Pyrrolidinium Ionic Liquids as Effective Solvents for Lignin Extraction and Enzymatic Hydrolysis of Lignocelluloses

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Lignocellulosic biomass as a renewable and valuable resource has been a very active research area during recent years. Ionic liquids (ILs) are an attractive approach for the pretreatment of lignocelluloses. In this work, a series of pyrrolidinium-based ionic liquids were prepared simply and were investigated for the pretreatment of corn stalk at 90 °C for 30 min. The characteristics of regenerated lignin and cellulose-rich materials were determined by Fourier transform infrared spectroscopy (FTIR) analysis. Both ILs and ILs/H_2O were studied. Notably, 10.17% lignin based on the original corn stalk was recovered with [Hmp]Cl-pretreatment; 21.34% and 15.83% reducing sugar based on the original corn stalk were observed during pretreatment with [Hmp]HSO_4/H_2O and [Hmp]Cl/H_2O pretreatment, respectively; 80.54% of reducing sugar was obtained based on the cellulose-rich materials with [Hmp]Cl/H_2O-pretreatment. Also, the glucose and cellobiose yields were 73.82% and 8.64%, respectively.

Keywords: Ionic liquids; Lignocelluloses; Pretreatment; Pyrrolidinium

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INTRODUCTION

The rapidly growing requirements for energy and the concern for global warming have directed researchers' focus on lignocellulosic biomass. Lignocelluloses, the most promising renewable resources, are obtained from various sources (Juturu and Wu 2014). The agricultural sources such as corn stalks and grasses have received much attention. Generally, lignocelluloses consist of cellulose, hemicelluloses, and lignin. Cellulose is formed *via* 1,4-β-D-glucose linkage of glucose units and contains several inter- and intramolecular hydrogen bonds (Zhang *et al*. 2005). Hemicelluloses consist of a polysaccharide backbone including xylan, mannan, betaglucan, and xyloglucan. Lignin is a complex polymer built up from three different phenylpropanoid monomers including guaiacyl (G), syringyl (S), and p-hydroxyphenyl (H) subunits. (Sun *et al.* 2009). Cellulose fibers are cross-linked to the matrix of lignin and hemicelluloses, becoming a recalcitrant structure (Moniruzzaman and Ono 2013). Pretreatment is necessary to open up the structure of the highly hydrolysis-resistant lignocellulosic matrix and to accelerate enzymatic hydrolysis (Ninomiya *et al*. 2014; Weerachanchai and Lee 2014).

In recent years, ionic liquids (ILs) have drawn a great deal of attention as they display several advantages such as wide range of fluidity, lack of flammability, and negligible vapor pressure (Nguyen *et al*. 2010; Ha *et al*. 2011; Haykir *et al*. 2013). Swatloski *et al*. (2002) reported the dissolution of cellulose in ILs. Following that initial study, pretreatment in ILs and fractionation of lignocelluloses in switchgrass (Singh *et al*. 2009; Cheng *et al*. 2011; Barr *et al*. 2012; Groff *et al*. 2013), wheat straw (Liu and Chen 2006; Li *et al*. 2009), rice straw (Liu *et al.* 2012; Hou *et al*. 2012, 2013), wood (Lee *et al*. 2009; Cheng *et al*. 2011; Barr *et al*. 2012; Goshadrou *et al.* 2013; Sun *et al*. 2013), and bagasse (Bian *et al*. 2014) have been examined. 1-Allyl-3-methylimidazolium chloride ([AMIM]Cl), 1-butyl-3-methyl imidazolium chloride ([BMIM]Cl), and 1-ethyl-3-methylimidazolium acetate ([EMIM]CH_3COO) are powerful ionic liquids. The pretreatment of cotton stalk with [AMIM]Cl, [BMIM]Cl, [EMIM]Cl, and [EMIM]CH_3COO at 150 °C for 30 min resulted in 46%, 31%, 38%, and 45% lignin removal (based on the lignin content), respectively (Haykir *et al.* 2013). High biomass digestibility (65%) was obtained with [EMIM]CH_3COO-pretreatment after 72 h of enzymatic saccharification (Haykir *et al*. 2013). Corn stalk was pretreated by [EMIM]CH_3COO at 140 °C for 3 h (Gao *et al*. 2014), resulting in 89.9% total lignin removal (based on the lignin content), and the yield of glucose was 91.6% after 72 h of enzymatic hydrolysis. Currently, more studies are focused on lignin extraction and recovery instead of just lignin removal. The pretreatment of corn stover with [EMIM]CH_3COO at 125 °C for 1 h resulted in 44% extraction of lignin (based on the lignin content). The yield of glucose was 80% or 50% glucose at 33% (w/w) or 50% (w/w) biomass loading (Li *et al*. 2009). Rice straw pretreated by cholinium argininate at 90 °C for 12 h resulted in 46% lignin recovery (based on the lignin content) and 74.6% glucose after 72 h of enzymatic saccharification (An *et al*. 2015).

