGY

4.1 Introduction

Lignocellulosic biomass is an abundant and prospective renewable resource that can be converted to biofuels and bioproducts. It is mainly composed of cellulose, hemicellulose and lignin. The complex and rigid structure is the main obstacle in lignocellulosic biomass degradation and bioconversion into fuels and chemicals (Himmel et al., 2007; Lee et al., 2009). Pretreatment aims to break down the carbohydrates-lignin complex to make cellulose and hemicellulose more susceptible to enzyme degradation (Hendriks and Zeeman, 2009). Long residence times, high energy demand, harsh processing conditions, high processing costs, and environment pollution remain as challenges in current biological, physical, chemical, and physicochemical pretreatment methods (Li et al., 2009; Shill et al., 2011; Zhao et al., 2009). Therefore, new approaches are needed in order to overcome such challenges and be able to develop pretreatment technologies that are efficient, green and cost-effective.

Ionic liquids (ILs) are a group of new organic salts that exist as liquids at relative low temperature (usually below 100 ℃). ILs exhibit excellent physical characteristics including the ability to dissolve polar and non-polar, organic, inorganic, and polymeric compounds (Lee and Lee, 2005). They are generally considered as green solvents which can be potential substitutes for traditional flammable and volatile solvents due to their desirable properties such as low volatility, high thermal stability, non-flammability, and good recyclability (Quijano et al., 2010).

Pretreatment with ILs can reduce the crystallinity of cellulose and partially remove hemicellulose and lignin while not generating degradation products which are inhibitory to enzymes and/or fermenting microorganisms (Dadi et al., 2007; Lee et al., 2009). Furthermore, pretreatment with ILs are less energy demanding, easier to handle and more environmentally friendly than other pretreatment methods such as mechanical milling, steam explosion, acid, base, or organic solvent processes (Rogers and Seddon, 2003; Zhao et al., 2009).

Energy cane, a hybrid of commercial and wild sugarcanes, is a relatively new energy crop being considered as an alternative source of biomass because of its high fiber content and low sucrose (Kim and Day, 2011). Unlike sugarcane, energy cane is more cold tolerant, requires less fertilizer and water input, and requires replanting only every ten years, as compared to every three years for sugarcane (Sierra et al., 2008). In our previous work, 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc])-treated energy cane bagasse resulted in significant lignin removal (32.0%) 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).

Response surface methodology (RSM) is a statistical modeling technique which utilizes quantitative data from an appropriate experimental design to determine a multivariate equation in order to obtain an optimal response (Maache-Rezzoug et al., 2011). RSM has been widely applied to various fields for parameters optimization including food processing and development, microbiology, biotechnology, and agriculture (Karunanithy and Muthukumarappan, 2010). The

main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple parameters and their interaction. Therefore, it requires less labor and time than other optimization approaches (Li et al., 2011a).

This study aimed to optimize ionic liquid ([EMIM][OAc]) pretreatment processing conditions (temperature, residence time and solids loading) and to evaluate the effect of the above mentioned pretreatment processing parameters on the enzymatic hydrolysis of [EMIM][OAc]-treated energy cane bagasse.

4.2 Materials and Methods

4.2.1 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 ℃.

4.2.2 Experimental design and statistical analysis

A Central Composite Design (CCD) was employed to assess the effect of three independent variables (pretreatment temperature, residence time and solids loading) on the responses (glucose yield). The experiments were designed by using the software Design-Expert 8.0.7.1 (State Ease Inc., Minneapolis, MN). The levels of variables are shown in Table 4.1. CCD consists of 2^k factorial points, 2k axial points ($\pm\alpha$), and 6 center points for replications, where k is the number of independent variables. Six replicates at the center point of the design were used to estimate the pure error sum of squares. A total of twenty experiments were performed as shown

in Table 4.2. A second-order polynomial equation (eq. 1) was assumed to approximate the true function and it is presented below:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j + \varepsilon_i \quad (eq.\ 1)$$

Where, Y is the response variable, X_i and X_j are the independent variables, β_0 is the constant coefficient, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, and β_{ij} is the two factors interaction coefficient, ε_i is the random error.

Table 4.1. Coded levels of the pretreatment condition variables tested in the CCD.

Variable	Unit	Coding	Coded level				
			-α*	-1	0	1	+α*
Temperature	℃	A	100.0	108.1	120	131.9	140.0
Residence time	min	B	20.0	28.1	40	51.9	60.0
Solids loading	% (w/w)	C	2.0	3.6	6	8.4	10.0

*α (axial distance) = $\sqrt[4]{N}$, where N is the number of experiments of the factorial design. In this case =1.6818.

The Design-Expert 8.0.7.1 was also used to analyze the CCD experimental results. Each coefficient in the second-order polynomial equation was calculated and the possible interaction effects of the independent variables on the response were obtained. The significance of each coefficient was checked by analysis of variance (ANOVA). Three dimensional response surfaces were drawn by using the same software to illustrate the effect of independent variables on the response.

Table 4.2. Experimental design matrix of CCD.

IL pretreatment run	Experimental variables		
	Temperature (℃)	Residence time (min)	Solids loading (%, w/w)
1	108.1	28.1	3.6
2	131.9	28.1	3.6
3	108.1	51.9	3.6
4	131.9	51.9	3.6
5	108.1	28.1	8.4
6	131.9	28.1	8.4
7	108.1	51.9	8.4
8	131.9	51.9	8.4
9	100.0	40.0	6.0
10	140.0	40.0	6.0
11	120.0	20.0	6.0
12	120.0	60.0	6.0
13	120.0	40.0	2.0
14	120.0	40.0	10.0
15	120.0	40.0	6.0
16	120.0	40.0	6.0
17	120.0	40.0	6.0
18	120.0	40.0	6.0
19	120.0	40.0	6.0
20	120.0	40.0	6.0

4.2.3 Ionic liquid pretreatment

Ionic liquid 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]) (IoLiTec Ionic Liquids Technologies Inc., Tuscsaloosa, AL) was mixed with biomass at different solids loading (%, biomass/ionic liquid) (2%-10%), and pretreated at various temperatures (100 ℃ to 140 ℃) and residence times (20 min to 60 min) as shown in Table 4.2. Post pretreatment, deionized water was added into the IL and biomass solution at a 5:1 (water: IL) ratio to recover the biomass. The ionic liquid/water mixture was separated from biomass 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 collected samples were oven-dried at 45 ℃ until constant weight was achieved.

4.2.4 Chemical composition analysis

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. The percent lignin removal, glucan loss and xlyan loss were calculated as described below:

$$\%\text{Lignin removal} = 1 - \frac{\%\text{Lignin in treated biomass} \times \%\text{Recovered solids}}{\%\text{Lignin in untreated biomass}} \quad (\text{eq. } 2)$$

$$\%\text{Glucan loss} = 1 - \frac{\%\text{Glucan in treated biomass} \times \%\text{Recovered solids}}{\%\text{Glucan in untreated biomass}} \quad (\text{eq. } 3)$$

$$\%\text{Xylan loss} = 1 - \frac{\%\text{Xylan in treated biomass} \times \%\text{Recovered solids}}{\%\text{Xylan in untreated biomass}} \quad (\text{eq. } 4)$$

4.2.5 Enzymatic hydrolysis

A combination of two commercially available enzymes, Spezyme CP (cellulases) (Genencor, Danisco US Inc., Rochester, NY,) and Novozyme 188 (cellobiases) (Sigma-Aldrich,

Inc., St. Louis, MO), were used for the hydrolysis studies. Enzymatic hydrolysis was conducted as described in NREL's LAP TP-510-43629. Briefly, hydrolysis was carried out with 1% (w/v) substrate at 50 ℃, 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.

4.2.6 Chemical analysis of hydrolyzed samples

Collected samples (0 h, 24 h, 48 h, and 72 h) were centrifuged (8000 rpm) with a Spectrafuge 24D (Labnet International Inc., Woodbridge, NJ), filtered (0.2 μm Syringe Filters, Environmental Express, Inc., Mt. Pleasant, SC) and diluted accordingly. Sugars (glucose, cellobiose, arabinose and xylose) from all collected samples were analyzed by high performance liquid chromatography (HPLC) (Agilent 1200 Series) with a BioRad Aminex HPX-87P, lead form, 3000 mm ×7.8 mm (ID), 9 μm column and a differential refractive index detector (G1362A Agilent). Percent theoretical cellulose digestibility was calculated using the equation (eq. 5) provided by NREL's LAP TP-510-43630 as described below:

$$\%\text{Theoretical Cellulose Digestibility} = \frac{[\text{Glucose}] + 1.053\,[\text{Cellobiose}]}{1.111\,f\,[\text{Biomass}]} \times 100\% \quad (\text{eq. } 5)$$

Where, [Glucose] is the residual glucose concentration (g/L), [Cellobiose] is the residual cellobiose concentration (g/L), 1.053 is the multiplication factor that converts cellobiose to equivalent glucose, [Biomass] is the dry biomass concentration at the beginning of the enzymatic

hydrolysis (g/L), f is the cellulose fraction in dry biomass (g/g), 1.111 is the factor that converts cellulose to equivalent glucose.

