Factors Governing the Pretreatment Process of Lignocellulosic Biomass in an Acidic Pyrrolidonium Ionic Liquid

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Ionic liquid (IL)-assisted pretreatment is an important step in biochemical conversion of lignocellulosic biomass into biofuels. Design for low-cost ILs that efficiently work at a relatively low pretreatment temperature with a short processing time is of great interest. In this work, a functional acidic ionic liquid, 1-H-N-methyl-2-pyrrolidonium chloride ([Hnmp]Cl), was prepared by a simple synthetic procedure in a cost-effective manner and was then investigated for use in the pretreatment of lignocellulosic biomass. Factors including temperature, time, ratio of biomass to ionic liquid, and water content were studied to determine their impact on the pretreatment of lignocellulose; 91.39% of lignin content was recovered when the corn stalk was pretreated by pure [Hnmp]Cl at 100 °C for 45 min with a biomass loading of 5%. The highest glucose yield attained was 93.20% and the highest cellobiose yield was 18.76%, when the ratio of biomass to water was kept at 1:4. The pretreatment efficacy was dependent on the pretreatment temperature and processing time, which was more efficient for pure ionic liquid with respect to lignin recovery from lignocelluloses. However, more efficient enzymatic saccharification of cellulose-rich materials was achieved with aqueous ionic liquid.

Keywords: Ionic liquids; Lignocellulosic Biomass; Pretreatment

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INTRODUCTION

The conversion of lignocellulosic biomass into useful chemical compounds is an important goal of green and sustainable chemistry. Lignocelluloses are primarily composed of cellulose, hemicelluloses, and lignin. Cellulose is a large linear polymer consisting of glucose units that are linked by 1,4-β-D-glycosidic bonds (Zhu *et al.* 2015). Hemicelluloses are a group of polysaccharides that includes xylose, mannose, and arabinose. Lignin is an aromatic polymer consisting of guaiacyl, syringyl, and *p*-hydroxyphenyl subunits (Sun *et al*. 2009). These three main components form a complex structure of lignocelluloses. In a typical biomass conversion process, the raw materials should be pretreated to remove lignin and to improve the accessibility of cellulose and hemicelluloses for further conversion into monosaccharides. A number of pretreatment processes, such as steam explosion, ammonia fiber expansion, acid hydrolysis, and ionic liquid (IL) pretreatment, are widely used.

Ionic liquids are generally defined as organic salts that melt below 100 °C and have received significant attention because of their advantages, such as widely accessible temperature range, low volatility, thermal stability, and lack of flammability (Ha *et al*. 2011). Consequently, ILs are considered to be of great interest as potential alternatives to traditional solvents for lignocellulosic pretreatment processes. A variety of ILs, like choline, imidazolium, pyridinium, ammonium, and phosphonium-based cations, along with alkyl, etheric, or allylic side chains coupled to a number of anions, such as chloride, bromide, acetate, or phosphonate, have been reported for the pretreatment of lignocellulosic biomass by various researchers (Lee *et al*. 2009; Haykir *et al*. 2013; Kumar *et al.* 2016). The ILs are regarded as applicable green solvents for lignocellulosic biomasses such as switchgrass (Singh *et al*. 2009; Cheng *et al*. 2011; Groff *et al*. 2013), wheat straw (Liu *et al*. 2006; Li *et al*. 2009), rice straw (Liu *et al*. 2012; Hou *et al*. 2013), corn straw (Zhang *et al*. 2015), wood (Cheng *et al*. 2011; Goshadrou *et al*. 2013; Sun *et al*. 2013), and bagasse (Bian *et al*. 2014).

Although the pretreatment of lignocelluloses by imidazolium-based ionic liquids has been reported, it requires high pretreatment temperatures and long retention times. In this work, the pretreatment of corn stalk was carried out in 1-H-N-methyl-2-pyrrolidonium chloride ([Hnmp]Cl) under various pretreatment conditions. [Hnmp]Cl worked efficiently at a relatively low temperature with a short processing time. Effects of experimental parameters such as pretreatment temperature, pretreatment time, ratio of biomass to ionic liquid, and water content on the recovery of lignin, as well as the enzymatic saccharification of lignocellulosic biomass, were studied (Tye *et al.* 2016).

EXPERIMENTAL

Materials

All chemicals and the cellulase (10,000 U/g) from *Aspergillus niger* were purchased from Aladin Company (China) and are used as received. The lignocellulosic biomass (corn stalk) was obtained from a local farm in Harbin City (China), ground in a cutter mill, passed through 40-mesh sieves, and then dried at 40 °C for 48 h. Corn stalk consisted of 42.95% cellulose, 19.88% hemicelluloses, and 22.19% lignin, which was determined according to the standard method from the National Renewable Energy Laboratory (NREL) (Sluiter *et al.* 2011).

Synthesis of 1-H-N-Methyl-2-Pyrrolidonium Chloride ([Hnmp]Cl)

Hydrochloric acid (0.1 mol) was added dropwise into N-methyl pyrrolidone (0.1 mol) kept in an ice bath under stirring. After the addition of HCL, the reaction was allowed to proceed at room temperature for 24 h under stirring. After that, the resulting product was washed three times with 20 mL of ethyl acetate. The ethyl acetate was then removed by rotary evaporation at 60 °C for 30 min, and the product was then dried in a vacuum at 60 °C for 24 h to give [Hnmp]Cl.