Most studies on lignocellulose pretreatment use imidazolium-based ILs, but new classes of ILs should be investigated. While the ionic liquids [AMIM]Cl, [BMIM]Cl, and [EMIM]CH_3COO can be used to pretreat lignocelluloses, high pretreatment temperatures and long pretreatment times are required (Haykir *et al*. 2013). In addition, lignin regeneration is not very efficient using [AMIM]Cl and [EMIM]CH_3COOH, which limits the enzymatic hydrolysis of lignocelluloses (Li *et al*. 2009). In this work, a series of pyrrolidinium-based ionic liquids were synthesized. Corn stalk was pretreated at 90 °C for 30 min in one of the ionic liquids.

EXPERIMENTAL

Materials

All chemicals and the cellulase (10000 U/g) from *Aspergillus niger* were purchased from Aladin Company (Shanghai, China) and used as received. The corn stalk, obtained from a local farm in Harbin City (China), was ground in a cutter mill, passed through 40 mesh sieves, and dried at 40 °C for 48 h. Corn stalk consisted of 42.95% cellulose, 19.88% hemicelluloses, and 22.19% lignin, which was determined as previously described (Sluiter *et al*. 2011).

Fourier transform infrared (FTIR) spectra were recorded on a FTIR-8400S spectrometer (Shimadzu, Kyoto, Japan) in the range of 4000 to 400 cm^{-1}. ^1H NMR and ^{13}C NMR spectra were recorded in Bruker Avance 400 MHZ spectrometer (Karlsruhe, Germany) with DMSO-d_6 as a solvent. Reducing sugar was analyzed by a UV-mini-1240 spectrometer (Shimadzu). Glucose, xylose, and cellobiose were analyzed by HPLC (2695, Waters, Massachusetts, America) equipped with an Agilent ZORBAX carbohydrate column.

Synthesis of Pyrrolidinium-based Ionic Liquids

N-methyl pyrrolidine (0.1 mol) was reacted with hydrochloric acid (0.1 mol) under stirring in an ice bath. The reaction was then carried out in an oil bath at 90 °C for 30 min under stirring. The resulting product was washed with 20 mL of ethyl acetate and dried under vacuum at 60 °C for 12 h to obtain [Hmp]Cl. Other ionic liquids including [Hmp]CH_3SO_3 and [Hmp]HSO_4 were prepared using a similar procedure. The analysis of ionic liquids shown in Table 1.

Table 1. Analysis of Ionic Liquids

Ionic Liquid	^{1}H NMR (400 MHz, DMSO-d_6)	^{13}C NMR (400 MHz, DMSO-d_6)
1-H-1-methyl-pyrrolidinium chloride ([Hmp]Cl)	1.853 (q, 2H, pyrrolidinium ring), 1.980 (q, 2H, pyrrolidinium ring), 2.720 (s, 3H, -CH_3), 2.908 (t, 2H, pyrrolidinium ring), 3.407 (t, 2H, pyrrolidinium ring), 11.284 (s, 1H, N-H)	17.612 (s, 1C, pyrrolidinium ring), 29.538 (s, 1C, pyrrolidinium ring), 30.502 (s, 1C, -CH_3), 49.140 (s, 1C, pyrrolidinium ring), 175.373 (s, 1C, pyrrolidinium ring)
1-H-1-methyl-pyrrolidinium methanesulfonate ([Hmp]CH_3SO_3)	1.870 (q, 2H, pyrrolidinium ring), 1.993 (q, 2H, pyrrolidinium ring), 2.356 (s, 3H, CH_3SO_3), 2.814 (s, 3H, -CH_3), 2.963 (t, 2H, pyrrolidinium ring), 3.487 (t, 2H, pyrrolidinium ring), 9.653(s, 1H, N-H)	17.473 (s, 1C, pyrrolidinium ring), 29.596 (s, 1C, pyrrolidinium ring), 30.381 (s, 1C, -CH_3), 39.685 (s, 1C, -CH_3SO_3), 49.463 (s, 1C, pyrrolidinium ring), 175.154 (s, 1C, pyrrolidinium ring)
1-H-2-methyl-pyrrolidinium hydrogen sulfate ([Hmp]HSO_4)	1.856 (q, 2H, pyrrolidinium ring), 1.984 (q, 2H, pyrrolidinium ring), 2.820 (s, 3H, -CH_3), 2.971 (t, 2H, pyrrolidinium ring), 3.491 (t, 2H, pyrrolidinium ring), 6.705 (s, 1H, HSO_4), 9.568 (s, 1H, N-H)	17.395 (s, 1C, pyrrolidinium ring), 29.751(s, 1C, pyrrolidinium ring), 30.385 (s, 1C, -CH_3), 49.731 (s, 1C, pyrrolidinium ring), 175.683 (s, 1C, pyrrolidinium ring)