Since significant solids losses after pretreatment were observed, the glucose yield was calculated based on the chemical composition of native biomass. Native biomass in this case refers to the biomass before pretreatment. Therefore, glucose yield was defined as the mass of glucose released via enzymatic hydrolysis per 100 g of native biomass as shown in the equation (eq. 6) below:

$$\text{Glucose yield} = 100 \times \%\text{Recovered solids} \times 1.111 f \times \%\text{Theoretical cellulose digestibility} \quad \text{(eq. 6)}$$

Where, 100 stands for 100 g of native biomass, %Recovered solids is the percentage of recovered solids after pretreatment, f is the cellulose fraction in dry biomass (g/g), 1.111 is the factor that converts cellulose to equivalent glucose, %Theoretical cellulose digestibility is the percentage of theoretical cellulose digestibility.

4.3 Results and Discussion

4.3.1 Effect of pretreatment on the composition of energy cane bagasse

The chemical composition of energy cane before and after IL pretreatment is summarized in Table 4.3. The initial chemical composition of untreated energy cane bagasse was 41.15% glucan, 21.13% xylan and 24.32% lignin. The results are comparable to those observed by Aita et al. (2011), Kim and Day (2011) and Qiu et al. (2012). Based on this composition and considering the mass increase after the conversion from polysaccharides to monosaccharides, the

maximal theoretical glucose and xylose yields were 45.72 g and 24.00 g per 100 g of energy cane bagasse on dry weight basis, respectively.

Table 4.3. Experimental data for composition analysis and glucose yields.

IL pretreatment run	Experimental variables			Recov-ered solids (%, w/w)[a]	Glucan (%, w/w)[a]	Xylan (%, w/w)[a]	Lignin (%, w/w)[a]	Cellulose digesti-bility[ab] (%, w/w)	Glucose yield[ab] (g/100 g native biomass)
	Temp. (℃)	Time (min)	Solids loading (%, w/w)[a]						
Untreated	N/A	N/A	N/A	100.00	41.15	21.13	24.32	7.93	3.63
1	108.1	28.1	3.6	93.43	41.08	21.74	22.03	68.98	29.42
2	131.9	28.1	3.6	67.94	48.93	12.87	25.94	97.17	35.89
3	108.1	51.9	3.6	89.33	43.25	20.95	21.40	72.78	31.24
4	131.9	51.9	3.6	59.72	52.38	13.29	24.19	97.88	34.02
5	108.1	28.1	8.4	95.45	41.63	21.08	22.03	68.15	30.08
6	131.9	28.1	8.4	74.91	45.88	14.46	27.45	94.17	35.96
7	108.1	51.9	8.4	92.08	43.55	21.39	20.30	76.02	33.87
8	131.9	51.9	8.4	70.69	44.66	15.29	29.64	99.39	34.86
9	100.0	40.0	6.0	93.41	41.97	21.83	22.57	60.41	26.32
10	140.0	40.0	6.0	63.36	55.09	12.59	22.49	88.96	34.49
11	120.0	20.0	6.0	86.02	47.47	21.33	21.44	72.33	32.82
12	120.0	60.0	6.0	70.25	53.13	13.58	21.94	84.61	35.09
13	120.0	40.0	2.0	72.60	55.03	16.37	17.01	76.62	34.01
14	120.0	40.0	10.0	83.27	49.24	19.58	21.57	71.88	32.75
15	120.0	40.0	6.0	77.74	50.53	16.61	22.75	82.17	35.86
16	120.0	40.0	6.0	77.47	50.59	16.27	20.41	80.39	35.00
17	120.0	40.0	6.0	80.18	48.82	16.60	20.13	78.03	33.94
18	120.0	40.0	6.0	80.41	48.81	16.73	23.35	79.66	34.73
19	120.0	40.0	6.0	76.44	51.08	16.48	21.78	80.46	34.91
20	120.0	40.0	6.0	79.22	48.98	17.35	22.80	83.38	35.95

a Dry weight basis.
b Enzymatic hydrolysis after72 h.
N/A= not applicable.

After pretreatment, significant mass loss was observed for all of the IL-treated samples. The linear effects of pretreatment temperature, time and solids loading had a significant impact (p-value < 0.001) on the recovered solids; however, no quadratic effects or interactive effects were observed. The contour plots (Fig.4.1 and Fig. 4.2) describe the effect of pretreatment temperature, time and solids loading on the percent of recovered solids. Each figure keeps one variable constant at central level. The percent of recovered solids increased with the decrease of both time and temperature, and with an increase in solids loading. Among all pretreatment runs, pretreatment at 131.9 ℃ for 51.9 min with 3.6% solids loading resulted in the lowest percent recovered solids (59.72%); whereas, pretreatment at 108.1 ℃ for 28.1 min with 8.4% solids loading resulted in the highest percent recovered solids (95.45%). Similar negative correlations between percent recovered solids and temperature or time was observed by Fu and Mazza (2011b) when pretreating wheat straw with aqueous [EMIM][OAc]. Weerachanchai et al. (2012) also indicated a negative correlation between pretreatment temperature and percent of recovered solids for both cassava pulp residue and rice straw pretreated by three types of ILs ([EMIM][OAc], [EMIM][$DEPO_4$] and [DMIM][$MESO_4$]).

The loss of biomass after pretreatment was mainly attributed to the loss of glucan, xylan and lignin as shown in Table 4.4. Generally, significant degradation of cellulose only takes place under harsh conditions such as high temperatures (Tan and Lee, 2012). The results of our study are in accordance with this observation. A significant positive correlation between temperature and glucan loss was observed. For the pretreatments at over 131 ℃, the glucan loss ranged from 15.18% to 23.98%; whereas, for the samples pretreated at lower temperatures the glucan loss was no more than 10%. However, no significant impact of pretreatment time and solids loading on glucan loss was observed. Fu and Mazza (2011b) also indicated that the pretreatment time

was not a statistically significant variable for the cellulose recovery of wheat straw pretreated by aqueous [EMIM][OAc]; whereas, the cellulose recovery increased with the decrease of pretreatment temperatures from 130 ℃ to 170 ℃. Tan and Lee (2012) pointed out that higher temperatures accelerate both cellulose dissolution and cellulose degradation processes during ionic liquid pretreatment, and the degradation products such as carbohydrate oligomers and monomers are all soluble in the anti-solvent such as water (Brennan et al., 2010). Therefore, higher pretreatment temperatures (> 130 ℃) favor glucan loss.

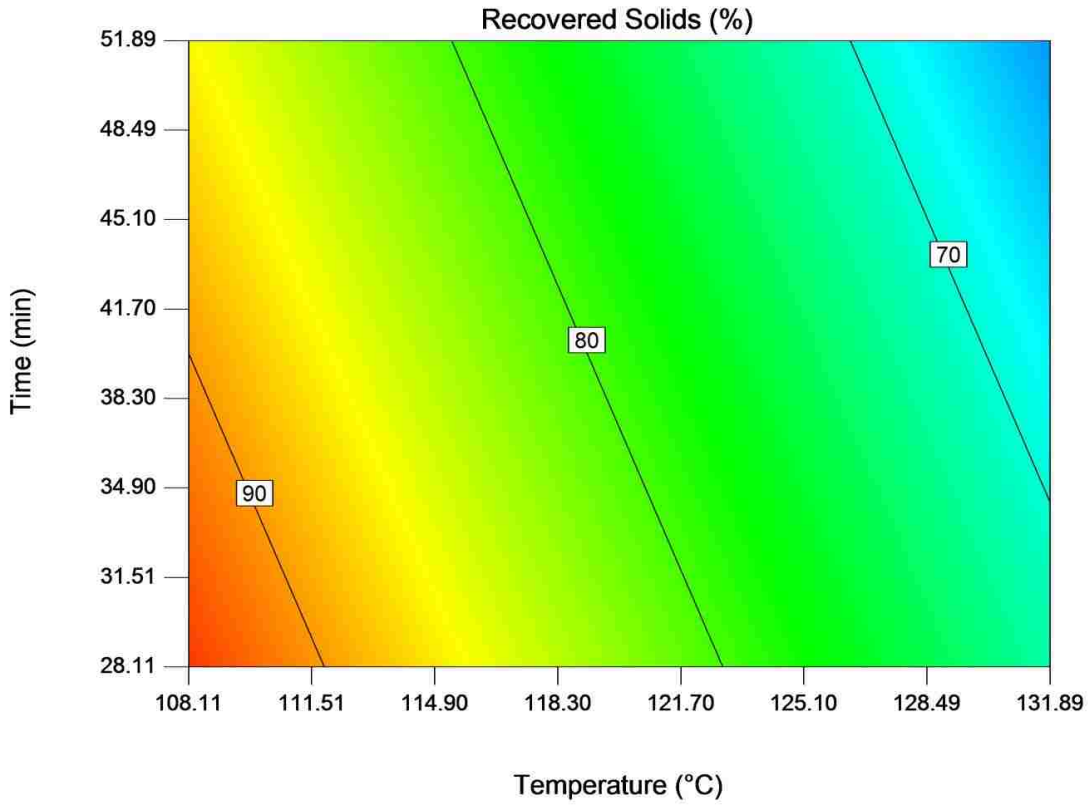

Fig. 4.1. Contour Plot of the Combined Effects of Pretreatment Temperature and Residence Time on Percent Recovered Solids at 6% (w/w) Solids Loading.

