BIORESOURCE TECHNOLOGY

Available online at www.sciencedirect.com

SciVerse ScienceDirect

(This is a sample cover image for this issue. The actual cover is not yet available at this time.)

Effect of ionic liquid pretreatment on the chemical composition, structure and enzymatic hydrolysis of energy cane bagasse

Zenghui Qiu, Giovanna M. Aita *, Michelle S. Walker

Audubon Sugar Institute, Louisiana State University Agricultural Center, Saint Gabriel, LA 70776, USA

HIGHLIGHTS

► 1-Ethyl-3-methylimidazolium acetate is an effective catalyst for pretreatment of energy cane bagasse.
► Significant lignin removal with slight glucan and xylan losses were observed.
► Cellulose and hemicellulose digestibilities were significant.
► Changes in cellulose crystallinity were observed.

GRAPHICAL ABSTRACT
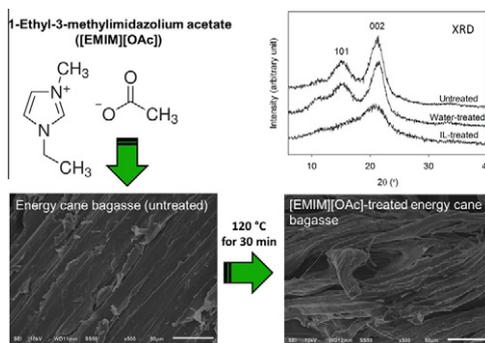
ARTICLE INFO

ABSTRACT

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 evaluated the effect of 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]) for the pretreatment of energy cane bagasse in terms of biomass composition, structural changes and enzymatic digestibility. Energy cane bagasse was pretreated with [EMIM][OAc] (5% (w/w)) at 120 °C for 30 min followed by hydrolysis with commercially available enzymes, Spezyme CP and Novozyme 188. IL-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% and 64.3%) than untreated (5.5% and 2.8%) or water-treated (4.0% and 2.1%) energy cane bagasse in terms of both cellulose and hemicellulose digestibilities, respectively. The enhanced digestibilities of IL-treated biomass can be attributed to delignification and reduction of cellulose crystallinity as confirmed by FTIR and XRD analyses.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Lignocellulose is a suitable and renewable energy resource that can be used for the generation of bio-based transportation fuels and chemicals. Current production of bioethanol (first generation biofuels) relies on the use of sugars from food crops. The sustain-able 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). The recently identified limitations of first generation biofuels have caused great emphasis on second generation biofuels produced from lignocellulosic biomass (Sims et al., 2010). The polysaccharides (hemicellulose and cellulose) present in native 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 to enzymatic hydrolysis. Processing shortages such as long residence time, high energy demand, high cost, and environment pollution exist in current biological, physical, 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).

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 easily 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 °C), 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 10 years, compared to every 3 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 aims 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. Methods

2.1. Biomass

Energy cane (L79-1002) was harvested at the Louisiana State University Agricultural Center Sugar Research Station located in St. Gabriel, LA. Leaves and roots were removed and the stalks were crushed in a roller press (Farrel Company, Ansonia, CT) three times to extract the juice. The remaining crushed fibers (bagasse) were stored at -20 °C.

2.2. Ionic liquid pretreatment

Ionic liquid 1-ethyl-3-methylimidazolium acetate ([EMIM]-[OAc]) (Sigma–Aldrich Inc., St. Louis, MO) was mixed with biomass at a 20:1 ratio and heated to 120 °C for 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 was prepared by combining water and bagasse at a 20:1 ratio and by heating the mixture to 120 °C for 30 min. Samples were oven dried at 45 °C and stored in sealed bags at ambient temperature.

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.

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.

2.5. 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 and 200 mA. The measurements were performed at the double crystal monochromator 7.5 T 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 Å 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 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 s 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):

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

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

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.

2.7. Enzymatic hydrolysis

A combination of two commercially available enzymes, Spezyme CP (cellulases) (Genencor, Danisco US Inc., Rochester, NY) and Novozyme 188 (cellobiases) (Sigma Aldrich, 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 °C, in 0.1 M sodium citrate buffer at pH 4.8 in a shaker incubator (Amerex Instruments Inc., Lafayette, CA) at 150 rpm. The substrates were hydrolyzed with Spezyme CP at 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.

2.8. Chemical analysis of hydrolyzed samples

Collected samples (0 h, 24 h, 48 h and 72 h) were centrifuged (8000 rpm) with 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 (P), lead form, 3000 × 7.8 mm (ID), 9 μm column and a differential refractive index detector (G1362A Agilent). Percent theoretical yields for cellulose and hemicellulose were calculated using the equations provided by NREL's LAP TP-510-43630 as described below.

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

$$\% \text{ Theoretical Hemicellulose Yield} = \frac{[\text{Xylose}]0.9 + 0.9[\text{Arabinose}]}{1.111f[\text{Biomass}]} \times 100\% \tag{1}$$

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

3. Results and discussion

3.1. Effect of IL pretreatment on biomass composition

The chemical composition of untreated, water-treated and IL-treated energy cane bagasse is summarized in Table 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

Table 1
Chemical composition of untreated, water-treated and [EMIM][OAc]-treated energy cane bagasse.

