

Research Article

An Efficient Process for Pretreatment of Lignocelluloses in Functional Ionic Liquids

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Background and Aims. The complex structure of the lignocelluloses is the main obstacle in the conversion of lignocellulosic biomass into valuable products. Ionic liquids provide the opportunities for their efficient pretreatment for biomass. Therefore, in this work, pretreatment of corn stalk was carried out in ultrasonic-assisted ionic liquid including 1-butyl-3-methylimidazolium chloride [BMIM]Cl, 1-H-3-methylimidazolium chloride [HMIM]Cl, and 1-(1-propylsulfonic)-3-imidazolium chloride [HSO₃-pMIM]Cl at 70°C for 2 h. We compared the pretreatments by ionic liquid with and without the addition of deionized water. Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) were employed to analyze the chemical characteristics of regenerated cellulose-rich materials. **Results.** [HMIM]Cl and [HSO₃-pMIM]Cl were effective in lignin extraction to obtain cellulose-rich materials. FTIR analysis and SEM analysis indicated the effective lignin removal and the reduced crystallinity of cellulose-rich materials. Enzymatic hydrolysis of cellulose-rich materials was performed efficiently. High yields of reducing sugar and glucose were obtained when the corn stalk was pretreated by [HMIM]Cl and [HSO₃-pMIM]Cl. **Conclusions.** Ionic liquids provided the ideal environment for lignin extraction and enzymatic hydrolysis of corn stalk and [HMIM]Cl and [HSO₃-pMIM]Cl proved the most efficient ionic liquids. This simple and environmentally acceptable method has a great potential for the preparation of bioethanol for industrial production.

1. Introduction

Biofuels produced from biomass have attracted much attention in recent years due to the awareness of energy crisis and global warming. Lignocellulosic biomass is increasingly promoted as environmentally and economically sustainable fuel with a high potential as it is abundant form of biomass and nonfood source for the bioethanol production [1]. During the process of lignocellulose-to-biofuel, the complex structure of the lignocelluloses is the main obstacle in lignocellulosic conversion [2]. Pretreatment of lignocelluloses in high temperature and high pressure is always required in order to break the lignocellulosic structure to make its cellulose available for enzymatic hydrolysis. It is necessary to develop alternative new techniques.

In recent years, ionic liquids (ILs) have gained wide popularity for their increasing applications as they possess a number of interesting properties such as low vapor pressure, high thermal stability, and lack of flammability. In 2002, Rogers group reported the dissolution of cellulose in ILs with the cation of 1-butyl-3-methylimidazolium ([BMIM]) and the anion of Cl⁻, Br⁻, or SCN⁻ at 100°C [3]. As follows, studies of cellulose in ILs with the cation of [BMIM] and the anion of chloride, bromide, acetate, or formate are reported. It is also described that the pretreatment with ILs can reduce the crystallinity of cellulose and partially remove hemicelluloses and lignin [2]. Currently, Nasirpour et al. reported the pretreatment of sugarcane bagasse by [BMIM]Cl at 130°C for 90 min [4]. Aver et al. reported the pretreatment of sugarcane bagasse by [EMIM]CH₃COO, [BMIM]CH₃COO,

[BMIM]Cl, [BMIM]Br, and [BMIM]CH₃SO₄ at 80°C and 120°C for a period of 24 h [5]. Ma et al. reported the combined use of ultrasound irradiation and [BMIM]Cl as a potential alternative method in the preparation of glutarates from sugarcane bagasse [1]. However, most studies focused on the pretreatment of lignocelluloses by ionic liquids including [AMIM]Cl, [BMIM]Cl, and [EMIM]CH₃COO. High pretreatment temperatures and long pretreatment time are always required in current studies. With the aim of producing more efficient ionic liquids for the treatment of agricultural lignocellulosic materials, ionic liquids including 1-butyl-3-methylimidazolium chloride [BMIM]Cl, 1-H-3-methylimidazolium chloride [HMIM]Cl, and 1-(1-propylsulfonic)-3-imidazolium chloride [HSO₃-pMIM]Cl were investigated.