^{1}H nuclear magnetic resonance spectroscopy (NMR) and ^{13}C NMR data for IL were recorded using a Bruker Avance 400 MHz spectrometer (Bruker, Germany) with DMSO-d6 as the solvent. [Hnmp]Cl: ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.881 (q, 2H), 2.173 (t, 2H), 2.678 (s, 3H), 3.293 (t, 2H), 9.822 (s, 1H) (Ma *et al*. 2016). [Hnmp]Cl: ^{13}C NMR (400 MHz, DMSO-d_6): δ 17.612 (s, 1C), 29.538 (s, 1C), 30.502 (s, 1C), 49.140 (s, 1C), 175.373 (s, 1C).

Ionic Liquid Pretreatment of Corn Stalk

Corn stalk was added portion-wise into an aqueous [Hnmp]Cl solution in a 100-mL round-bottomed flask. The mixture was stirred at various temperatures for an appropriate amount of time. After that time, 20 mL of acetone/deionized water (1/1, v/v) was added to the mixture as an anti-solvent. The cellulose-rich materials were then filtered and washed with 20 mL of acetone/deionized water (1/1, v/v), followed by 20 mL of deionized water, and then dried at 60 °C for 24 h. After acetone was removed from the filtrate by rotary evaporation at room temperature, the precipitated lignin from the solution was washed again with 20 mL of deionized water and dried at 60 °C for 24 h. The filtrate was then neutralized to pH 7.0 by the addition of an aqueous solution of sodium hydroxide. The reducing sugar content was measured by the 3,4-dinitrosalicylic acid (DNS) method (Miller 1959). The concentration of reducing sugar was calculated by employing a standard curve prepared using glucose, and the absorbance of glucose was measured at 540 nm using an ultraviolet (UV)-mini-1240 spectrophotometer (Shimadzu, Japan). The yields of different residues were calculated according to Eqs. 1 and 2. The ionic liquid was then concentrated and dried in vacuum for 24 h for reuse (Montalbo-Lomboy and Grewell 2015). The alternative way is to separate sugars and the ionic liquid using an alumina column. Ionic liquid was eluted by a methanol/acetonitrile mixture (v/v, 1/1), followed by deionized water to elute the sugars.

$$\text{Recovered residue (\%)} = \text{Residue (mg)/ Untreated biomass subjected to pretreatment (mg)} \quad (1)$$

$$\text{Lignin content (\%)} = \text{Recovered lignin (\%)/ Original lignin in biomass (\%)} \quad (2)$$

Enzymatic Hydrolysis

Enzymatic hydrolysis was performed with a biomass loading of 1% (g/mL) and 0.02 g of cellulase in 0.1 M sodium citrate buffer (pH 4.8) on a shaker at 150 rpm, 50 °C for 72 h. After that time, the mixture was heated at 100 °C for 10 min. The reducing sugars were measured by the DNS method (Miller 1959). The yields of glucose and cellobiose were measured by high-performance liquid chromatography (HPLC). The yields of reducing sugar, glucose, xylose, or cellobiose after enzymatic hydrolysis were calculated according to Eq. 3.

$$\text{Sugar (\%)} = \text{Sugar (mg)/ cellulose-rich materials subjected to enzymatic hydrolysis (mg)} \quad (3)$$

FTIR Analysis

FTIR analysis was performed on a FTIR-8400S spectrophotometer (Shimadzu, Japan). Spectrum software (OPUS 6.5) was used. The spectra were acquired within the 4000 to 400 cm^{-1} region with a total of 16 scans and a resolution of 4 cm^{-1}.

HPLC Analysis

The HPLC analysis was performed on an Agilent 1100 (Agilent, America) device equipped with an Agilent Zorbax carbohydrate column. Deionized water containing 63% acetonitrile (v/v) was used as a mobile phase. The column temperature was kept at 30 °C, and the flow rate was 0.5 mL/min. The injection volume was 10 μL, and the acquisition time was 20 min. Glucose, xylose, and cellobiose standards were prepared at concentrations of 0.25, 1.25, 2.50, 3.75, and 5.00 mg/mL to construct the calibration curve.

RESULTS AND DISCUSSION

The corn stalk was treated with [Hnmp]Cl under a variety of pretreatment conditions. Lignin precipitated out of the filtrate after acetone solvent was evaporated. The yields of the different fractions were calculated according to Eqs. 1 and 2. The yields of various sugars were calculated according to Eq. 3.

Effect of Pretreatment Temperature

Previous studies have shown that ILs can break down the complex network of lignocellulose and thus partially remove lignin, which is important to enhance the enzymatic saccharification of cellulose (Haykir *et al*. 2013; Zhang *et al*. 2015). Haykir *et al*. (2013) described the pretreatment of cotton stalk by 1-allyl-3-methylimidazolium chloride ([AMIM]Cl), 1-butyl-3-methylimidazolium chloride ([BMIM]Cl), 1-ethyl-3-methylimidazolium chloride ([EMIM]Cl), and 1-ethyl-3-methylimidazolium acetate ([EMIM]CH_3COO) at 150 °C for 30 min. Total lignin removal was 46%, 31%, 38%, and 45%, respectively, using various ILs (based on the lignin content) from cotton stalk. The highest biomass digestibility, approximately 65%, was obtained *via* [EMIM]CH_3COO pretreatment after 72 h of enzymatic hydrolysis. In this study, a high yield of 14.53% lignin was recovered after [Hnmp]Cl treatment at 110 °C for 30 min, which corresponded to a level of 65.48% lignin (based on lignin content) (Fig. 1). A low pretreatment temperature was used to obtain a high yield of lignin in this work. Enzymatic hydrolysis of cornstalk gave the high yield of 85.6% glucose and 16.36% cellobiose when it was pretreated at 100 °C (Table 1).