Biomass component (%, dry 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.

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, C–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 °C 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 °C for 2 h. Another study with switchgrass reported a 69.2% total lignin removal using [EMIM][OAc] at 160 °C for 3 h (Li et al., 2010). 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 and 160 °C) and longer retention times (1.5 and 3 h) used.

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 °C for 30 min. Li et al. (2010) reported that the loss of xylan in pretreated switchgrass was 62.6% at 160 °C for 3 h. Xylan losses of 6% and 26% had been reported in Maple wood flour pretreated at 110 and 130 °C for 1.5 h, respectively (Lee et al., 2009).

3.2. Effect of IL pretreatment on lignocellulose structure

3.2.1. Fourier transform infrared spectroscopy (FTIR)

FTIR analysis was conducted to examine the cellulose structure of untreated, water-treated and IL-treated samples (Fig. A1). Two infrared ratios related to cellulose structure were calculated: (1)

Table 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.

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) and (2) α 1373 cm^{-1}/α 2917 cm^{-1}, the ratio of peak areas at 1373 and 2917 cm^{-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. LOI decreased from 0.9593 to 0.3718 and TOI decreased from 0.4057 to 0.1937 in IL-treated bagasse; whereas, water-treated samples just had a slightly 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), switch grass (Li et al., 2010), straw (Fu and Mazza, 2011), sugar cane (Yoon et al., 2011) and kenaf powder (Ninomiya et al., 2012) have also been reported after pretreatment with ILs.

3.2.2. X-ray powder diffraction (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 (1 0 1) and (0 0 2) lattice planes of crystalline cellulose type I (Fig. A2). After IL pretreatment, the peak (1 0 1) disappeared and the peak (0 0 2) became broader and weaker (Fig. A2C). 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. 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 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 anion and cation 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.

3.2.3. Scanning electron microscopy (SEM)

SEM images of untreated, water-treated and IL-treated energy cane bagasse are shown in Fig. A3. Both untreated and water-treated biomass showed compact, ordered and rigid fibril structures (Fig. A3A and A3B). After pretreatment of energy cane bagasse with ionic liquid, the structure became loose, disordered and curly (Fig. A3C). This was probably due to the removal of lignin and decrease of cellulose crystallinity, which have already been confirmed by FTIR and XRD analysis.

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. 1. Significantly higher cellulose digestibility (64.6%, 68.4% and 68.9%) was 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 Spezyme CP at 30 FPU/g glucan and Novozyme 188 at 30 CBU/g glucan at 24 h, 48 h and 72 h, respectively. 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) even obtained 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
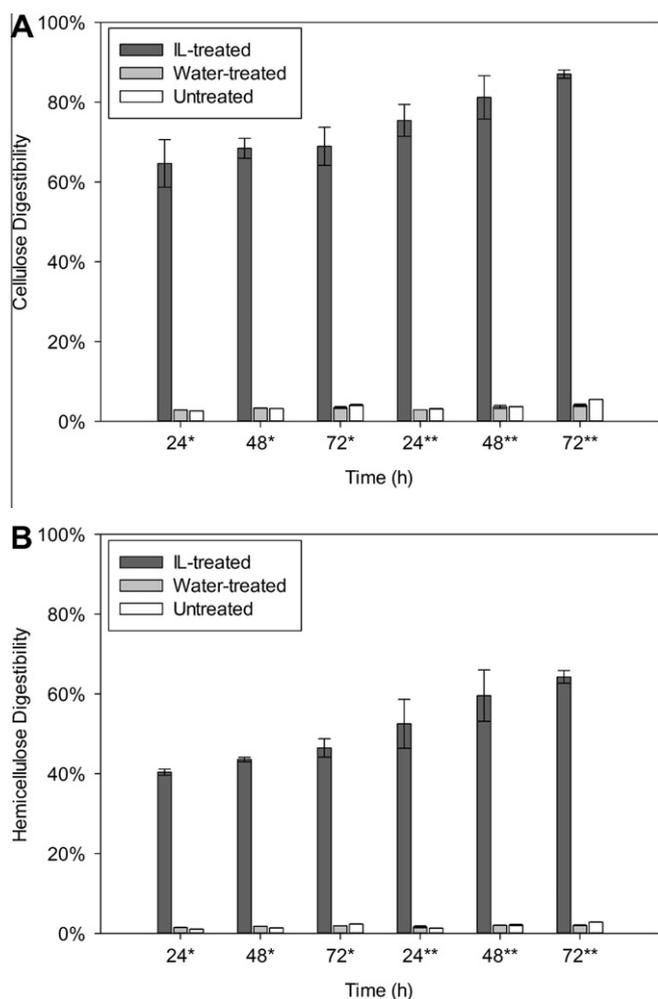
Fig. 1. (A) Cellulose and (B) hemicellulose digestibility of [EMIM][OAc]-treated, water-treated and untreated energy cane bagasse at two enzyme loadings. *15 FPU Spezyme CP/g glucan and 15 CBU Novozyme 188/g glucan. **30 FPU Spezyme CP/g glucan and 30 CBU Novozyme 188/g glucan.