2. Materials and Methods

2.1. Materials. All chemicals were purchased from Aladdin Company (China) and used as received. Cellulase (10000 U/mg) was purchased from Aladdin Company. The corn stalk supplied by local farm in Harbin City (China) was milled to pass through 40 mesh sieves and dried at 60°C for 24 h prior to use. [BMIM]Cl, [HMIM]Cl, and [HSO₃-pMIM]Cl were prepared as described [6, 7]. Treatment of corn stalk was performed in KQ-400KDE ultrasound (Kunshan). Analysis of reducing

sugar was performed on UV-mini-1240 spectrometer (SHIMADZU). Fourier transform infrared spectroscopy (FTIR) was analyzed on FTIR-8400S spectrometer (SHIMADZU, Japan) in the range of 4000–400 cm⁻¹. Scanning electron microscopy (SEM) was performed on S-3400N (HITACHI). HPLC analysis was performed on WATERS 2695. The composition of corn stalk was determined according to the standard NREL method [8]. There are 21.34% of lignin, 20.31% of hemicelluloses, 43.11% of cellulose, and 0.75% of ash.

2.2. Ionic Liquid Treatment. The treatment of corn stalk in ionic liquids was conducted in 50 mL vessels placed under ultrasound irradiation (400 W). About 0.2 g of cotton stalk with or without 1 mL of deionized water was rapidly added into 5 g of one of the ionic liquids for 120 min at 70°C. When [BMIM]Cl was used, 2 mL of DMSO was added into [BMIM]Cl. After pretreatment by ionic liquid, 20 mL of an antisolvent (deionized water/acetone, 1/1, v/v) was added into the mixture. The filtrate was measured for sugar analysis. The solid was filtered out, washed with 30 mL of water/acetone (1/1, v/v), and then dried in a vacuum at 60°C for 24 h to give the cellulose-rich materials. When acetone was evaporated, lignin precipitated out in IL/H₂O solution. The regenerated lignin was filtered out, washed with deionized water, and dried under vacuum at 60°C for 24 h:

$$\text{The cellulose-rich materials or lignin (\%)} = \left(\frac{\text{the weight of cellulose-rich materials or lignin (g)}}{\text{the weight of untreated corn stalk subjected to pretreatment (g)}} \right) \times 100\%. \quad (1)$$

2.3. Determination of the Reducing Sugar. The filtrate from 2.2 was neutralized by the addition of solid of sodium hydroxide. The amount of reducing sugar was measured using the 3,4-dinitrosalicylic acid (DNS) method [9]. The

concentration of reducing sugar was calculated by employing a standard curve prepared using glucose and the absorbance was measured at 540 nm using UV spectrometer:

$$\text{Reducing sugar during pretreatment (\%)} = \left(\frac{\text{the weight of reducing sugar in the filtrate (g)}}{\text{the weight of untreated corn stalk subjected to pretreatment (g)}} \right) \times 100\%. \quad (2)$$

2.4. Enzymatic Hydrolysis. Enzymatic hydrolysis of untreated or IL-treated samples was carried out with a biomass loading of 1% (g/mL) in 0.05 M sodium citrate buffer at pH 4.8 and 50°C in a water shaker incubator at 200 rpm. 5 mL of cellulase was used at a loading of 4000 U/g. The enzymatic reaction

proceeded for 12 h and was then quenched after stirring at 100°C for 10 min. After that, the mixture was filtered out and the filtrate was neutralized by the addition of solid of sodium hydroxide. The reducing sugars were analyzed via DNS method [9]:

$$\text{Enzymatic reducing sugar (\%)} = \left(\frac{\text{the weight of reducing sugar after enzymatic hydrolysis (g)}}{\text{the weight of cellulose-rich materials subjected to pretreatment (g)}} \right) \times 100\%. \quad (3)$$