Generally, increasing temperature accelerates the swelling and dissolution rates of lignocellulose in ILs (Sun *et al*. 2009; Wang *et al*. 2011). As shown in Fig. 1, when the temperature was increased from 70 to 110 °C, the yield of lignin removed was increased from 2.87% to 14.53%. Thus, high temperatures were beneficial for lignin extraction in ILs. However, the yield of reducing sugar during [Hnmp]Cl pretreatment was reduced as the temperature increased, probably because of the further degradation of monosaccharides. High temperatures can lead to compound degradation: Sant'Ana Da Silva *et al*. (2011) reported that the xylose yield decreased as temperature increased from 100 to 120 °C for the pretreatment of sugarcane bagasse with [EMIM]CH_3COO.

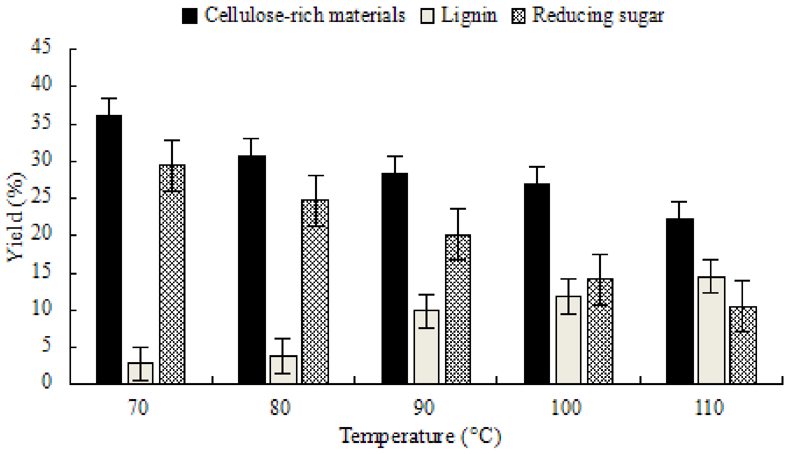


Fig. 1. Effect of temperature on the yields of residues during IL pretreatment (0.25 g corn stalk, 5 g IL, 1 mL H_2O, 30 min)

Therefore, higher temperature could effectively disrupt the recalcitrant structure of lignocelluloses to release lignin and accelerate the hemicellulose hydrolysis, but it also induced further degradation of monosaccharides. The high energy demand when using elevated temperature is also a drawback for sustainable and economic processes. To achieve the maximal enzymatic saccharification of cellulose-rich materials, the temperature was set at 100 °C for the subsequent studies.

Table 1. Effect of Temperature on the Yield of Sugars after Enzymatic Hydrolysis

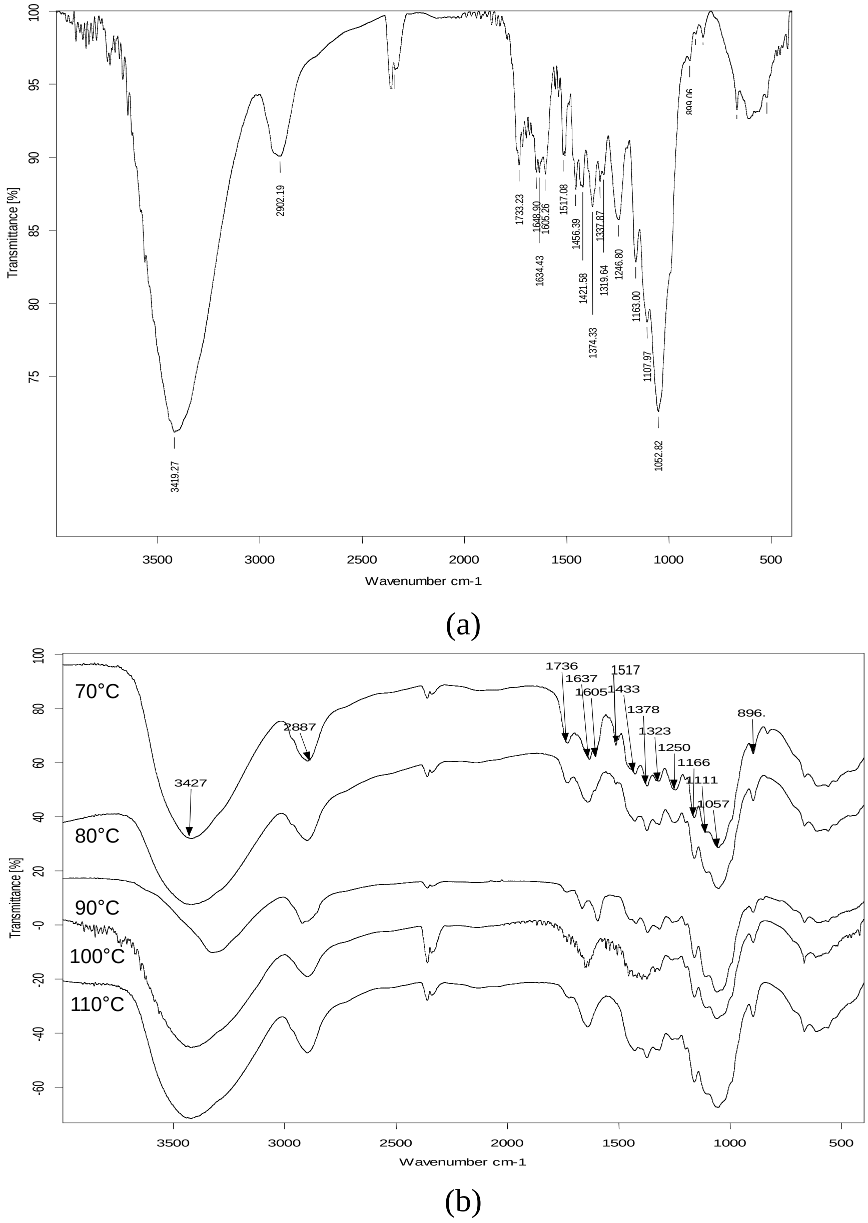
Temperature (°C)	Reducing sugar (%)		Xylose (%)		Glucose (%)		Cellobiose (%)	
	Ave(%)	Std.	Ave(%)	Std	Ave(%)	Std	Ave(%)	Std.
70	65.70	1.04	4.90	0.96	57.12	1.26	2.14	1.13
80	77.38	1.26	3.34	1.05	58.26	0.74	6.37	0.88
90	91.81	0.94	0	0	79.63	1.20	13.66	1.07
100	97.34	0.57	0	0	85.60	0.63	16.36	0.61
110	88.08	0.81	0	0	61.70	0.91	9.55	0.76