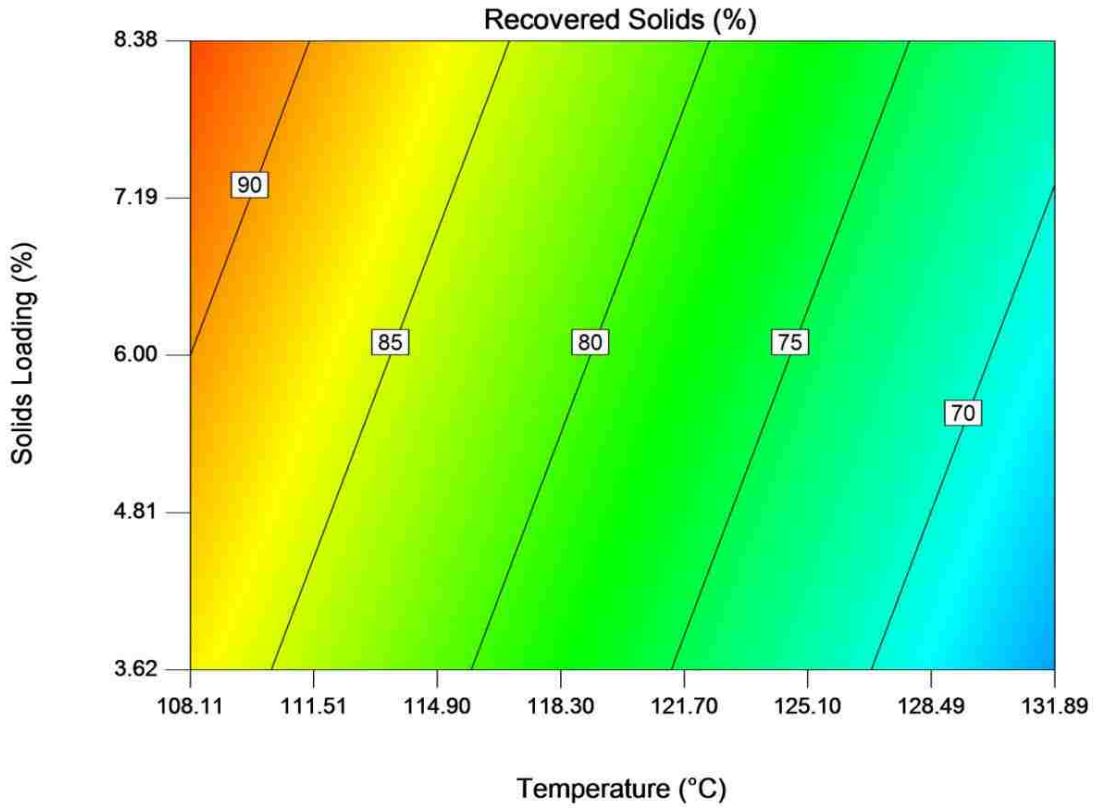

Fig. 4.2. Contour Plot of the Combined Effects of Pretreatment Temperature and Solids Loading on Percent Recovered Solids at Pretreatment time of 40 min.

Pretreatment temperature and residence time had a strong positive correlation with xylan loss in the design space; whereas, solids loading had a relatively weak negative correlation with xylan loss. Xylan loss (48.72% to 62.44%) was more severe than glucan loss for pretreatments placed at over 131 ℃. Fu and Mazza (2011b) reported similar observation where severe xylan loss (83.2%) was observed when high pretreatment temperature (150 ℃) was used for pretreating wheat straw with [EMIM][OAc] for 3 h. Tan and Lee (2012) also found that a significant amount of xylan was loss when high temperature (120 ℃) and longer residence times (1 h - 3 h) were employed for the pretreatment of oil palm frond with [BMIM]Cl. Arora et al. (2010) observed that partial hemicellulose was depolymerized into oligosaccharides after

Table 4.4. Lignin removal, glucan loss and xylan loss in IL-treated biomass.

IL pretreatment run	Experimental variables			Glucan loss (%, w/w)[a]	Xylan loss (%, w/w)[a]	Lignin removal (%, w/w)[a]
	Temp. (℃)	Time (min)	Solids loading (%, w/w)[a]			
1	108.1	28.1	3.6	6.72	3.88	15.35
2	131.9	28.1	3.6	19.22	58.61	27.53
3	108.1	51.9	3.6	6.10	11.41	21.38
4	131.9	51.9	3.6	23.98	62.44	40.61
5	108.1	28.1	8.4	3.44	4.76	13.52
6	131.9	28.1	8.4	16.48	48.72	15.46
7	108.1	51.9	8.4	2.55	6.77	23.14
8	131.9	51.9	8.4	23.28	48.84	13.84
9	100.0	40.0	6.0	4.72	3.49	13.32
10	140.0	40.0	6.0	15.18	62.26	41.41
11	120.0	20.0	6.0	0.76	13.16	24.16
12	120.0	60.0	6.0	9.30	54.86	36.63
13	120.0	40.0	2.0	2.91	43.75	49.21
14	120.0	40.0	10.0	0.36	22.84	26.15
15	120.0	40.0	6.0	4.53	38.90	27.29
16	120.0	40.0	6.0	4.76	40.35	34.97
17	120.0	40.0	6.0	4.86	36.99	33.64
18	120.0	40.0	6.0	4.62	36.35	22.81
19	120.0	40.0	6.0	5.11	40.37	31.54
20	120.0	40.0	6.0	5.69	34.96	25.72

a Dry weight basis.

pretreatment of switchgrass with [EMIM][OAc] at longer residence time (≥ 3 h). Yoon et al. (2012) indicated that IL pretreatment at high temperature (> 135 C °) for a long residence time (> 30 min) might lead to the depolymerization of polysaccharides into their respective monomers, which could not be recovered after pretreatment. Therefore, in this study significant glucan and xylan losses were observed at pretreatments with higher temperatures and longer residence times.

Pretreatment temperature and solids loading had a significant impact on lignin removal, while the correlation between residence time and lignin removal was not significant in the design space. Lignin is one of the main obstacles during enzymatic hydrolysis, which 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). Generally, high lignin removal is favorable. Lignin removal increased with the increase of pretreatment temperature, and decreased with the increase of solids loading. Shill et al. (2011) indicated that the π-π interactions of the IL cation with lignin assisted in lignin solubilization. Tan and Lee (2012) pointed out that dissolved lignin would remain in the ionic liquid when the anti-solvent such as water was added for biomass recovery. Higher temperature should accelerate the solubilization of lignin in ionic liquid, and pretreatment temperatures above the glass transition of lignin (T_g =130-150 ℃) result in better delignification (Li et al., 2011b). Therefore, better delignification was achieved at higher temperatures (> 120 ℃) as observed in this study. Solids loading had a negative correlation with lignin removal. The highest lignin removal (49.21%) was obtained at the lowest solids loading (2%) at 120 ℃. Samples with more than 8% solids loading exhibited less lignin removal (13.52%-26.15%) regardless of pretreatment temperature and residence time. High solids loading can decrease the probability of biomass mixing with the ionic liquid thus limiting heat and mass transfer (Tan and Lee, 2012). Furthermore, the [EMIM][OAc] with dissolved biomass at high solids loading (> 8%) turned into a solid phase after pretreatment with a residence time of more than 20 min, which significantly reduced the mixing rate and limited lignin solubilization. [EMIM][OAc] with dissolved biomass at lower solids loading remained in the liquid phase, which is favorable for lignin extraction. Therefore, higher solids loading resulted in lower lignin removal. Previous studies by Samayam and Schall (2010), Ninomiya et

al. (2012) and Qiu et al. (2012) have suggested that a higher delignification is not necessarily a prerequisite for a higher cellulose digestibility, so high solids loading samples with relative low lignin removal may still result in high hydrolysis yields.