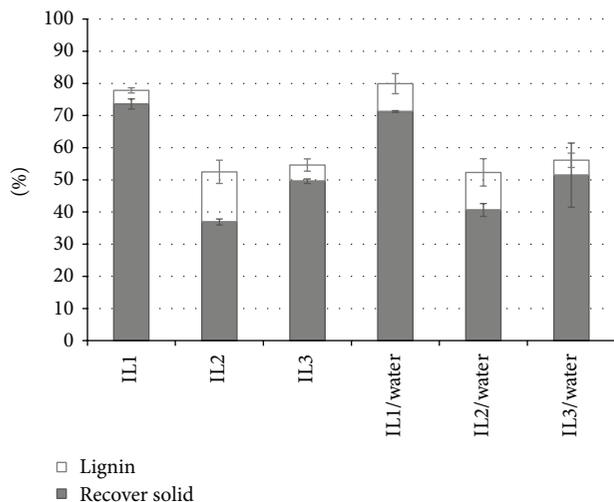


FIGURE 1: Yields of IL-isolated lignin and cellulose-rich materials. IL1: [BMIM]Cl/DMSO, IL2: [HMIM]Cl, and IL3: [HSO₃-pMIM]Cl.

3. Results and Discussion

In this work, pretreatment of corn stalk performed in IL and IL/H₂O was investigated. Due to the solid form of [BMIM]Cl at reaction conditions, DMSO was added into [BMIM]Cl, which can decrease the viscosity of ionic liquid and benefit the pretreatment process [10]. After pretreatment with one of the ionic liquids, a solution of water/acetone (1:1) was added into the mixture and the solid was filtered out to give cellulose-rich materials. As presented in Figure 1, about 73% of cellulose-rich materials were obtained when corn stalk was treated by [BMIM]Cl. About 37% and 50% of cellulose-rich materials were achieved when the corn stalk was treated by [HMIM]Cl and [HSO₃-pMIM]Cl, respectively, which indicated that most lignin was isolated by [HMIM]Cl.

The presence of lignin limits cellulase reaching to cellulose; thus, lignin removal is important to conduct an efficient cellulose conversion. As shown in Figure 1, higher lignin was obtained when corn stalk was pretreated with only IL compared to that treated with IL/H₂O, which was probably because the dissolution of lignin from corn stalk was decreased when water was added. A high yield of lignin was obtained when the corn stalk was pretreated with [HMIM]Cl and [HSO₃-pMIM]Cl. A low yield was observed using [BMIM]Cl. As reported, ionic liquids including [BMIM]Cl, [EMIM]COOH have been successfully employed for their ability to remove lignin. Li et al. reported 69.2% total lignin removal from switchgrass using [EMIM]COOH at 160 °C for 3 h [11]. Liu et al. described that the lignin of untreated corn stalk was 17.71% and it reduced to 12.35% after being treated by [BMIM]Cl at 145 °C for 15 min [12]. In this work, 15.59% of lignin was obtained when the corn stalk was pretreated by only [HMIM]Cl without deionized water at 70 °C for 2 h, corresponding to 73% in the original lignin of corn stalk.

Actually, when water was added into the pretreatment process, hydrolysis of cellulose and hemicelluloses occurred. As shown in Figure 2, a high yield of reducing sugar was obtained with the pretreatment by IL/H₂O. A low yield of

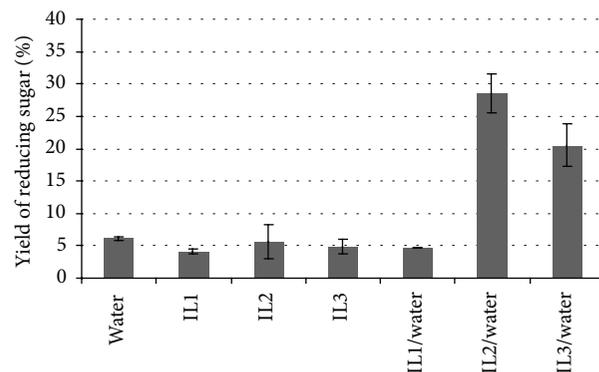


FIGURE 2: Yields of reducing sugar in the pretreatment. IL1: [BMIM]Cl/DMSO, IL2: [HMIM]Cl, and IL3: [HSO₃-pMIM]Cl.

reducing sugar was observed with the pretreatment by only IL. High yields of reducing sugar specially were obtained when corn stalk was treated by [HMIM]Cl/H₂O or [HSO₃-pMIM]Cl/H₂O. Poor yield was found when corn stalk was pretreated by [BMIM]Cl/DMSO/H₂O, probably due to the weak basic property of [BMIM]Cl, which could not induce the effective hydrolysis of the corn stalk. Reducing sugar was comparable when the corn stalk was treated by only water or ionic liquid.