Effect of Temperature by FTIR Analysis

The FTIR spectra of cellulose-rich materials obtained from [Hnmp]Cl pretreatment at various temperatures are illustrated in Fig. 2. The absorbances at 3427, 2887, 1433, 1378, 1323, 1166, 1111, 1057, and 896 cm^{-1} were associated with the cellulose fraction of corn stalk. The absorption at 3427 cm^{-1} was assigned to O-H stretching. The peak at 2887 cm^{-1} band was assigned to -C-H stretching. A peak at 1433 cm^{-1} was related to the bending vibration of $-CH_2$. The 1378 cm^{-1} band was due to -C-H bending. The absorbance at 1323 cm^{-1} originated from the $-CH_2$ bending. The peak at 1166 cm^{-1} was related to -C-O antisymmetric stretching. A band at 1111 cm^{-1} was attributed to -C-OH skeletal vibration. A band at 1057 cm^{-1} was assigned to the -C-O-C pyranose ring skeletal vibration. The peak at 896 cm^{-1} corresponded to the vibration of glycosidic C_1-H deformation with contribution from ring vibration, a characteristic feature of β-glycosidic bonds between glucose in cellulose (Pappas *et al*. 2002).

As shown in Fig. 2, the prominent peak at 1736 cm^{-1} (corn) was attributed to the presence of unconjugated carbonyl and acetyl groups of the hemicelluloses in corn stalk. This peak intensity decreased considerably after [Hnmp]Cl pretreatment at 100 and 110 °C, which indicated that hemicelluloses were reduced dramatically. The intensity of the peak at 1250 cm^{-1} assigned to -C-O stretching in lignin and hemicelluloses decreased noticeably when the corn stalk was pretreated at 100 and 110 °C, which indicated that lignin and hemicelluloses were reduced.

The intensity of absorption bands at 1605 and 1517 cm^{-1} were related to the aromatic skeletal vibrations from lignin. These bands disappeared, especially when the corn stalk was pretreated with [Hnmp]Cl at 100 °C, and 110 °C, indicating that most of the lignin was removed. Lignin removal was 87% based on lignin content at 110 °C, as checked by NREL method. In addition, the peak at 896 cm^{-1} was apparent after the [Hnmp]Cl pretreatment, which indicated that the crystallinity of cellulose was reduced after pre-treatment.



(a)

(b)

Fig. 2. FTIR analysis of corn stalk (a) cellulose-rich materials (b) at various pretreatment temperatures

Effect of Pretreatment Time

Generally, the pretreatment time is related to the applied temperature required to accomplish an efficient pretreatment (Da Costa Lopes *et al.* 2013). With decreasing temperature, longer times are required for efficient swelling and pretreatment of biomass. Ninomiya *et al.* (2015) investigated the use of choline acetate for the pretreatment and fractionation of bagasse at 110 °C for 16 h. Almost all of the carbohydrates with approximately 50% of lignin in the original bagasse were recovered as carbohydrate-rich material, approximately 20% of the lignin in the original bagasse was fractionated as lignin-rich material, and the cellulose saccharification percentage reached over 95% in the case of carbohydrate-rich material. As shown in Fig. 3, a high yield of 15.63% lignin was recovered after treatment by [Hnmp]Cl at 100 °C for 75 min, corresponding to 70.44%

lignin (based on lignin content). A short processing time was used and a high yield of lignin was obtained in this work. Enzymatic hydrolysis of cellulose-rich materials gave a high yield of 93.2% glucose and 18.76% cellobiose.

Long pretreatment time has been reported to improve lignin extraction (Tan *et al*. 2009). In this study, as the processing time was increased from 15 min to 75 min, the quantity of lignin extracted in corn stalk was improved from 40% to 71% based on lignin content, which is consistent with the results of Lee *et al.* (2009). They reported that when wood flour was pretreated by [EMIM]CH_3COO at 90 °C, 40% and 85% of lignin (based on lignin content) was extracted from wood flour after 5 h and 70 h, respectively. Another study showed that the pretreatment of triticale straw at 90 °C resulted in 21.1% of lignin extraction within 5 h and the increase in lignin extraction was up to 30.3% after 24 h of treatment (Fu *et al*. 2010). Therefore, lignin extraction was improved as the pretreatment time increased. However, the yield of reducing sugar was reduced as the temperature increased because of the further degradation of monosaccharides. Because the goal was to obtain maximal yields of sugars after enzymatic hydrolysis, the processing time was set at 45 min for the following experiments, and the yield of lignin extracted was 12.29% at this stage.