Determination of the Acidity of Ionic Liquids

The acidity of ILs was determined using the Hammett method with UV-VIS spectroscopy (UV-mini-1240, Shimadzu, Kyoto, Japan) at room temperature. 4-Nitroaniline was used as an indicator, and deionized water was used as a solvent. The acidity was measured by evaluating the protonation extent of uncharged indicator bases (named I) in a solution, in terms of the measurable ratio [I]/[IH$^+$] (Duan *et al.* 2006; Xing *et al.* 2007). The Hammett function (H_0) is defined by Eq.1,

$$H_0 = \mathrm{pK}[I]_{\mathrm{aq}} + \log [I]/[\mathrm{IH}^+] \tag{1}$$

where the pK$[I]_{\mathrm{aq}}$ of 4-nitroaniline is 0.99, and [I] and [IH$^+$] are the molar concentrations of unprotonated and protonated form of indicators in the solution, respectively.

IL Pretreatment of Corn Stalk

Corn stalk (0.25 g) was mixed with one of the ILs (5 g) both with and without deionized water (1 mL) at 90 °C for 30 min in an oil bath. When the pretreatment was completed, 20 mL of acetone/deionized water (1/1, v/v) was added to the mixture. The solid was filtered and washed with 20 mL of acetone/deionized water (1/1, v/v), followed by 20 mL of deionized water to remove any residual ionic liquid, and then it was dried at 60 °C for 24 h to obtain regenerated cellulose-rich materials. Acetone was evaporated from the filtrate by rotary evaporation at room temperature for 30 min, and the resulting precipitates were washed with deionized water and dried at 60 °C for 24 h to give regenerated lignin. As [Hmp]CH_3SO_3 is solid at 90 °C, DMSO was added into [Hmp]CH_3SO_3 to prepare a liquid solution. The yields of lignin or cellulose-rich materials were determined according to Eq. 2. The ionic liquids were concentrated under rotary evaporation and dried in a vacuum for 12 h for reuse.

$$\begin{aligned}&\text{Lignin or cellulose-rich materials (\%)} = \text{lignin or cellulose-rich materials (mg)/corn stalk}\\&\text{subjected to pretreatment (mg)}\end{aligned} \tag{2}$$

Determination of Reducing Sugar during IL Pretreatment

After lignin was removed, the filtrate was neutralized to pH 7.0 by the addition of aqueous sodium hydroxide. The reducing sugar was measured by the 3,4-dinitrosalicylic acid (DNS) method (Miller *et al*. 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. The yield of reducing sugar was determined by Eq. 3.

$$\text{Reducing sugar (\%)} = \text{reducing sugar (mg)/corn stalk subjected to pretreatment (mg)} \tag{3}$$

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 and 50 °C for 72 h. When the enzymatic reaction was completed, it was stopped by shaking the mixture at 100 °C for 10 min. The reducing sugars were again measured by the DNS method (Miller *et al*. 1959). The yields of glucose, xylose, and cellobiose were measured by HPLC (2695, Waters, Massachusetts, America) and were calculated according to Eq. 4.

$$\text{Sugar (\%)} = \text{Sugar (mg)/ cellulose-rich materials subjected to enzymatic hydrolysis (mg)} \tag{4}$$

RESULTS AND DISCUSSION

IL Pretreatment of Corn Stalk

In this work, the pretreatment of corn stalk in IL and IL/H_2O was investigated at 90 °C for 30 min. The yield of cellulose-rich materials was calculated according to Eq. 2. As shown in Fig.1, slightly higher yields of cellulose-rich materials were obtained by use of IL than IL/H_2O. A significant difference in the yields of cellulose-rich materials was observed by difference ILs. A yield of 22 to 23% of cellulose-rich materials was achieved with [Hmp]Cl-pretreatment. About 50% and 40% of cellulose-rich materials were obtained with [Hmp]CH_3SO_3-pretreatment and [Hmp]HSO_4-pretreatment, respectively.

Lignin analysis

Most studies on the application of ionic liquids for biomass concentrated on the conversion of cellulose fraction. However, the lignin fraction is potentially valuable as lignin could serve as a source of aromatic chemicals. Previously, 8.2% lignin removal from cotton stalk was achieved (based on the original cotton stalk) after [BMIM]CH_3COO-pretreatment at 120 °C for 5 h (Poornejad *et al*. 2013), and 44% lignin extraction from corn stover was observed with [EMIM]CH_3COO-pretreatment at 125 °C for 1 h (based on the lignin content) (Li *et al*. 2009). 46% of lignin recovery from rice straw was obtained when rice straw was pretreated by cholinium argininate at 90 °C for 12 h (based on the lignin content) (An *et al*. 2015).