3.1. SEM Analysis. SEM was employed to examine the structural change of untreated and pretreated corn stalk in morphology before and after IL pretreatment. As shown in Figure 3, the compact ordered and rigid fibril structure was observed in untreated corn stalk. After pretreatment of corn stalk with ionic liquids, the structure became loose disordered, which was probably due to the removal of lignin and decrease of cellulose crystallinity. Pretreatment with IL and IL/H₂O caused slight modifications on the structure of corn stalk. Pretreatment with [HMIM]Cl and [HSO₃-pMIM]Cl induced considerable changes (Figure 3).

3.2. FTIR Analysis. FTIR analysis was conducted to examine the structure of untreated, IL-treated, and IL/H₂O-treated corn stalk. As presented in Figure 4, the peaks at 3422, 2918, 1430, 1371, 1318, 1162, 1112, 1069, and 897 cm⁻¹ are associated with cellulose. The peak at 3422 cm⁻¹ is attributed to O-H stretching. The absorption at 2918 cm⁻¹ is attributed to C-H stretching. A peak at 1430 cm⁻¹ is associated with CH₂ symmetric bending. 1371 cm⁻¹ is associated with C-H bending. 1318 cm⁻¹ is associated with CH₂ bending of carbohydrates. The peak at 1162 cm⁻¹ is attributed to C-O antisymmetric stretching. A band at 1112 cm⁻¹ is related to C-OH skeletal vibration. A peak at 1069 cm⁻¹ is due to the C-O-C pyranose ring skeletal vibration. The peak at 897 cm⁻¹ corresponds to the glycosidic C-H deformation with ring vibration contribution, which is a characteristic of β-glycosidic linkages between glucose molecules in cellulose.

As shown in Figure 4, a strong peak at 1733 cm⁻¹ (Line 1) was attributed to the presence of unconjugated carbonyl and acetyl groups of hemicelluloses in untreated corn stalk. This