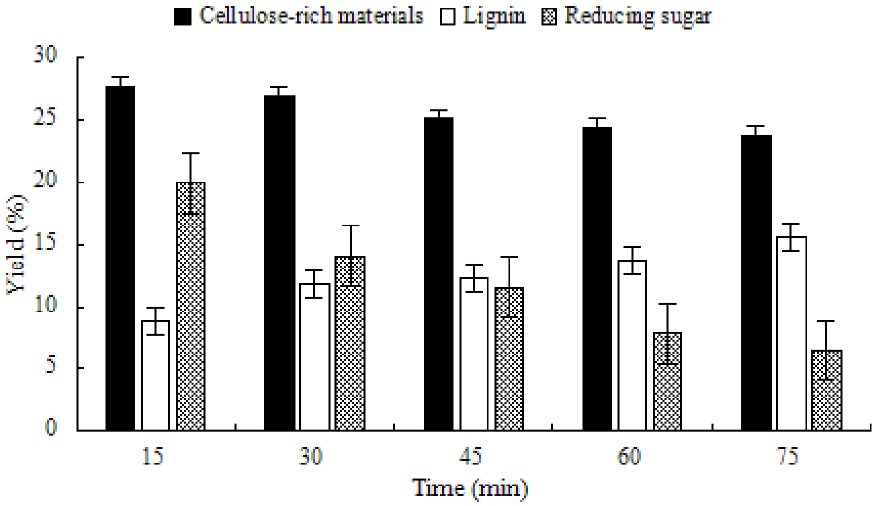


Fig. 3. Effect of pretreatment time on the yields of various residues with IL pretreatment (0.25 g corn stalk, 5 g IL, 1 mL H_2O, 100 °C)

Table 2. Effect of Time on the Yield of Sugars after Enzymatic Hydrolysis

Time (min)	Reducing sugar (%)		Xylose (%)		Glucose (%)		Cellobiose (%)	
	Ave(%)	Std.	Ave(%)	Std.	Ave(%)	Std.	Ave(%)	Std.
15	76.21	0.97	5.53	0.57	61.04	0.67	8.56	0.57
30	97.34	1.05	0	0	85.60	1.24	16.36	0.91
45	110.36	1.32	0	0	93.20	1.10	18.76	1.28
60	90.30	0.68	0	0	78.71	0.32	14.92	0.62
75	72.72	0.84	0	0	65.58	0.76	10.55	0.57

Effect of Pretreatment Time by FTIR Analysis

The FTIR analysis of cellulose-rich materials after [Hnmp]Cl pretreatment for various processing times is summarized in Fig. 4. The prominent peaks at 1736 and 1250 cm^{-1} were attributed to hemicelluloses. These peaks decreased noticeably after [Hnmp]Cl pretreatment for 30 min, which indicated that the hemicelluloses contents were reduced dramatically. In addition, the peaks at 1605 and 1517 cm^{-1}, related to the aromatic skeletal vibrations from lignin, disappeared in all spectra, specifically, lignin was removed by [Hnmp]Cl pretreatment after 45 min. Lignin removal was 90% based on lignin content after 75 min as checked by the NREL method.

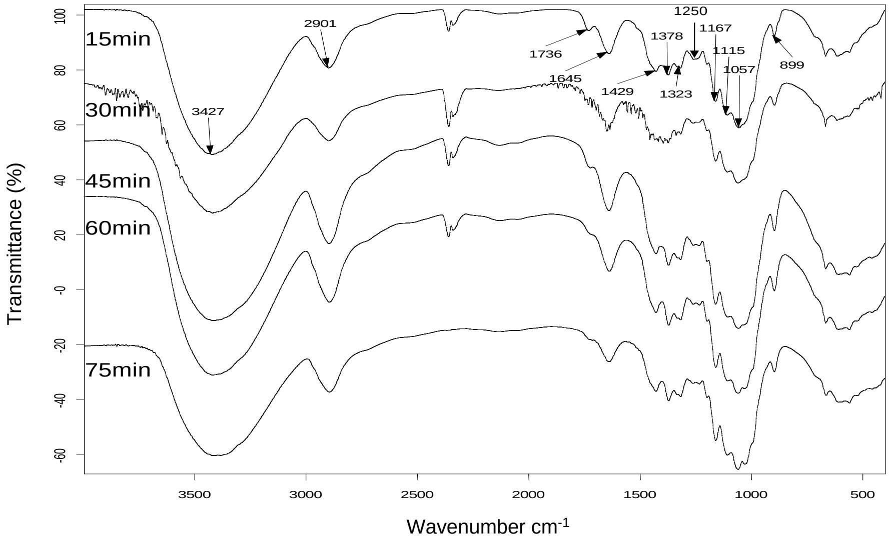


Fig. 4. FTIR analysis of cellulose-rich materials with various processing times

Effect of Biomass Loading

The ratio of biomass weight to IL weight (biomass loading) is an important economical aspect to be considered. Wang *et al.* (2011) reported that a reduction in dissolution rate was observed (from 35% to 26%) when biomass loading increased from 1% to 5%. Wu *et al.* (2011) increased the biomass loading from 4.8% to 50% for the pretreatment of corn stover in [EMIM]CH_3COO at 125 °C for 1 h, and the lignin removal decreased from 44% to 8%. In our study (Fig. 5), a high yield of 12.29% lignin recovery was achieved, which corresponded to 55.39% (based on lignin content), after [Hnmp]Cl treatment at 100 °C for 45 min with a biomass loading of 5%.