4.3.2 Statistical analysis and model fitting

The experimental data of glucose yield per 100 g of native biomass presented in Table 4.3 was used to fit the model. Based on sequential adjusted sum of square type I, lack of fit tests, adjusted R-squared, and predicted R-squared, the quadratic model was selected. The quadratic model fitted the data significantly but not aliased, and had an insignificant lack of fit. Thus, a second order polynomial equation was used to fit the response as shown in eq. 1. Coefficients β_0, β_i, $\beta_{ii,}$ and β_{ij} were estimated by using Design-Expert 8.0.7.1 software. Analysis of variance (ANOVA) was performed to test the significance of the developed model and the effects of each linear, quadratic and interaction terms on the response. The stepwise selection method was used to choose significant model terms at 95% confidence interval. The significance of each coefficient was determined by the F test. A reduced quadratic model was obtained as shown in the ANOVA table (Table 4.5).

A model is considered significant if its p-value is < 0.05, in this model, the p-value was <0.0001, which indicated that there was only a 0.01% chance that a "Model F-Value" could occur due to noise. The p-value of A and A^2 were both lower than 0.0001, which suggested that pretreatment temperature had great impact on the glucose yield in terms of both linear and quadratic effects. The p-value (0.0063) of AB indicated that the interactive effect of pretreatment temperature and time had significant effect on the glucose yield. Although the linear effect of residence time (B, p-value = 0.0877) did not have a significant effect on the glucose yield at 95%

confidence interval, this term was retained in the model in order to support the model hierarchy because the interactive effect of temperature and residence time (AB) was significant. The linear effect of solids loading (C), quadratic effect of residence time (B^2) and solids loading (C^2), the interactive effects of temperature and solids loading (AC), and residence time and solids loading (BC) were excluded from the model due to statistical insignificance. The final equation (eq. 7) obtained in terms of actual factors after removing the insignificant model terms was as follow:

$$\text{Glucose yield} = -171.17089 + 2.91457 \times \text{Temperature} + 0.95036 \times \text{Residence Time} - 0.00758841 \times \text{Temperature} \times \text{Residence Time} - 0.010113 \times \text{Temperature}^2 \quad \text{(eq. 7)}$$

Table 4.5. Analysis of variance (ANOVA) table for reduced quadratic model.

Source	Sum of square	Degree of freedom	Mean square	F value	p-value Prob > F	
Model	107.56	4	26.89	29.41	< 0.0001	significant
A-Temperature	65.28	1	65.28	71.39	< 0.0001	
B-Residence time	3.05	1	3.05	3.34	0.0877	
AB	9.21	1	9.21	10.08	0.0063	
A^2	30.02	1	30.02	32.83	< 0.0001	
Residual	13.72	15	0.91			
Lack of fit	10.89	10	1.09	1.93	0.2429	not significant
Pure error	2.83	5	0.57			
Corrected total	121.28	19				

Std. dev.	0.96	R-squared	0.8869
Mean	33.56	Adj R-squared	0.8567
C.V. %*	2.85	Pred R-squared	0.8096
PRESS	23.09	Adeq precision	18.9882

* Coefficient of variation.

The p-value (0.2429) of the lack of fit test with an F-value of 1.93 was not significant, which indicated that there was a 24.29% chance that a "Lack of Fit F-value" this large could occur due to noise. The determination coefficient (R^2) was 0.8869, which implied a high correlation between the actual and predicted values as shown in Fig. 4.3. Only 11.31% of the total variance could not be explained by the model. The predicted determination coefficient (Pred R^2) of 0.8096 was in reasonable agreement with the adjusted determination coefficient (Adj R^2) of 0.8567, which also confirmed the significance of the model. The coefficient of variation (C.V.%) was low (2.85%), which indicated the experiments conducted were precise and reliable. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 18.9882 in this model indicated an adequate signal, which implied that this model can be used

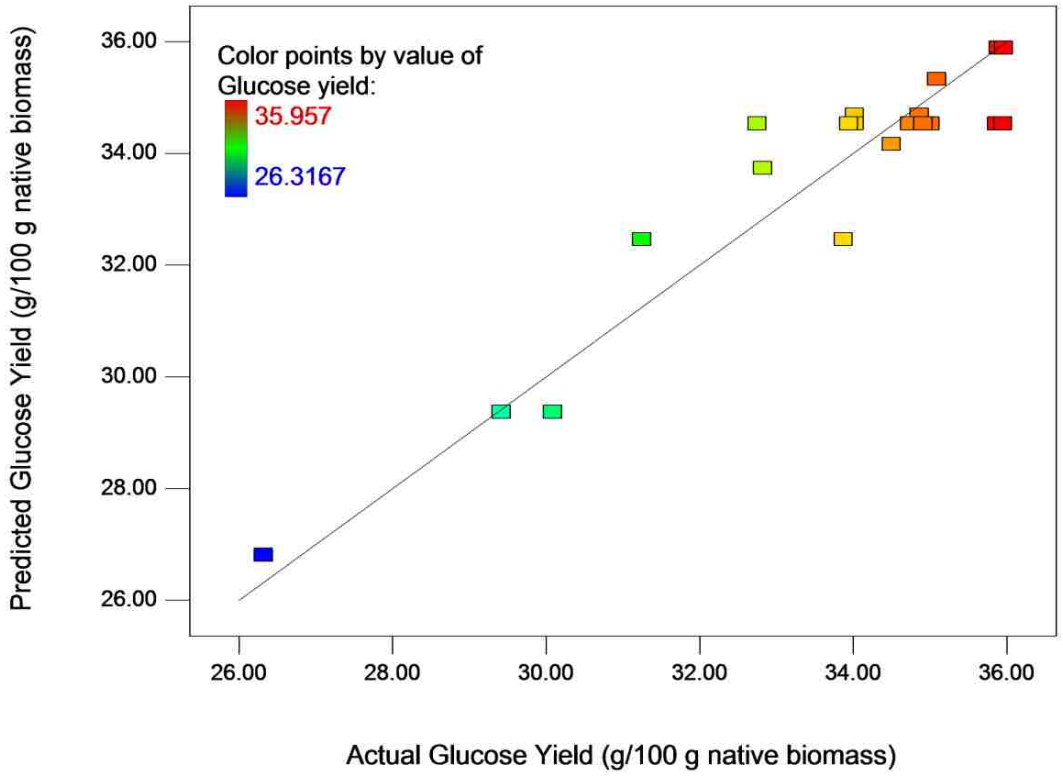

Fig. 4.3. Predicted versus Actual Glucose Yield.

to navigate the design space. The normality of residuals was confirmed by the normal probability plot of the studentized residuals as shown in Fig. 4.4.

These statistical tests demonstrated that the reduced quadratic model presents a decent description of the correlation between the processing variables and the response, and it is adequate to predict the glucose yield under different combinations of pretreatment conditions with the range of design space.

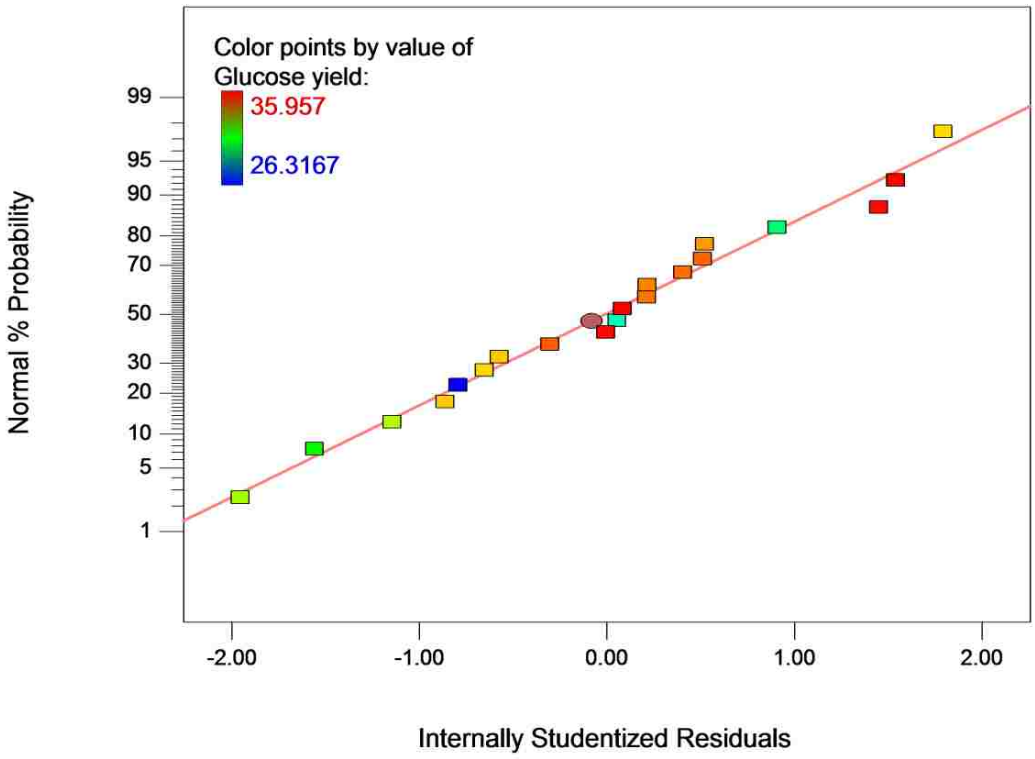

Fig. 4.4. Normal Probability Plot of the Studentized Residuals.