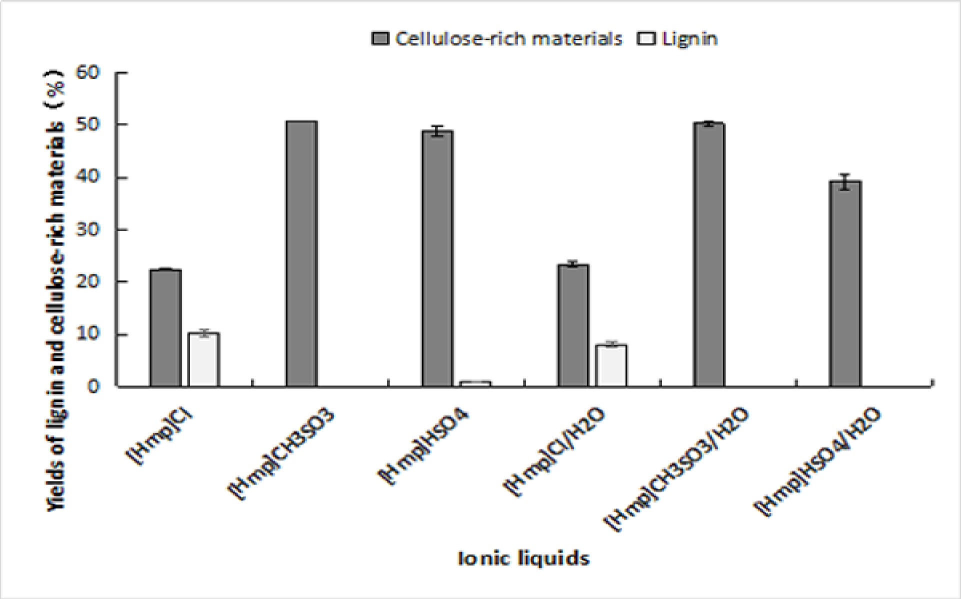

Fig. 1. Yields of lignin and cellulose-rich materials extracted from corn stalk with IL and IL/H_2O. (0.25 g corn stalk, 5 g IL, 1 ml H_2O, T=90 °C , t=30 min)

As shown in Fig. 1, when the corn stalk was pretreated by [Hmp]Cl at 90 °C for 30 min, 10.17% lignin in corn stalk was recovered, corresponding to 46% of lignin (based on lignin content). 8% lignin in corn stalk was obtained with [Hmp]Cl/H_2O-pretreatment corresponding to 36% of lignin (based on lignin content).

Compared with the recovery yield of lignin in current studies, as shown in Table 2, lower pretreatment time and shorter pretreatment time were employed in this work.

Table 2. Comparison of the Recovered Yield of Lignin

Ionic liquid	Biomass	Temp.	time	Precipitation solvent	Quantity of precipitate	Reference
$[C_2C_1im][MeCO_2]$	Pine	110 ℃	16h	Water	10%	Sun *et al.* 2009
	Spruce	115 ℃	72h	Water	36%	Viell *et al.* 2011
	Beech	115 ℃	72h	Water	43%	Viell *et al.* 2011
[EMIM]$[CH_3COO]$	Corn stover	125 ℃	1h	Water	44%	Li *et al.* 2009
$[C_2C_1im][MeCO_2]$(20 %Water）	Miscanthus	120℃	22h	Water	18%	Brandt *et al.* 2011
$[C_4C_1im][HSO_4]$(20% Water）	Miscanthus	120℃	22h	Water	47%	Brandt *et al.* 2011
$[C_4C_1im][MeSO_4]^a$(20 %Water）	Miscanthus	120℃	26h	Water	43%	Brandt *et al.* 2011
$[C_4C_1im][MeSO_3]$(20% Water）	Miscanthus	120℃	22h	Water	30%	Brandt et al. 2011

The yield of lignin was higher when corn stalk was pretreated by IL than by IL/H_2O, as the addition of water caused the reduced dissolution of lignin. However, no isolated yield of lignin was observed with [Hmp]CH_3SO_3/DMSO-pretreatment. The addition of DMSO or water could potentially explain the dramatic reduction of lignin fraction.