Generally, a higher dissolution rate at lower biomass loading could be attributed to the efficient dispersion of biomass in IL. As the mass ratio of biomass to IL decreased from 1:5 to 1:20, the lignin recovery improved from 9.65% to 12.29%. Interestingly, when the ratio decreased from 1:20 to 1:30, the yield of recovered lignin was found to be reduced from 12.29% to 9.83%. As reported by Wei *et al.* (2012), when legume straw was pretreated in [BMIM]Cl with a water content of 20%, the lignin extraction was 48.2% based on lignin content. However, only 32.4% was recovered by adding water as a precipitant solvent, which indicated that some lignin was dissolved in IL and could not precipitate out. Thus as the quantity of IL was increased, more lignin would be dissolved in IL and a lower yield of lignin was recovered. Therefore, the yield of lignin recovery reported from the literature (Ninomiya *et al.* 2015) was commonly lower than that from lignin removal.

In addition, the decrease in biomass loading resulted in a low yield of reducing sugar content during pretreatment, possibly because of the increase in the quantity of IL. The acidity of reaction medium was thus increased, which induced a high degradation of monosaccharides.

Furthermore, an appropriate biomass loading also was found to be important for the enzymatic accessibility of cellulose-rich materials. As shown in Table 3, the enzymatic hydrolysis of cellulose-rich materials gave a high yield of 93.2% glucose and 18.76% cellobiose with a biomass loading of 5% (biomass to ionic liquid, 1:20), which was consistent with the findings of Sun *et al.* (2009). They studied a 4% to 10% (w/w) wood load in [EMIM]CH_3COO at 110 °C for 16 h and found an optimum concentration of 5% (w/w) biomass loading.

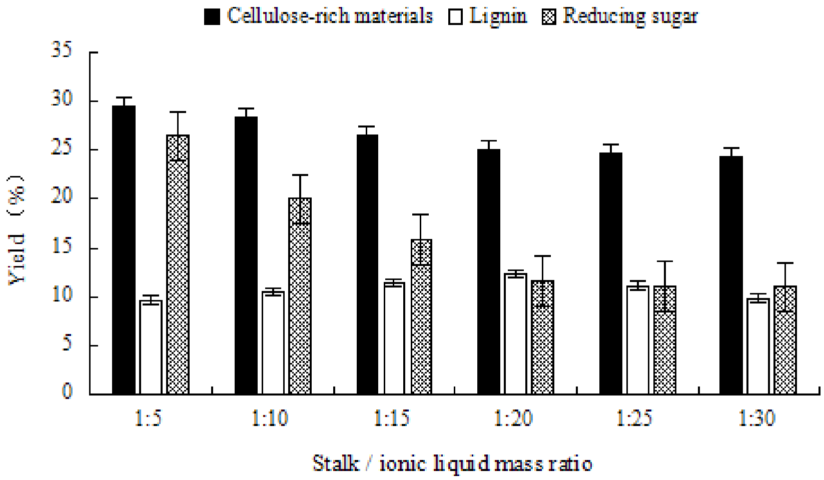


Fig. 5. Effect of biomass loading on the yields of various constituents with IL pretreatment (0.25 g corn stalk, 1 mL H_2O, 100 °C, and 45 min)

Table 3. Effect of Biomass Loading on the Yield of Sugars after Enzymatic Hydrolysis

Ratio of biomass to ionic liquid (wt/wt)	Reducing sugar (%)		Glucose (%)		Cellobiose (%)	
	Ave(%)	Std.	Ave(%)	Std.	Ave(%)	Std.
1:5	82.75	1.16	67.09	0.83	12.14	0.50
1:10	95.73	0.99	79.36	1.22	14.27	0.88
1:15	100.89	1.01	88.99	0.74	16.34	1.13
1:20	110.36	0.82	93.20	0.85	18.76	0.66
1:25	109.23	1.27	91.69	1.21	17.64	0.42
1:30	110.30	0.73	90.16	0.38	17.67	1.09

Effect of Biomass Loading by FTIR Analysis

The FTIR analysis of cellulose-rich materials after [Hnmp]Cl pretreatment with various biomass loading levels is summarized in Fig. 6. The peaks at 1736 and 1258 cm^{-1}

that were attributed to the hemicelluloses decreased noticeably with [Hnmp]Cl pretreatment. When the ratio of biomass to IL was less than 1:5, the hemicellulose contents were reduced dramatically. Moreover, the peaks at 1605 and 1517 cm^{-1}, related to the aromatic skeletal vibrations from lignin, disappeared in all spectra, which indicated that lignin was removed completely by [Hnmp]Cl pretreatment with a biomass loading less than 20%.