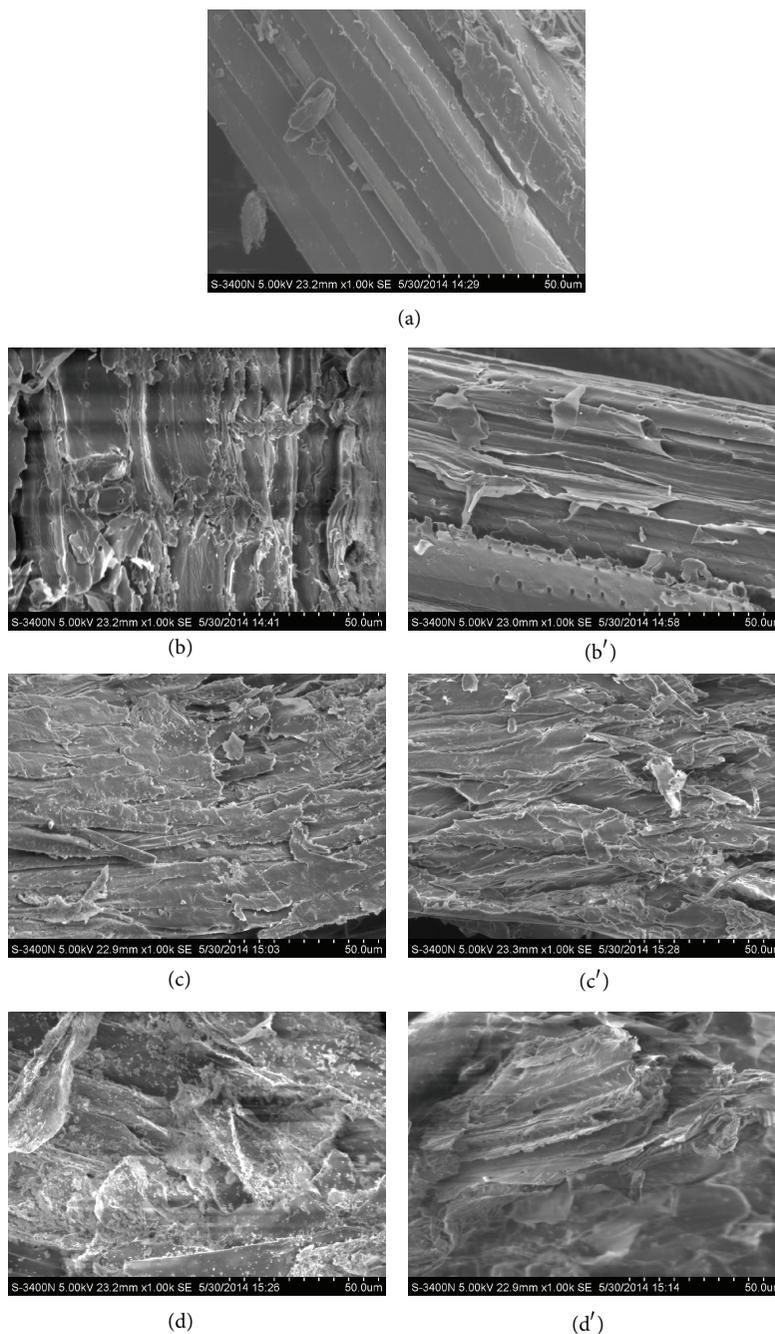


FIGURE 3: SEM analysis of corn stalk and cellulose-rich materials. (a) Untreated corn stalk; (b) [BMIM]Cl/DMSO; (c) [HMIM]Cl; (d) [HSO₃-pMIM]Cl; (b') [BMIM]Cl/DMSO/H₂O; (c') [HMIM]Cl/H₂O; (d') [HSO₃-pMIM]Cl/H₂O.

peak intensity was significantly reduced after the pretreatment by [HMIM]Cl and [HSO₃-pMIM]Cl, which indicated that hemicelluloses were decreased effectively; however, no obvious reduction was observed with the pretreatment by [BMIM]Cl. The peak at 1430 cm⁻¹ (Line 2) can be assigned to bending vibration of CH₂ that is strong in crystalline cellulose and weak in amorphous cellulose [13]. The CH₂ band was weaker after the sample was pretreated by [HMIM]Cl and [HSO₃-pMIM]Cl, which implied that the crystallinity of

cellulose-rich materials was reduced. The intensity of peak at 1250 cm⁻¹ (Line 3) assigned as C-O stretching signal in lignin and hemicelluloses was also reduced indicating the decreased lignin and hemicelluloses [11]. In addition, the peak at 897 cm⁻¹ (Line 4) corresponds to the glycosidic C-H deformation with ring vibration contribution, which is a characteristic of β-glycosidic linkages between glucose molecules in cellulose [14]. This peak was obvious after the pretreatment by ionic liquids, especially [HMIM]Cl