Sugar analysis

When water was added into the pretreatment process, the hydrolysis of hemicelluloses and cellulose occurred. The yield of reducing sugar during the pretreatment process was calculated according to Eq. 3. As shown in Fig. 2, a higher yield of reducing sugar was obtained when corn stalk was pretreated by IL/H_2O. Specifically, the yield of reducing sugar was 21.34% and 15.83% when corn stalk was pretreated by [Hmp]HSO_4/H_2O and [Hmp]Cl/H_2O, respectively, which was probably caused by different acidic capability of ionic liquids. Generally, pretreatment and acidolysis of biomass were processed with an ionic liquid containing acidic catalyst. In our previous study (Zhang *et al*. 2015), the yield of reducing sugar was 13.56% with 1-H-3-methyl-imidazolium hydrogen sulfate/H_2O pretreatment assisted with ultrasound irradiation at 70 °C for 3 h.

The presence of water induced the hydrolysis of hemicelluloses and cellulose. The acidity of ionic liquids was then measured. The ratio of [I]/[IH^+] was determined and then the Hammett function (H_0) values of ionic liquid were calculated according to Eq. 1. As shown in Table 3, the order of acidity was as follows: [Hmp]HSO_4 > [Hmp]Cl > [Hmp]CH_3SO_3. Higher acidity was observed with [Hmp]HSO_4 and [Hmp]Cl. As a result, a higher yield of reducing sugar was obtained with [Hmp]HSO_4/H_2O and [Hmp]Cl/H_2O pretreatments (Fig. 2).

Table 3. The Acidity of Ionic Liquids

Ionic Liquid	A_{max}	[I]/%	[IH^+]/%	H_0
[Hmp]Cl	1.4569	96.46	3.54	2.4253
[Hmp]CH_3SO_3	1.4984	99.21	0.79	3.0889
[Hmp]HSO_4	1.2534	82.99	17.01	1.6783

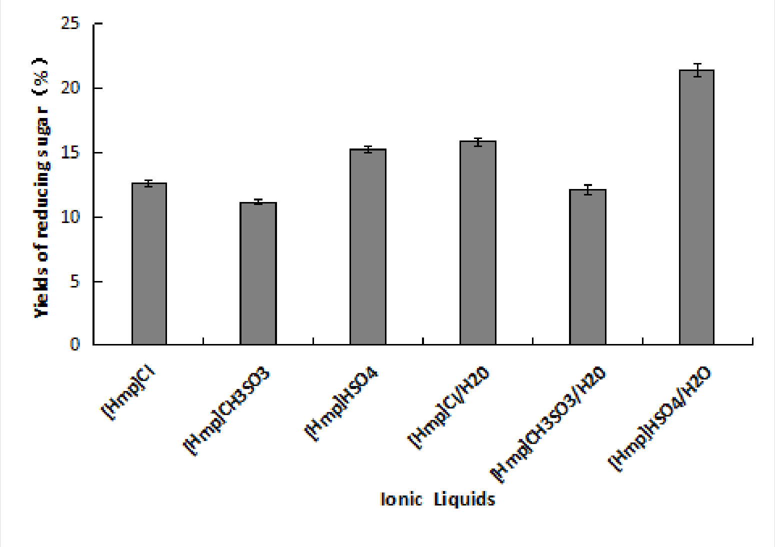

Fig. 2. Yields of reducing sugar in the pretreatment with IL. (0.25 g corn stalk, 5 g IL, 1 ml H_2O, *T*=90 °C, *t*=30 min)