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 and the reduction in cellulose crystallinity.

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 digestibility (87.0% and 64.3%) than untreated (5.5% and 2.8%) or water (4.0% and 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.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2012.04.070.

References

Aita, G.A., Salvi, D.A., Walker, M.S., 2011. Enzyme hydrolysis and ethanol fermentation of dilute ammonia pretreated energy cane. Bioresour. Technol. 102, 4444–4448.

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. Bioresour. Technol. 101, 4851–4861.

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

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? Adv. Biochem. Eng./Biotechnol. 108, 67–93.

Cheng, G., Varanasi, P., Li, C., Liu, H., Melnichenko, Y.B., Simmons, B.A., Kent, M.S., Singh, S., 2011. Transition of cellulose crystalline structure and surface morphology of biomass as a function of ionic liquid pretreatment and its relation to enzymatic hydrolysis. Biomacromolecules 12, 933–941.

Dadi, A., Schall, C., Varanasi, S., 2007. Mitigation of cellulose recalcitrance to enzymatic hydrolysis by ionic liquid pretreatment. Appl. Biochem. Biotechnol. 137–140, 407–421.

Feng, L., Chen, Z.-l., 2008. Research progress on dissolution and functional modification of cellulose in ionic liquids. J. Mol. Liq. 142, 1–5.

Fu, D., Mazza, G., 2011. Aqueous ionic liquid pretreatment of straw. Bioresour. Technol. 102, 7008–7011.

Fu, D., Mazza, G., Tamaki, Y., 2010. Lignin extraction from straw by ionic liquids and enzymatic hydrolysis of the cellulosic residues. J. Agric. Food Chem. 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 Chem. 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. J. Plant Reg. 3, 248–252.

Gremos, S., Zarafeta, D., Kekos, D., Kolisis, F., 2011. Direct enzymatic acylation of cellulose pretreated in BMIMCl ionic liquid. Bioresour. Technol. 102, 1378–1382.

Hurtubise, F.G., Krassig, H., 1960. Classification of fine structural characteristics in cellulose by infrared spectroscopy. Use of potassium bromide pellet technique. Anal. Chem. 32, 177–181.

Kim, M., Day, D., 2011. Composition of sugar cane, energy cane, and sweet sorghum suitable for ethanol production at Louisiana sugar mills. J. Ind. Microbiol. Biotechnol. 38, 803–807.

Kim, T.H., Kim, J.S., Sunwoo, C., Lee, Y.Y., 2003. Pretreatment of corn stover by aqueous ammonia. Bioresour. Technol. 90, 39–47.

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. Chem. Commun. 27, 3469–3471.

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. Biotechnol. Bioeng. 102, 1368–1376.

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. Bioresour. Technol. 100, 3570–3575.

Li, C., Knierim, B., Manisseri, C., Arora, R., Scheller, H.V., Auer, M., Vogel, K.P., Simmons, B.A., Singh, S., 2010. Comparison of dilute acid and ionic liquid pretreatment of switchgrass: biomass recalcitrance, delignification and enzymatic saccharification. Bioresour. Technol. 101, 4900–4906.

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. J. Appl. Polym. Sci. 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. J. Appl. Polym. Sci. 8, 1311–1324.

Ninomiya, K., Kamide, K., Takahashi, K., Shimizu, N., 2012. Enhanced enzymatic saccharification of kenaf powder after ultrasonic pretreatment in ionic liquids at room temperature. Bioresour. Technol. 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. Text. Res. J. 28, 382–392.

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

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. Bioresour. Technol. 101, 3561–3566.

Sheldon, R.A., Lau, R.M., Sorgedrager, M.J., van Rantwijk, F., Seddon, K.R., 2002. Biocatalysis in ionic liquids. Green Chem. 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. Biotechnol. Bioeng. 108, 511–520.

Sierra, R., Smith, A., Granda, C., Holtzapple, M.T., 2008. Producing fuels and chemicals from lignocellulosic biomass. Chem. Eng. Prog. 104, 10.

Sims, R.E.H., Mabee, W., Saddler, J.N., Taylor, M., 2010. An overview of second generation biofuel technologies. Bioresour. Technol. 101, 1570–1580.

Yang, B., Wyman, C.E., 2008. Pretreatment: the key to unlocking low-cost cellulosic ethanol. Biofuels, Bioprod. Biorefin. 2, 26–40.

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. J. Chem. Technol. Biotechnol. 86, 1342–1348.

Zhao, H., Baker, G.A., Song, Z., Olubajo, O., Crittle, T., Peters, D., 2008. Designing enzyme-compatible ionic liquids that can dissolve carbohydrates. Green Chem. 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. J. Biotechnol. 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. Bioresour. Technol. 99, 3817–3828.