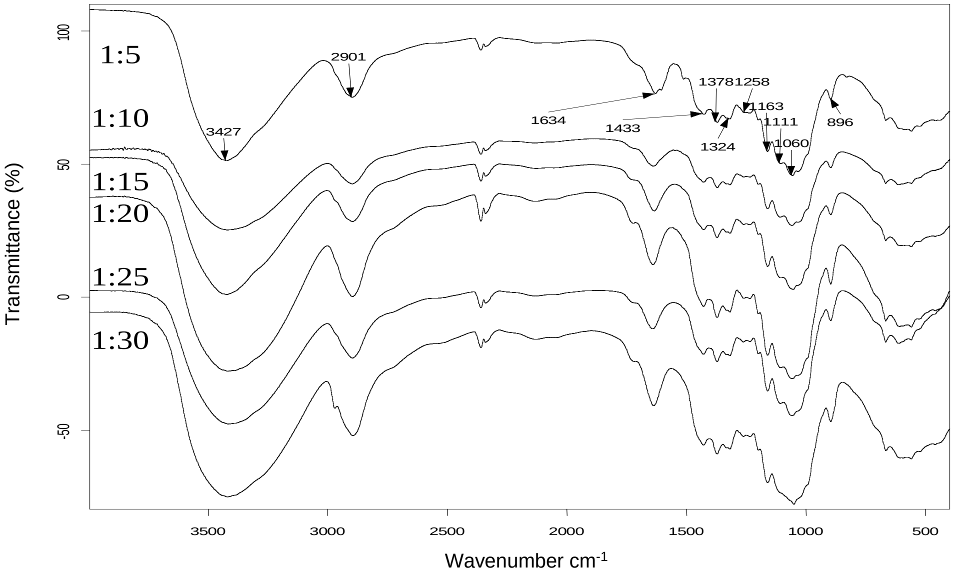


Fig. 6. FTIR analysis of cellulose-rich materials with different biomass loading

Effect of Water Content

As reported before, an increased amount of lignin was removed when the water content was decreased from 95% to 0% (w/w) for the pretreatment of triticale straw in aqueous [EMIM]CH_3COO solution. Cellulose digestibility was 67.4% in the case of pure [EMIM]CH_3COO pretreatment, which was further enhanced to 81.0% with 50% water content (w/w) achieving the highest sugar yield (Fu *et al.* 2010). In another study, on the pretreatment of wheat straw in aqueous IL solutions, the effects of pretreatment temperature (130 to 170 °C), time (0.5 to 5.5 h), and water content (0% to 100%) were studied. The optimized conditions were determined to be 158 °C for 3.6 h with 50.5% water content (w/w), and 71.4% of sugars were obtained (Fu and Mazza 2011; Da Costa Lopes *et al.* 2013).

In this study, a high yield of 20.28% lignin was achieved after it was treated by pure [Hnmp]Cl at 100 °C for 45 min with a biomass loading of 5%, which corresponded to 91.39% lignin (based on lignin content). As the ratio of biomass to water was increased from 1:0 to 1:6, lignin recovery was reduced from 20.28% to 7.39% (91.39% to 33.3% based on lignin content).

However, as the water content was increased, the yield of saccharification of cellulose-rich materials improved. With a ratio of biomass to water of 1:4, the highest glucose yield obtained was up to 93.20%, with a cellobiose yield of 18.76%.

Water competes with the anions of ILs to form hydrogen bonds with lignin. In the case of chloride-based ILs, water would coordinate with chloride ions (Shill *et al.* 2011). Thus, the presence of water in ILs would reduce the capabilities of hydrogen bond acceptor and thus decrease the solubility of lignin. Additionally, when the water content was increased, the acidity of the reaction mixture was reduced, which resulted in a higher yield of reducing sugars during pretreatment because of the inhibition of further degradation of monosaccharides.

Therefore, the pretreatment efficiency is higher for pure IL with respect to lignin recovery. Enzymatic hydrolysis of cellulose was more efficient with aqueous IL. The considerably reduced lignin and hemicelluloses contents after [Hnmp]Cl pretreatment resulted in the highest enzyme digestibility.

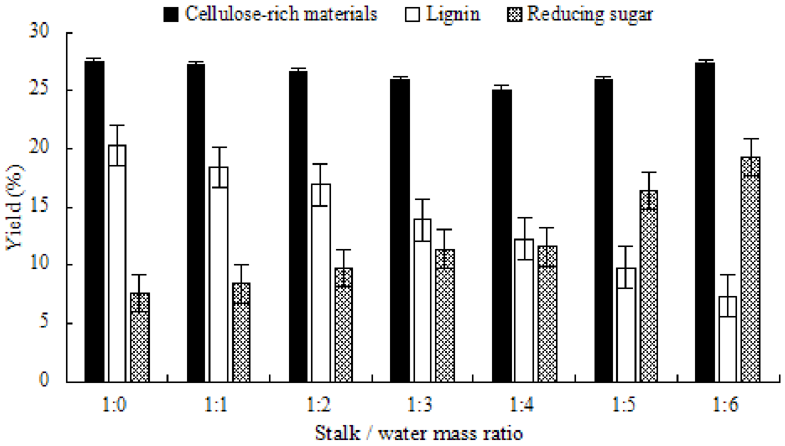


Fig. 7. Effect of water content on the yields of various residues with IL pretreatment (0.25 g corn stalk, 5 g IL, 100 °C, and 45 min)

Table 4. Effect of Water Content on the Yield of Sugars after Enzymatic Hydrolysis

Ratio of biomass to water (wt/wt)	Reducing sugar (%)		Glucose (%)		Cellobiose (%)	
	Ave(%)	Std.	Ave(%)	Std.	Ave(%)	Std.
1:0	75.43	0.51	63.47	0.41	12.58	0.59
1:1	82.23	0.89	68.28	1.05	14.60	1.33
1:2	90.26	1.34	73.39	1.11	16.09	1.07
1:3	99.91	1.12	82.28	0.86	16.13	0.94
1:4	110.36	0.75	93.20	0.49	18.76	0.62
1:5	100.19	0.33	84.43	0.52	16.98	0.51
1:6	98.62	0.67	83.29	0.77	16.27	1.04

Effect of Water Content by FTIR Analysis

The FTIR analysis of cellulose-rich materials after [Hnmp]Cl pretreatment with various water content levels is summarized in Fig. 8. The prominent peaks at 1736 and 1258 cm^{-1} were attributed to hemicelluloses. The intensity of both peaks decreased substantially with [Hnmp]Cl pretreatment as the ratio of biomass to water decreased from 1:1 to 1:6. This indicated that hemicelluloses were reduced significantly. In addition, the peaks at 1605 and 1517 cm^{-1}, related to aromatic skeletal vibrations from lignin, disappeared when the ratio decreased from 1:0 to 1:4. This observation further revealed that the lignin was removed completely. However, a peak at 1517 cm^{-1} was observed with ratios of 1:5 and 1:6. Thus, the water content has a crucial effect on the lignin recovery of lignocelluloses during IL pretreatment.