4.3.3 Effect of processing variables on glucose yield

Based on the quadratic model developed, three-dimensional response surface graph were plotted to investigate the interactive effect of pretreatment temperature and residence time with

the range of design space on the glucose yield, while keeping the solids loading constant at central level (6%). The effect of pretreatment temperature and residence time on the glucose yield at 6% solids loading is shown in Fig. 4.5. Pretreatment temperature had significant impact on the glucose yield, and an interactive effect of pretreatment and residence time was also observed. At lower pretreatment temperatures, the glucose yield increased with the increase of pretreatment residence time. At 108.1 ℃, the glucose yield almost linearly increased from 29.37 g to 32.46 g per 100 g native biomass, while the residence time increased from 28.1 min to 51.9 min. This trend was reversed at temperatures higher than 125 ℃. A negative correlation between residence time and glucose yield was observed. The glucose yield decreased from 35.89 g to 34.69 g/100 g native biomass as the residence time increased from 28.1 min to 51.9 min at 131.9 ℃. Temperature had a greater impact on glucose yield, which was also confirmed by previous statistical analysis. With shorter pretreatment residence times, the glucose yield increased significantly with the increase of temperature. When the residence time was set at 28.1 min, the glucose yield increased from 29.37 to 35.89 g/100 g native biomass as the temperature increased from 108.1 ℃ to 131.9 ℃. However, if the residence time was extended to more than 40 min, the plot of temperature to glucose yield changed to a convex shape, and the highest glucose yield shifted to a lower temperature around 121 ℃ to 125 ℃ (Fig. 4.5). Therefore, the highest glucose yield is expected at higher pretreatment temperatures and shorter residence times within the range of the design space.

Tan et al. (2011) also reported that improvement of glucose recovery was observed when oil palm was pretreated with [BMIM]Cl at higher temperature (100 ℃) for shorter residence time (15 min); whereas, prolonged residence time to 60 min at higher temperature led to reduction in glucose recovery. Similarly, Yoon et al. (2012) observed that the reducing sugar

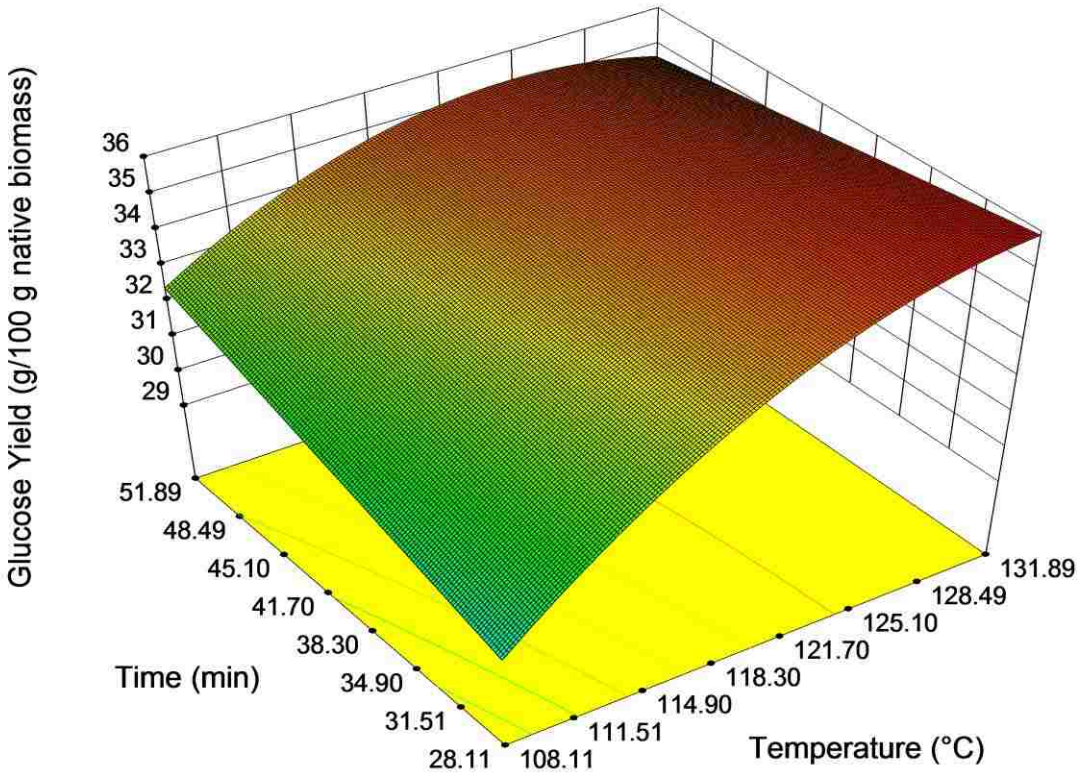

Fig. 4.5. Three-Dimensional Response Surface Plot of the Effects of Pretreatment Temperature and Residence Time on Glucose Yield at 6% (w/w) Solids Loading.

yield of [EMIM][OAc]-treated sugar cane bagasse increased with the increase of residence time at lower temperature (120 ℃); whereas, an increase in residence time resulted in lower reducing sugar yields at higher temperatures (>135 ℃). The higher glucose yield observed at higher pretreatment temperature and shorter residence time can be attributed to several factors: (1) the rate of cellulose dissolution in ILs can be accelerated by increasing the temperature during pretreatment, (2) higher temperature can reduce the viscosity of ILs, which is conducive to the mixing of biomass and ILs, and (3) higher pretreatment temperature results in a destabilization of the hydrogen bonds which tighten the three-dimensional structure of cellulose (Tan et al., 2011; Yoon et al., 2012; Zavrel et al., 2009). However, it has been discussed before that pretreating

biomass at higher temperature for a long residence time could lead to the degradation and loss of polysaccharides (Brennan et al., 2010; Tan and Lee, 2012; Yoon et al., 2012). Higher temperature and longer residence time would also produce overcooked biomass with burnt surface, which has a negative effect on enzymatic hydrolysis (Teramoto et al., 2008). Moreover, pretreatment of energy cane bagasse with [EMIM][OAc] tends to coagulate mixture when pretreatment takes place at high temperatures (> 120 ℃) with a long residence time (> 40 min). This is probably because the increased hydrogen bonds between cellulose and ionic liquid immobilized the ionic liquid. It was difficult to recover the biomass from the coagulated mixture after pretreatment, which not only reduced the amount of recovered solids, but also might have left small amounts of [EMIM][OAc] residual in the recovered biomass even after thorough washing. Turner et al. (2003) and Zhao et al. (2009) indicated that ionic liquid residual can inactivate cellulases and inhibit enzymatic hydrolysis, which would directly result in a decrease of glucose yield. Yoon et al. (2012) reported similar coagulate formation after pretreatment of sugar cane bagasse with [EMIM][OAc] at high temperature and long residence time. Another possible reason for the decrease of glucose yield after pretreatment at high temperature and long residence time is thermal degradation of [EMIM][OAc] at high temperature. Li et al. (2011b) observed that dealkylation occurred upon heating of [EMIM][OAc], and more dealkylation took place at high temperature even for short times. Therefore, in our study more [EMIM][OAc] decomposed at longer residence times (> 30 min) and higher temperatures (> 130 ℃).

Solids loading (3.6%-8.4%) had no significant impact on the glucose yield within the range of design space. Generally, a better pretreatment efficiency is expected at low solids loading, as it has no mixing problem and provides better heat and mass transfer. Tan et al. (2011) argued that high biomass concentration under agitation allowed for more frequent contacts and

collisions between biomass particles. This frequent collision probably compensated for the heat and mass transfer limitation. Therefore, in this study no considerable differences in glucose yields between low solids loading and high solids loading were observed.