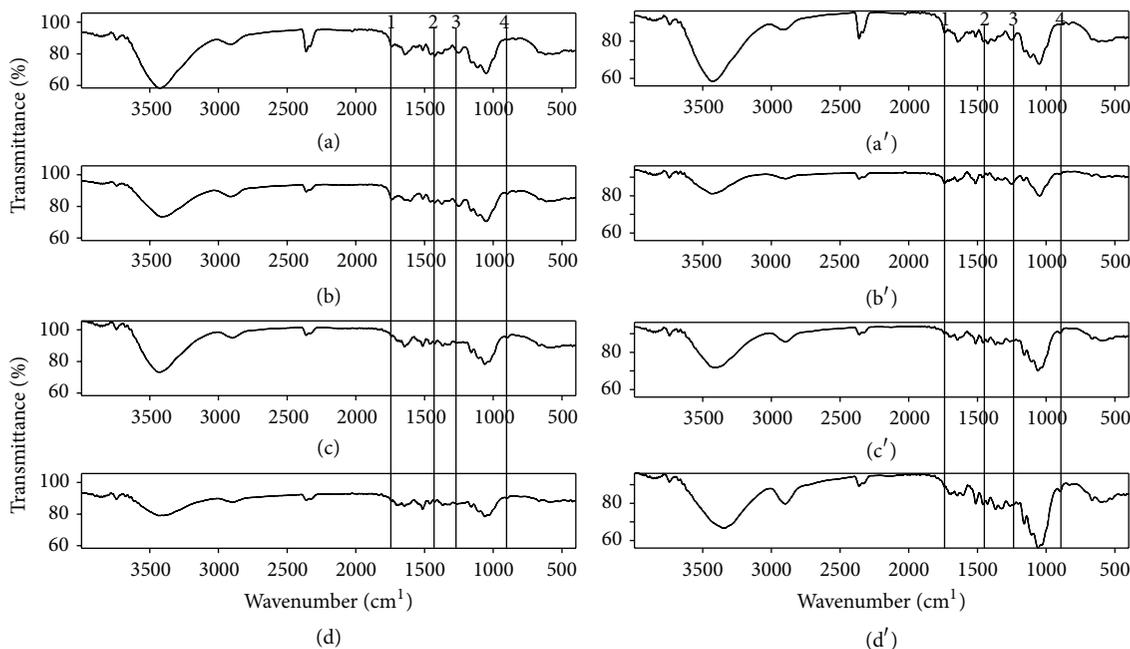


FIGURE 4: FTIR spectra of corn stalk and cellulose-rich materials: (a) untreated corn stalk; (b) [BMIM]Cl/DMSO; (c) [HMIM]Cl; (d) [HSO₃-pMIM]HSO₃; (a') untreated corn stalk; (b') [BMIM]Cl/DMSO/H₂O; (c') [HMIM]Cl/H₂O; (d') [HSO₃-pMIM]Cl/H₂O.

and [HSO₃-pMIM]Cl indicating the reduced crystallinity of cellulose.

3.3. Enzymatic Hydrolysis of Corn Stalk. Enzymatic hydrolysis of untreated, IL-treated, and IL/H₂O-treated corn stalk and cellulose-rich materials after 12 h was summarized in Figure 5. Significantly high yield of reducing sugar was observed in the samples treated by [HMIM]Cl and [HSO₃-pMIM]Cl, possibly because most of lignin was removed and the crystalline structure was decreased when corn stalk was pretreated by ionic liquid. After 12 h, a yield of 42% and 39% of reducing sugar was obtained when the corn stalk was pretreated by [HMIM]Cl and [HSO₃-pMIM]Cl, respectively. Then enzymatic hydrolysis time was increased to 72 h. After 72 h, 85% and 81% of reducing sugar were achieved for [HMIM]Cl and [HSO₃-pMIM]Cl, respectively. Sugar analysis was conducted by HPLC. There are 75% of glucose and 12% of cellobiose for the pretreated sample by [HMIM]Cl after 72 h. The considerable lignin removal and the reduction of cellulose crystalline of corn stalk benefited the effective enzymatic digestibility.

4. Conclusion

In conclusion, ionic liquids provided the ideal environment for lignin extraction and enzymatic hydrolysis for corn stalk. [HMIM]Cl and [HSO₃-pMIM]Cl were proved as the most efficient ionic liquids as pretreated solvents to remove lignin and give the recovery biomass for enzymatic hydrolysis. This simple and environmentally acceptable method has a great potential for the preparation of bioethanol for industrial production.

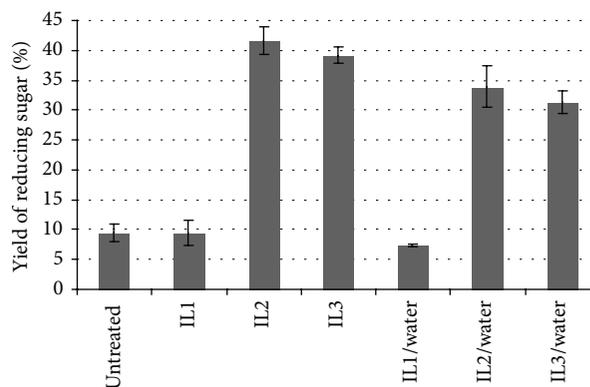


FIGURE 5: Yields of reducing sugar after enzymatic hydrolysis. IL1: [BMIM]Cl/DMSO, IL2: [HMIM]Cl, and IL3: [HSO₃-pMIM]Cl.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Bi-Xian Zhang and Shi-Jia Dong contributed equally to this work; both of them are first authors.

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