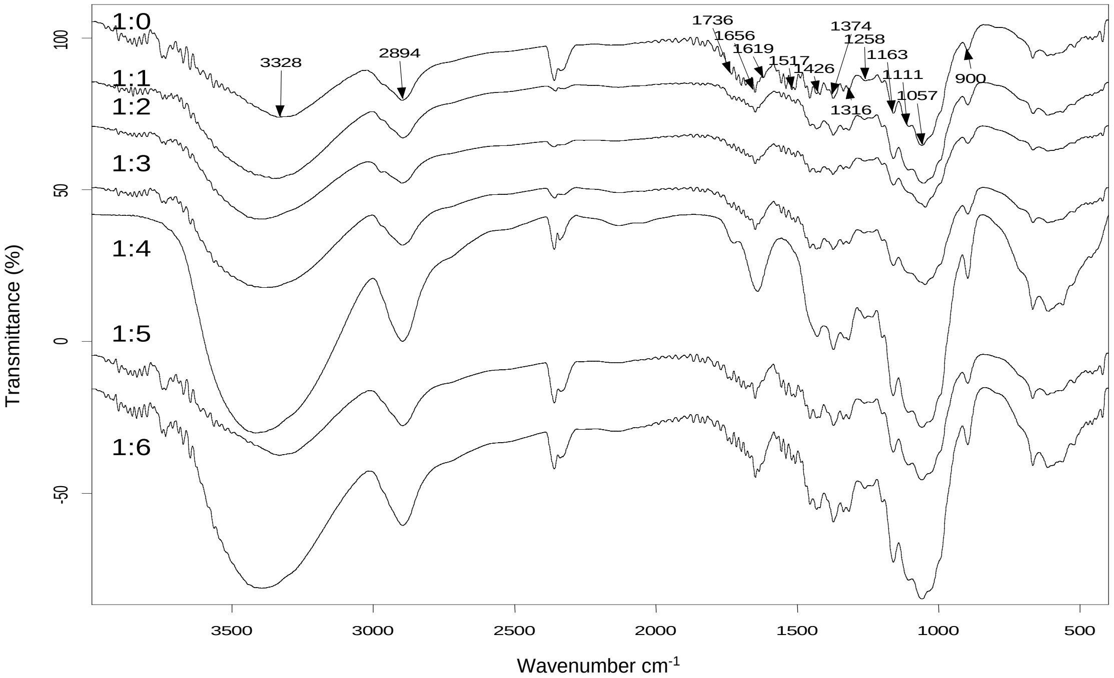


Fig. 8. FTIR analysis of cellulose-rich materials with various water content levels

Reduction of the high cost of ILs is one of the key issues for the scale-up process. In this work, [Hnmp]Cl was synthesized by N-methyl pyrrolidone with hydrochloric acid in one step. The cost of N-methyl pyrrolidone is much lower than N-methylimidazole. For example, 1L of N-methyl pyrrolidone costs 1196 RMB, while 1L of N-methylimidazole costs 3893RMB as ordered from Sigma Aldrich. Moreover, 1-ethyl-3-methylimidazolium has been widely reported to be prepared in two steps. However, it is still a challenging task to synthesize desirable "low-cost" ILs that will have potential for application to lignocellulosic biomass at a large scale.

CONCLUSIONS

1. The pretreatment of corn stalk with [Hnmp]Cl can be considered to be an effective disruption of the intricate network of non-covalent interactions. The strong hydrogen bonding capability removed the lignin content effectively. The acidity of ionic liquids assisted the hydrolysis of the hemicelluloses to achieve high enzyme digestibility. The significantly reduced lignin and hemicelluloses after [Hnmp]Cl pretreatment were also confirmed by FTIR analysis.
2. 91.39% of lignin was effectively removed when the corn stalk was pretreated by pure [Hnmp]Cl at 100 °C for 45 min with a biomass loading of 5%, The highest glucose yield was up to 93.20%, and that for cellobiose was 18.76%, when the ratio of biomass to water was at 1:4.
3. The pretreatment efficacy was quite dependent on the pretreatment temperature and processing time. High temperature and a long period of time were found to favor lignin recovery. However, reducing sugar during pretreatment was decreased as the

temperature and time were increased. Water content was quite important for lignin recovery. Pure IL was more efficient for lignin recovery and aqueous IL was more effective for reducing sugar during pretreatment and enzymatic saccharification of cellulose-rich materials.

4. [Hnmp]Cl was synthesized by N-methyl pyrrolidone with hydrochloric acid in one step. This "low-cost" ionic liquid will have potential for application to lignocellulosic biomass.

LIST OF ABBREVIATIONS

IL	ionic liquid
[Hnmp]Cl	1-H-N-methyl-2-pyrrolidonium chloride
NREL	National Renewable Energy Laboratory
NMR	nuclear magnetic resonance spectroscopy
UV	Ultraviolet
HPLC	high-performance liquid chromatography
FTIR	Fourier Transform infrared
[AMIM]Cl	1-allyl-3-methylimidazolium chloride
[BMIM]Cl	1-butyl-3-methylimidazolium chloride
[EMIM]Cl	1-ethyl-3-methylimidazolium chloride
[EMIM]CH_3COO	1-ethyl-3-methylimidazolium acetate

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