4.3.4 Optimization of processing conditions

Design-Expert 8.0.7.1 software was used to predict optimal processing conditions. As discussed in section 4.3.3, the highest glucose yield could be expected at higher pretreatment temperature and shorter residence time with the range of the design space. The pretreatment combination of 131.9 ℃, 28.1 min and solids loading from 3.6% to 8.4% was predicted to have the highest glucose yield of 35.89 g/100 g native biomass with a 95% prediction interval from 33.54 g to 38.24 g/100 g native biomass. Since solids loading did not have a significant impact on the glucose yield, the highest solids loading of 8.4% in the design space was selected as higher solids loadings are preferred during processing. Therefore, the combination of 131 ℃, 28.1 min and 8.4% solids loading was selected as the optimal processing condition for the pretreatment of energy cane bagasse with [EMIM][OAc]. The experimental glucose yield for the optimal processing conditions was 35.96 g/100 g native biomass. There is only a 0.19% difference between the predicted value and the experimental value, which suggests that this model is applicable and accurate with the range of design space.

4.4 Conclusions

The effect of temperature, residence time and solids loading on glucose yields of [EMIM][OAc]-treated energy cane bagasse was assessed in this study by response surface methodology (RSM). A reduced quadratic model was built based on statistical tests. The model presented a suitable description of the correlation between the processing variables and the

response. The model predicted that higher glucose yields could be obtained at higher pretreatment temperatures with shorter residence times regardless of the solids loading. The pretreatment combination of 131.9 ℃, 28.1 min and 8.4% of solids loading was selected as the optimal process condition, which resulted in a glucose yield of 35.96 g glucose per 100 g of native biomass as compared to the predicted value of 35.89. The consistency observed between the predicted value and the experimental value suggests that this model is adequate and accurate to predict the glucose yield with the range of design space. Furthermore, this model can also provide valuable insight for further industry application.

CHAPTER 5

SUMMARY AND FUTURE WORK

Lignocellulosic biomass appears to be a prospective renewable energy resource that can be used for the generation of biofuels and bioproducts. The major concern in lignocellulose conversion is overcoming biomass recalcitrance through pretreatment while still maintaining a green, energy efficient and cost-effective process. Energy cane is a promising energy crop with high fiber content and good cold tolerance traits that requires less fertilizer and water input than sugarcane. This study assessed the use of ionic liquid 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]) as solvent for the pretreatment of energy cane bagasse.

[EMIM][OAc]-treated energy cane bagasse resulted in significant lignin removal (32.0%) with slight glucan and xylan losses (8.8% and 14.0%, respectively), and exhibited significant higher enzymatic digestibility (87.0%, 64.3%) than untreated (5.5%, 2.8%) or water (4.0%, 2.1%) treated energy cane bagasse in terms of both cellulose and hemicellulose yields, respectively. SEM images revealed a loose and disordered structure of biomass post pretreatment. FTIR analysis indicated that IL treated biomass exhibited a significant loss of native cellulose crystalline structure. XRD analysis also confirmed that IL pretreatment resulted in a decrease of crystallinity index from 0.5628 to 0.2452.

The energy cane bagasse pretreated with recycled [EMIM][OAc] resulted in a decrease of lignin removal as compared to bagasse pretreated with the original ionic liquid. Pretreatment with recycled IL at 120 ℃ for 0.5 h removed more lignin than pretreatment with recycled IL at 100 ℃ for 2 h. However, the lignin removal efficiency at 120 ℃ for 0.5 h decreased with increasing number of IL recycles; whereas, relatively low pretreatment temperature (100 ℃) and

longer residence time (2 h) resulted in fixed lignin removal efficiency. The energy cane bagasse pretreated with recycled IL at 100 ℃ for 2 h retained more than 90% of both glucan and xylan in each recycle. The enzymatic digestibility decreased with increasing numbers of IL recycles for pretreatments at 120 ℃ for 0.5 h and at 100 ℃ for 2 h. However, higher digestibilities in terms of both cellulose (70.09%, 68.81% and 66.91%) and hemicellulose (47.71%, 50.90% and 49.13%) were observed with 1st, 2nd and 3rd recycled IL at 100 ℃ for 2 h, respectively, as compared to cellulose digestibilities (39.55% and 30.34%) and hemicellulose digestibilities (29.38% and 14.99%) of 1^{st} and 2^{nd} recycled IL-treated biomass at 120 ℃ for 0.5 h. Decreasing pretreatment temperatures from 120 ℃ to 100 ℃ and extending the residence time from 0.5 h to 2 h resulted in significant improvements to the pretreatment efficiency of recycled [EMIM][OAc] on energy cane bagasse.

Response surface methodology (RSM) model indicated that higher glucose yields could be obtained at higher pretreatment temperatures with shorter residence times regardless of the solids loading. The pretreatment combination of 131.9 ℃, 28.1 min and 8.4% of solids loading was selected as the optimal process condition, which resulted in a glucose yield of 35.96 g glucose per 100 g of native biomass as compared to the predicted value of 35.89. The consistency observed between the predicted value and the experimental value suggests that this model is adequate and accurate to predict the glucose yield with the range of design space.

The work discussed in this thesis presented a systematic study on the use of [EMIM][OAc] as solvent during the pretreatment of energy cane bagasse and demonstrated that [EMIM][OAc] pretreatment for energy cane bagasse has great potential as a pretreatment method. However, further research is still needed to improve the efficiency of this pretreatment on energy crops. Future research work will investigate the optimal pretreatment conditions with

extended design space, assess the efficiency of recycled ionic liquid pretreatment at optimal processing conditions, evaluate hydrolysis yields with a combination of both cellulases and xylanases, and calculate the cost for the entire conversion process and energy returned on energy invested (EROEI).

REFERENCES

Aita, G.A., Salvi, D.A., Walker, M.S. 2011. Enzyme hydrolysis and ethanol fermentation of dilute ammonia pretreated energy cane. *Bioresource Technology*, 102, 4444-4448.

Aita, G.M., Kim, M. 2010. Pretreatment Technologies for the Conversion of Lignocellulosic Materials to Bioethanol. in: *Sustainability of the Sugar and Sugar-Ethanol Industries*, Vol. 1058, American Chemical Society, pp. 117-145.

Ajanovic, A. 2011. Biofuels versus food production: Does biofuels production increase food prices? *Energy*, 36, 2070-2076.

Alvira, P., Tomás-Pejó, E., Ballesteros, M., Negro, M.J. 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. *Bioresource Technology*, 101, 4851-4861.

Beg, Q.K., Kapoor, M., Mahajan, L., Hoondal, G.S. 2001. Microbial xylanases and their industrial applications: a review. *Applied Microbiology And Biotechnology*, 56, 326-338.

Bischoff, K., Gravois, K., Reagan, T., Hoy, J., Kimbeng, C., LaBorde, C., Hawkins, G. 2008. Registration of 'L 79-1002'sugarcane. *Journal of Plant Registrations*, 2, 211-217.

Boddiger, D. 2007. Boosting biofuel crops could threaten food security. *The Lancet*, 370, 923-924.

Brennan, T.C., Datta, S., Blanch, H.W., Simmons, B.A., Holmes, B.M. 2010. Recovery of Sugars from Ionic Liquid Biomass Liquor by Solvent Extraction. *BioEnergy Research*, 3.

Brodeur, G., Yau, E., Badal, K., Collier, J., Ramachandran, K.B., Ramakrishnan, S. 2011. Chemical and Physicochemical Pretreatment of Lignocellulosic Biomass: A Review. *Enzyme Research*, 2011.

Chandra, R.P., Bura, R., Mabee, W.E., Berlin, A., Pan, X., Saddler, J.N. 2007. Substrate pretreatment: the key to effective enzymatic hydrolysis of lignocellulosics? *Advances in biochemical engineering/biotechnology*, 108, 67-93.

Dadi, A., Schall, C., Varanasi, S. 2007. Mitigation of cellulose recalcitrance to enzymatic hydrolysis by ionic liquid pretreatment. *Applied Biochemistry and Biotechnology*, 137-140, 407-421.

Feng, L., Chen, Z.-l. 2008. Research progress on dissolution and functional modification of cellulose in ionic liquids. *Journal Of Molecular Liquids*, 142, 1-5.

Fu, D., Mazza, G. 2011a. Aqueous ionic liquid pretreatment of straw. *Bioresource Technology*, 102, 7008-7011.

Fu, D., Mazza, G. 2011b. Optimization of processing conditions for the pretreatment of wheat straw using aqueous ionic liquid. *Bioresource Technology*, 102, 8003-8010.

Fu, D., Mazza, G., Tamaki, Y. 2010. Lignin Extraction from Straw by Ionic Liquids and Enzymatic Hydrolysis of the Cellulosic Residues. *Journal of Agricultural and Food Chemistry*, 58, 2915-2922.

Fukaya, Y., Hayashi, K., Wada, M., Ohno, H. 2008. Cellulose dissolution with polar ionic liquids under mild conditions: required factors for anions. *Green Chemistry*, 10, 44-46.

Gravois, K., Bischoff, K., Hoy, J., Reagan, T., Laborde, C., Kimbeng, C., Hawkins, G., Pontif, M. 2009. Registration of 'L 99–233'sugarcane. *Journal of Plant Registrations*, 3, 248-252.

