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

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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.

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-
lignin structure and disrupting the crystalline structure of cellulose
to make cellulose and hemicellulose available to enzymatic hydro-
lysis. Processing shortages such as long residence time, high energy
demand, high cost, and environment pollution exist in current bio-
logical, physical, chemical and physicochemical pretreatment
methods (Shill et al., 2011; Zhao et al., 2009). Therefore, the major
concern in lignocellulose conversion is overcoming biomass recal-
citrance 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 advan-
tages 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, for-
mate, 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, devel-
oped in collaboration by the United States Department of Agricul-
ture–Agricultural Research Service (USDA–ARS) in Houma, LA
and stored in sealed bags at ambient temperature.

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 re-
cover 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, mannann, lignin,
ethanol extractives and ash content following Laboratory Analyti-
cal Procedures (LAP TP-510-42618, 42619, 42622) as documented
by the National Renewable Energy Laboratory (NREL). NREL refer-
ence material (8491 sugarcane bagasse) was analyzed as an inter-
 nal 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 monochro-
mator 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 A 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-back-
ground plates (50 μm depth) coated with a very thin layer of vac-
uum 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 (Crl) was calculated using the formula as de-
scribed by Cheng et al. (2011):

\[ CRI = (I_{002} - I_{am}) / I_{002} \]

where \( I_{002} \) is the scattered intensity at the main peak for cellul-
ose I; \( I_{am} \) is the scattered intensity due to the amorphous portion
evaluated as the minimum intensity between the main and sec-
ondary 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 (celllobiases) (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, celllobiose, 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 column and a differential refractometer 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.

\[
\text{% Theoretical Cellulose Yield} = \left( \frac{\text{Glucose} + 1.053[\text{Celllobiose}]}{1.111[\text{Biomass}]} \right) \times 100\%
\]

\[
\text{% Theoretical Hemicellulose Yield} = \left( \frac{\text{Xylose}:0.9 + 0.9[\text{Arabinose}]}{1.111[\text{Biomass}]} \right) \times 100\%
\]

where [Glucose], residual glucose concentration (g/L); [Celllobiose], residual celllobiose concentration (g/L); 1.053, multiplication factor that converts celllobiose 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); [x], residual xylene 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 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 extract 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).

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)
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 CH₂ scissoring motion (Nelson and O’Connor, 1964b); the 896 cm⁻¹ band indicates the vibrational mode involving C₁ 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 CH₂ 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 0.4057 and TCI 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), switchgrass (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 2θ = 15° and 21°, which correspond to (101) and (002) lattice planes of crystalline cellulose type I (Fig. A2). After IL pretreatment, the peak (101) disappeared and the peak (002) 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 qualitative 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.

### 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 crystal-line 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 and Novozyme 188 at 30 CBUs 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.

Hemicellulosic digestibilities were lower than those observed for cellulose since the enzyme mixture used in this study contained mostly cellulase-degrading enzymes. Hemicellulosic 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

### Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>LOI (1426/896 cm⁻¹)</th>
<th>TCI (1373/2917 cm⁻¹)</th>
<th>CrI (XRD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0.9593</td>
<td>0.4057</td>
<td>0.5628</td>
</tr>
<tr>
<td>Water-treated</td>
<td>0.8174</td>
<td>0.3747</td>
<td>0.5338</td>
</tr>
<tr>
<td>IL-treated</td>
<td>0.3718</td>
<td>0.1937</td>
<td>0.2452</td>
</tr>
</tbody>
</table>

LOI: lateral order index or crystallinity index based on FTIR.
TCI: total crystallinity index based on FTIR.
CrI: crystallinity index based on XRD.
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.

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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