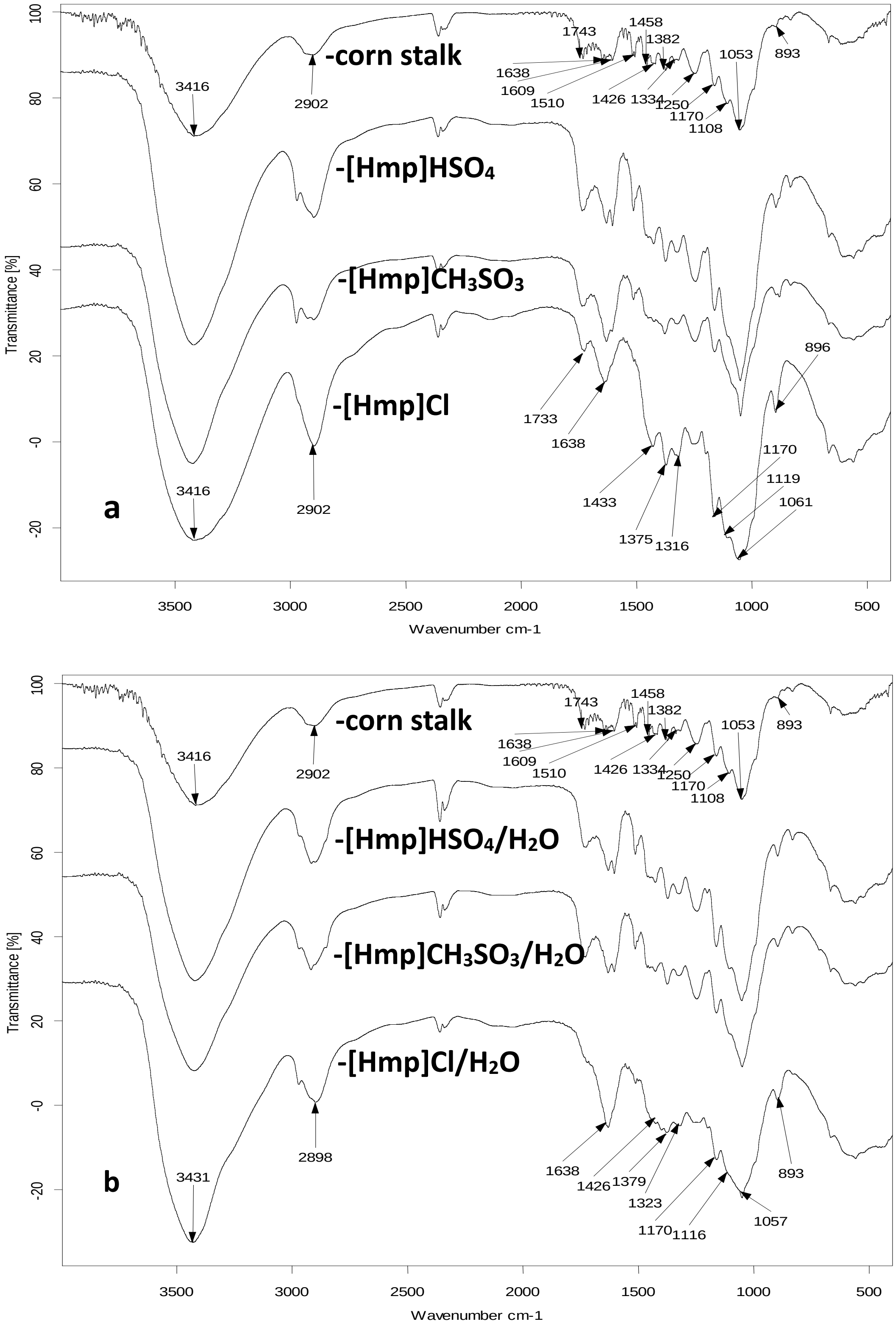

Fig. 3. FTIR spectra of untreated corn stalk and cellulose-rich materials extracted from corn stalk with IL (a) and IL/H_2O (b). (0.25 g corn stalk, 5 g IL, 1 mL H_2O, T=90 °C, t=30 min)

FTIR Analysis

FTIR analysis of cellulose-rich materials

FTIR spectra of untreated corn stalk and cellulose-rich materials are illustrated in Fig. 3. The absorbance peaks at 3416, 2902, 1433, 1375, 1316, 1170, 1119, 1061, and 896 cm^{-1} are associated with cellulose. The peak at 3416 cm^{-1} signifies O-H stretching (Nasirpour *et al*. 2014). The peaks at 2902 cm^{-1} and 1433 cm^{-1} are related to C-H stretching and the bending vibration of CH_2, respectively. The peak at 1375 cm^{-1} is associated with C-H bending. The peak at 1316 cm^{-1} is associated with CH_2 bending. The peak located at 1170 cm^{-1} is related to C-O antisymmetric stretching. The peak at 1119 cm^{-1} is attributed to C-OH skeletal vibration. The peak at 1061 cm^{-1} is attributed to the C-O-C pyranose ring skeletal vibration. The peak at 896 cm^{-1} is attributed to the vibration of glycosidic C_1-H deformation with ring vibration contribution. Compared with untreated corn stalk with cellulose-rich materials, the prominent peak at 1733 cm^{-1} (corn) was attributed to the presence of unconjugated carbonyl and acetyl groups of the hemicelluloses in the original corn stalk. This peak was missing in cellulose-rich materials with [Hmp]Cl/H_2O pretreatment, which indicated that hemicelluloses were removed completely.