Gremos, S., Zarafeta, D., Kekos, D., Kolisis, F. 2011. Direct enzymatic acylation of cellulose pretreated in BMIMCl ionic liquid. *Bioresource Technology*, 102, 1378-1382.

Hendriks, A.T.W.M., Zeeman, G. 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology*, 100, 10-18.

Himmel, M.E., Ding, S.Y., Johnson, D.K., Adney, W.S., Nimlos, M.R., Brady, J.W., Foust, T.D. 2007. Biomass recalcitrance: engineering plants and enzymes for biofuels production. *Science*, 315, 804-807.

Holm, J., Lassi, U. 2011. Ionic liquids in the pretreatment of lignocellulosic biomass. *Kokorin, A. Ionic Liquids: Applications and Perspectives. Finland: InTech*, 551.

Hu, G., Heitmann, J.A., Rojas, O.J. 2008. Feedstock pretreatment strategies for producing ethanol from wood, bark, and forest residues. *BioResources*, 3, 270-294.

Huddleston, J.G., Visser, A.E., Reichert, W.M., Willauer, H.D., Broker, G.A., Rogers, R.D. 2001. Characterization and comparison of hydrophilic and hydrophobic room temperature ionic liquids incorporating the imidazolium cation. *Green Chemistry*, 3, 156-164.

Hurtubise, F.G., Krassig, H. 1960. Classification of Fine Structural Characteristics in Cellulose by Infared Spectroscopy. Use of Potassium Bromide Pellet Technique. *Analytical Chemistry*, 32, 177-181.

Karunanithy, C., Muthukumarappan, K. 2010. Application of response surface methodology to optimize the alkali concentration, corn stover particle size and extruder parameters for maximum sugar recovery. *Biofuel*, 978-953.

Kim, M., Day, D. 2011. Composition of sugar cane, energy cane, and sweet sorghum suitable for ethanol production at Louisiana sugar mills. *Journal of Industrial Microbiology & Biotechnology*, 38, 803-807.

Kim, T.H., Kim, J.S., Sunwoo, C., Lee, Y.Y. 2003. Pretreatment of corn stover by aqueous ammonia. *Bioresource Technology*, 90, 39-47.

Lee, S.H., Doherty, T.V., Linhardt, R.J., Dordick, J.S. 2009. Ionic liquid-mediated selective extraction of lignin from wood leading to enhanced enzymatic cellulose hydrolysis. *Biotechnology and Bioengineering*, 102, 1368-1376.

Lee, S.H., Lee, S.B. 2005. The Hildebrand solubility parameters, cohesive energy densities and internal energies of 1-alkyl-3-methylimidazolium-based room temperature ionic liquids. *Chemical Communications*, 3469-3471.

Li, B., Asikkala, J., Filpponen, I., Argyropoulos, D.S. 2010a. Factors Affecting Wood Dissolution and Regeneration of Ionic Liquids. *Industrial & Engineering Chemistry Research*, 49, 2477-2484.

Li, C., Knierim, B., Manisseri, C., Arora, R., Scheller, H.V., Auer, M., Vogel, K.P., Simmons, B.A., Singh, S. 2010b. Comparison of dilute acid and ionic liquid pretreatment of switchgrass: Biomass recalcitrance, delignification and enzymatic saccharification. *Bioresource Technology*, 101, 4900-6.

Li, J., Nie, S., Yang, C., Qiu, Z., Xie, M. 2011a. Extraction optimization, characterization and bioactivity of crude polysaccharides from Herba Moslae. *Carbohydrate Polymers*, 83, 1201-1206.

Li, Q., He, Y.-C., Xian, M., Jun, G., Xu, X., Yang, J.-M., Li, L.-Z. 2009. Improving enzymatic hydrolysis of wheat straw using ionic liquid 1-ethyl-3-methyl imidazolium diethyl phosphate pretreatment. *Bioresource Technology*, 100, 3570-3575.

Li, W.L.W., Sun, N., Rogers, R.D. 2011b. Rapid dissolution of lignocellulosic biomass in ionic liquids using temperatures above the glass transition of lignin. *Green Chemistry*, 13, 2038-2047.

Liu, C.-Z., Wang, F., Stiles, A.R., Guo, C. 2012. Ionic liquids for biofuel production: Opportunities and challenges. *Applied Energy*, 92, 406-414.

Lynd, L.R., Weimer, P.J., Van Zyl, W.H., Pretorius, I.S. 2002. Microbial cellulose utilization: fundamentals and biotechnology. *Microbiology And Molecular Biology Reviews*, 66, 506-577.

Maache-Rezzoug, Z., Pierre, G., Nouviaire, A., Maugard, T., Rezzoug, S. 2011. Optimizing thermomechanical pretreatment conditions to enhance enzymatic hydrolysis of wheat straw by response surface methodology. *Biomass and Bioenergy*, 35, 3129-3138.

Macrae, R., Robinson, R.K., Sadler, M.J. 1993. *Encyclopaedia of food science, food technology and nutrition*. Academic Press, London.

Mäki-Arvela, P., Anugwom, I., Virtanen, P., Sjöholm, R., Mikkola, J.P. 2010. Dissolution of lignocellulosic materials and its constituents using ionic liquids—A review. *Industrial Crops And Products*, 32, 175-201.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M., Ladisch, M. 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology*, 96, 673-686.

Mueller, S.A., Anderson, J.E., Wallington, T.J. 2011. Impact of biofuel production and other supply and demand factors on food price increases in 2008. *Biomass and Bioenergy*, 35, 1623-1632.

Nelson, M.L., O'Connor, R.T. 1964a. Relation of certain infrared bands to cellulose crystallinity and crystal lattice type. Part II. A new infrared ratio for estimation of crystallinity in celluloses I and II. *Journal of Applied Polymer Science*, 8, 1325-1341.

Nelson, M.L., O'Connor, R.T. 1964b. Relation of certain infrared bands to cellulose crystallinity and crystal latticed type. Part I. Spectra of lattice types I, II, III and of amorphous cellulose. *Journal of Applied Polymer Science*, 8, 1311-1324.

Nguyen, T.-A.D., Kim, K.-R., Han, S.J., Cho, H.Y., Kim, J.W., Park, S.M., Park, J.C., Sim, S.J. 2010. Pretreatment of rice straw with ammonia and ionic liquid for lignocellulose conversion to fermentable sugars. *Bioresource Technology*, 101, 7432-7438.

Ninomiya, K., Kamide, K., Takahashi, K., Shimizu, N. 2012. Enhanced enzymatic saccharification of kenaf powder after ultrasonic pretreatment in ionic liquids at room temperature. *Bioresource Technology*, 103, 259-265.

O'Connor, R.T., DuPré, E.F., Mitcham, D. 1958. Applications of Infrared Absorption Spectroscopy to Investigations of Cotton and Modified Cottons. *Textile Research Journal*, 28, 382-392.

Olivier-Bourbigou, H., Magna, L., Morvan, D. 2010. Ionic liquids and catalysis: Recent progress from knowledge to applications. *Applied Catalysis A: General*, 373, 1-56.

Park, S., Baker, J., Himmel, M., Parilla, P., Johnson, D. 2010. Cellulose crystallinity index: measurement techniques and their impact on interpreting cellulase performance. *Biotechnology for Biofuels*, 3, 10.

Perlack, R.D., Wright, L.L., Turhollow, A.F., Graham, R.L., Stokes, B.J., Erbach, D.C. 2005. Biomass as feedstock for a bioenergy and bioproducts industry: the technical feasibility of a billion-ton annual supply. DTIC Document.

Qiu, Z., Aita, G.M., Walker, M.S. 2012. Effect of ionic liquid pretreatment on the chemical composition, structure and enzymatic hydrolysis of energy cane bagasse. *Bioresource Technology*, 117, 251-256.

Quijano, G., Couvert, A., Amrane, A. 2010. Ionic liquids: Applications and future trends in bioreactor technology. *Bioresource Technology*, 101, 8923-8930.

Rogers, R.D., Seddon, K.R. 2003. Ionic Liquids--Solvents of the Future? *Science*, 302, 792-793.

Samayam, I.P., Schall, C.A. 2010. Saccharification of ionic liquid pretreated biomass with commercial enzyme mixtures. *Bioresource Technology*, 101, 3561-3566.

Sheldon, R.A., Lau, R.M., Sorgedrager, M.J., van Rantwijk, F., Seddon, K.R. 2002. Biocatalysis in ionic liquids. *Green Chemistry*, 4, 147-151.

Shill, K., Padmanabhan, S., Xin, Q., Prausnitz, J.M., Clark, D.S., Blanch, H.W. 2011. Ionic liquid pretreatment of cellulosic biomass: Enzymatic hydrolysis and ionic liquid recycle. *Biotechnology And Bioengineering*, 108, 511-520.

Sierra, R., Smith, A., Granda, C., Holtzapple, M.T. 2008. Producing fuels and chemicals from lignocellulosic biomass. *Chemical Engineering Progress*, 104, 10.

Sims, R.E.H., Mabee, W., Saddler, J.N., Taylor, M. 2010. An overview of second generation biofuel technologies. *Bioresource Technology*, 101, 1570-1580.

Sun, N., Rodriguez, H., Rahman, M., Rogers, R.D. 2011. Where are ionic liquid strategies most suited in the pursuit of chemicals and energy from lignocellulosic biomass? *Chemical Communications*, 47, 1405-1421.

Sun, Y., Cheng, J. 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource Technology*, 83, 1-11.

Tan, H.T., Lee, K.T. 2012. Understanding the impact of ionic liquid pretreatment on biomass and enzymatic hydrolysis. *Chemical Engineering Journal*, 183, 448-458.

Tan, H.T., Lee, K.T., Mohamed, A.R. 2011. Pretreatment of lignocellulosic palm biomass using a solvent-ionic liquid [BMIM]Cl for glucose recovery: An optimisation study using response surface methodology. *Carbohydrate Polymers*, 83, 1862-1868.

Teramoto, Y., Lee, S.H., Endo, T. 2008. Pretreatment of woody and herbaceous biomass for enzymatic saccharification using sulfuric acid-free ethanol cooking. *Bioresource Technology*, 99, 8856-8863.

Turner, M.B., Spear, S.K., Huddleston, J.G., Holbrey, J.D., Rogers, R.D. 2003. Ionic liquid salt-induced inactivation and unfolding of cellulase from Trichoderma reesei. *Green Chemistry*, 5, 443-447.

Weerachanchai, P., Leong, S.S.J., Chang, M.W., Ching, C.B., Lee, J.-M. 2012. Improvement of biomass properties by pretreatment with ionic liquids for bioconversion process. *Bioresource Technology*, 111, 453-459.

Wu, J., Zhang, J., Zhang, H., He, J., Ren, Q., Guo, M. 2004. Homogeneous acetylation of cellulose in a new ionic liquid. *Biomacromolecules*, 5, 266-268.

Yang, B., Wyman, C.E. 2008. Pretreatment: the key to unlocking low-cost cellulosic ethanol. *Biofuels, Bioproducts and Biorefining*, 2, 26-40.

Yoon, L.W., Ang, T.N., Ngoh, G.C., Chua, A.S.M. 2012. Regression analysis on ionic liquid pretreatment of sugarcane bagasse and assessment of structural changes. *Biomass and Bioenergy*, 36, 160-169.

Yoon, L.W., Ngoh, G.C., May Chua, A.S., Hashim, M.A. 2011. Comparison of ionic liquid, acid and alkali pretreatments for sugarcane bagasse enzymatic saccharification. *Journal of Chemical Technology and Biotechnology*, 86, 1342-1348.

Zavrel, M., Bross, D., Funke, M., Büchs, J., Spiess, A.C. 2009. High-throughput screening for ionic liquids dissolving (ligno-)cellulose. *Bioresource Technology*, 100, 2580-2587.

Zhao, H., Baker, G.A., Song, Z., Olubajo, O., Crittle, T., Peters, D. 2008. Designing enzyme-compatible ionic liquids that can dissolve carbohydrates. *Green Chemistry*, 10, 696-705.

Zhao, H., Jones, C.L., Baker, G.A., Xia, S., Olubajo, O., Person, V.N. 2009. Regenerating cellulose from ionic liquids for an accelerated enzymatic hydrolysis. *Journal of Biotechnology*, 139, 47-54.

Zhu, L., O'Dwyer, J.P., Chang, V.S., Granda, C.B., Holtzapple, M.T. 2008. Structural features affecting biomass enzymatic digestibility. *Bioresource Technology*, 99, 3817-3828.

Zhu, S., Wu, Y., Chen, Q., Yu, Z., Wang, C., Jin, S., Ding, Y., Wu, G. 2006. Dissolution of cellulose with ionic liquids and its application: a mini-review. *Green Chemistry*, 8, 325-327.

APPENDIX A

SUPPLEMENTARY DATA (1) FOR CHAPTER 2

Table A1. Cellulose digestibility data for chapter 2.

Pretreatment time (h) / Pretreatment type	% Cellulose digestibility					
	24*	48*	72*	24**	48**	72**
IL treated	64.63	68.43	68.93	75.41	81.19	87.01
Std. dev.	5.96	2.48	4.79	3.99	5.42	1.01
Water treated	2.89	3.33	3.47	2.90	3.66	4.04
Std. dev.	0.01	0.06	0.26	0.01	0.41	0.30
Untreated	2.64	3.23	4.12	3.17	3.73	5.49
Std. dev.	0.01	0.02	0.15	0.09	0.03	0.01

* 15 FPU Spezyme CP/g glucan and 15 CBU Novozyme 188/g glucan.

** 30 FPU Spezyme CP/g glucan and 30 CBU Novozyme 188/g glucan.

APPENDIX B

SUPPLEMENTARY DATA (2) FOR CHAPTER 2

Table A2. Hemicellulose digestibility data for chapter 2.

Pretreatment time (h) / Pretreatment type	% Hemicellulose digestibility					
	24*	48*	72*	24**	48**	72**
IL treated	40.38	43.58	46.47	52.51	59.56	64.25
Std. dev.	0.77	0.54	2.29	6.11	6.42	1.57
Water treated	1.47	1.78	1.90	1.61	2.03	2.06
Std. dev.	0.04	0.01	0.01	0.25	0.04	0.10
Untreated	1.07	1.38	2.35	1.31	2.12	2.84
Std. dev.	0.05	0.05	0.05	0.06	0.17	0.03

* 15 FPU Spezyme CP/g glucan and 15 CBU Novozyme 188/g glucan.

** 30 FPU Spezyme CP/g glucan and 30 CBU Novozyme 188/g glucan.

APPENDIX C

SUPPLEMENTARY DATA (1) FOR CHAPTER 3

Table A3. Cellulose digestibility data for chapter 3.

Biomass component (%, dry weight basis) Pretreatment conditions			% Cellulose digestibility					
Solvent	Temp. (℃)	Time (h)	24 h	Std. dev.	48 h	Std. dev.	72 h	Std. dev.
None	120	0.5	3.17	0.09	3.73	0.03	5.49	0.01
Water	120	0.5	2.90	0.01	3.66	0.41	4.04	0.30
IL	120	0.5	75.41	3.99	81.19	5.42	87.01	1.01
1st recycled IL	120	0.5	30.35	0.55	38.43	2.93	39.55	1.81
2nd recycled IL	120	0.5	26.63	0.17	30.75	2.84	30.34	0.96
IL	100	0.5	55.10	1.21	55.49	2.02	59.81	0.98
IL	100	1	54.67	3.31	64.30	1.55	73.45	2.73
IL	100	2	56.35	0.79	61.64	2.11	98.39	4.73
IL	100	4	77.63	2.49	75.79	2.49	99.80	13.43
1st recycled IL	100	2	43.83	0.72	49.78	2.29	70.09	4.32
2nd recycled IL	100	2	38.07	3.21	43.77	2.93	68.81	2.73
3rd recycled IL	100	2	40.98	0.88	44.99	1.57	66.91	15.44

APPENDIX D

SUPPLEMENTARY DATA (2) FOR CHAPTER 3

Table A4. Hemicellulose digestibility data for chapter 3.

Biomass component (%, dry weight basis) Pretreatment conditions			% Hemicellose digestibility					
Solvent	Temp. (℃)	Time (h)	24 h	Std. dev.	48 h	Std. dev.	72 h	Std. dev.
None	120	0.5	1.31	0.06	2.12	0.17	2.84	0.03
Water	120	0.5	1.61	0.25	2.03	0.04	2.06	0.10
IL	120	0.5	52.51	6.11	59.56	6.42	64.25	1.57
1st recycled IL	120	0.5	19.04	0.65	26.42	1.57	28.73	1.91
2nd recycled IL	120	0.5	9.49	0.06	13.47	0.82	14.66	0.23
IL	100	0.5	27.33	0.58	29.07	1.64	32.94	1.68
IL	100	1	31.48	1.68	37.21	1.63	45.10	1.68
IL	100	2	39.10	0.56	44.79	2.24	73.88	5.90
IL	100	4	59.41	0.25	61.15	0.43	82.77	13.22
1st recycled IL	100	2	29.45	0.04	34.68	0.91	46.66	7.48
2nd recycled IL	100	2	25.25	1.45	30.65	1.32	49.78	0.77
3rd recycled IL	100	2	27.95	0.75	31.76	1.48	48.05	11.52

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