The intensity of the peak at 1250 cm^{-1} was associated to C-O stretching signal in lignin and hemicelluloses. This peak decreased noticeable with [Hmp]Cl and [Hmp]Cl/H_2O pretreatment, suggesting a dramatic reduction of hemicelluloses and lignin (Li *et al*. 2010). The peaks at 1609 cm^{-1} and 1510 cm^{-1} were related to aromatic skeletal vibrations from lignin. These peaks were less noticeable following [Hmp]Cl and [Hmp]Cl/H_2O pretreatment, suggesting that most lignin was removed by [Hmp]Cl. The peak at 898 cm^{-1} is a characteristic of β-glycosidic bonds between glucose in cellulose (Pappas *et al*. 2002), which was obvious, after [Hmp]Cl and [Hmp]Cl/H_2O pretreatment, indicating that the crystallinity of cellulose was reduced (Perez-Pimienta *et al*. 2015)

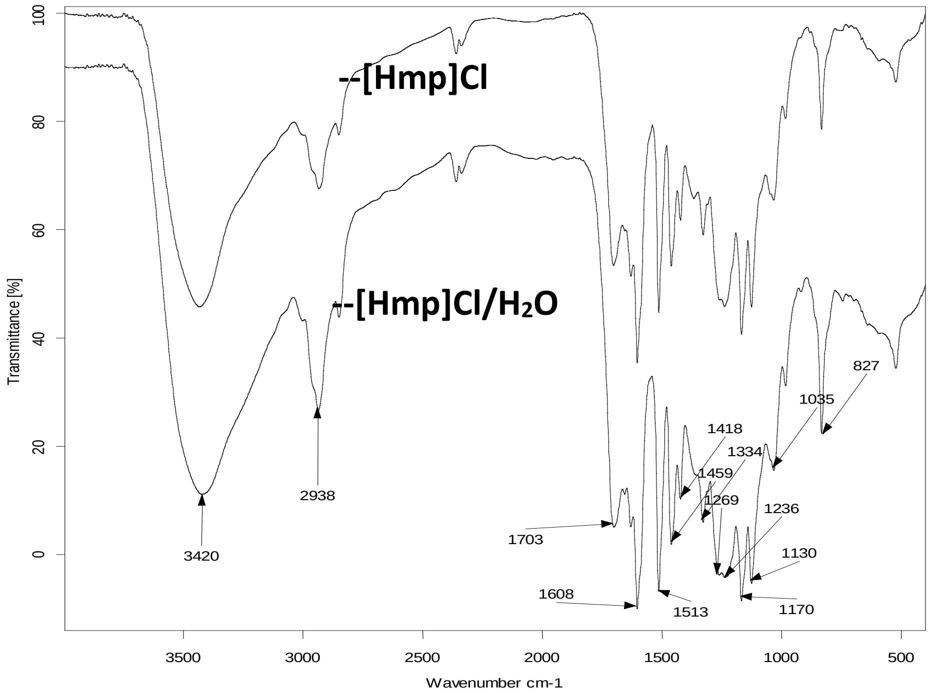

Fig. 4. FTIR spectra of lignin extracted from corn stalk with [Hmp]Cl and [Hmp]Cl/H_2O. (0.25 g corn stalk, 5 g [Hmp]Cl, 1 mL H_2O, T=90 °C , t=30 min)

FTIR analysis of lignin

A high yield of lignin was achieved by [Hmp]Cl. The FTIR spectra of lignin that was extracted by [Hmp]Cl and [Hmp]Cl/H_2O are presented in Fig. 4. The peaks at 3420, 2938, 1703, 1608, 1513, 1459, 1418, 1334, 1269, 1236, 1170, 1130, and 827 cm^{-1} are attributed to lignin. Typically, the peak at 1703 cm^{-1} is associated with C=O stretching, indicating the presence of hydroxycinnamates, *e.g.*, p-coumarate. p-Coumarate is usually esterified with lignin and hemicelluloses in cell walls (Costa Lopes *et al*. 2013). The peaks at 1608 cm^{-1}, 1513 cm^{-1}, and 1418 cm^{-1} are related to aromatic skeletal vibrations. The peak at 1459 cm^{-1} is related to C-H deformations (Costa Lopes *et al*. 2013). The peak at 1334 cm^{-1} is associated with C=O stretching in S units (Ninomiya *et al*. 2015). The peak at 1269 cm^{-1} is related to C=O stretching in G units (Casas *et al*. 2012). The peak at 827 cm^{-1} is attributed to C-H deformations of S units (Casas *et al*. 2012). There is no noticeable difference for the lignin that was obtained from [Hmp]Cl and [Hmp]Cl/H_2O.

Enzymatic Hydrolysis

IL pretreatment accelerates the enzymatic hydrolysis of lignocelluloses (Haykir *et al.* 2013; Aver *et al.* 2014; An *et al.* 2015). Cotton stalk was pretreated in [EMIM]CH_3COO at 150 °C for 30 min (Haykir *et al*. 2013). A total of 65% of reducing sugars were released after 72 h of enzymatic hydrolysis. Rice straw was pretreated by cholinium argininate at 90 °C for 12 h (An *et al*. 2015), and 74.6% of glucose was achieved after 72 h of enzymatic hydrolysis. Wheat straw was pretreated with 1-ethyl-3-methylimidazolium diethylphosphate at 130 °C for 30 min; 54.8% reducing sugar was observed (Sathitsuksanoh *et al*. 2012). The enzymatic hydrolysis of the cellulose-rich materials in this work is summarized in Table 4. The yield of reducing sugar was calculated according to Eq. 4. After enzymatic hydrolysis, a higher yield of reducing sugar was obtained when the corn stalk was pretreated by IL/H_2O compared with IL. Specifically, a high yield (80.54%) of reducing sugar was achieved for [Hmp]Cl/H_2O-treated samples. Sugars were determined by HPLC analysis. The highest yields of glucose and cellobiose were 73.82% and 8.64%, respectively, with [Hmp]Cl/H_2O pretreatment.

Table 4. Enzymatic Hydrolysis of Cellulose-Rich Materials

Entry	Reducing Sugar	Xylose	Glucose	Cellobiose
[Hmp]Cl	52.21%	1.17%	52.25%	5.96%
[Hmp]CH_3SO_3	21.02%	1.21%	29.89%	0.88%
[Hmp]HSO_4	30.67%	2.33%	37.50%	1.02%
[Hmp]Cl/H_2O	80.54%	0	73.82%	8.64%
[Hmp]CH_3SO_3/H_2O	22.58%	0.97%	32.09%	0.51%
[Hmp]HSO_4/H_2O	33.70%	2.72%	40.95%	1.03%

An Insight into IL Pretreatment of Corn Stalk

Generally, ILs with strong hydrogen bond basicity are effective in weakening the hydrogen-bonding network of the polymer chains (Abe *et al*. 2010; Muhammad *et al*. 2011). In this work, a higher yield of lignin was isolated by [Hmp]Cl. ILs containing the anion Cl^- could be more efficient at disrupting inter- and intra-molecular hydrogen bonding in polymers. Additionally, a smaller anion was preferable because it diffuses faster within the lignocellulosic matrix, for example, Cl^- (Mäki-Arvela *et al*. 2010; Hu *et al*. 2013). The small sized cation ($[Hmp]^+$) with a small sized anion (Cl^-) being a strong hydrogen-bond acceptor made [Hmp]Cl an effective pretreatment solvent for lignocelluloses.

However, water competes with the anions of ILs to form the hydrogen bonds with lignin. For chloride-based ILs, the presence of water should coordinate with chloride ions (Shill *et al.* 2011). In this work, 10.17% of lignin was achieved by [Hmp]Cl, and 8% of lignin was obtained in [Hmp]Cl/H_2O. The presence of water in ILs reduces hydrogen bonding between ILs and lignin, thus decreasing the solubility of lignin.

Moreover, higher acidity was observed with [Hmp]HSO_4 and [Hmp]Cl; as a result, a higher yield of reducing sugar was obtained when corn stalk was pretreated in [Hmp]HSO_4/H_2O and [Hmp]Cl/H_2O (Fig. 2). The acidity of ionic liquids could catalyze the hydrolysis of hemicelluloses.

Therefore, the hydrogen bond capability of ionic liquids could remove the lignin content effectively, and the acidity of ionic liquids could assist to hydrolyze hemicelluloses. The noticeable decrease of lignin and hemicelluloses resulted in the high enzyme digestibility of cellulose-rich materials with [Hmp]Cl/H_2O pretreatment. The reduced lignin and hemicelluloses in cellulose were also confirmed by FTIR analysis. Both the hydrogen bond capability and the acidity of [Hmp]Cl contributed to the high yield of IL-isolated lignin and high enzymatic digestibility of cellulose-rich materials.

CONCLUSIONS

1. A series of pyrrolidinium-based ionic liquids with a simple protocol was used as effective pretreatment solvents for lignocelluloses. Both ionic liquids alone and ionic liquid with deionized water were studied. Short pretreatment temperature and pretreatment time were employed.
2. [Hmp]Cl was effective for lignin extraction, as 10.17% of the original lignin of corn stalk was recovered with [Hmp]Cl pretreatment at 90 °C for 30 min. [Hmp]Cl/H_2O was effective for enzymatic digestibility of the recovery lignocelluloses; 80.54% of reducing sugar was obtained after enzymatic hydrolysis, containing 73.82% glucose and 8.64% cellobiose.
3. FTIR analysis indicated the characteristics of regenerated lignin and cellulose-rich materials. A dramatic reduction of hemicelluloses and lignin for corn stalk after [Hmp]Cl and [Hmp]Cl/H_2O pretreatment was observed.
4. The pretreatment efficiency is higher for pure IL in respect to lignin removal. The pretreatment with IL/H_2O could enhance the enzymatic saccharification of lignocelluloses.

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