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Selective Hydrolysis of Lignocelluloses from Corn Stalk in an Ionic Liquid

Wei-ping Yin,[1,2] Xin Li,[1] Yun-lai Ren,[1] Shuang Zhao,[1,2] Jian-ji Wang[1,2]

[1]School of Chemical Engineering and Pharmaceutics, Henan University of Science and Technology, Luoyang, Henan 471003, People's Republic of China

[2]The Key Laboratory for Biomass Clean Energy, Henan University of Science and Technology, Luoyang, Henan 471003, People's Republic of China

Correspondence to: W.-P. Yin (E-mail: ywpq@163.com); J.-J. Wang (E-mail: jwang@henannu.edu.cn)

ABSTRACT: In this work, a selective two-stage hydrolysis of lignocelluloses from corn stalk was developed through pH adjustment in 1-*n*-butyl-3-methylimidazolium chloride ([C_4mim]Cl). In the first stage, the lignin–hemicelluloses matrix of corn stalk was disrupted and hydrolyzed in the ionic liquid at pH 4.5 and 90°C to obtain xylose with 23.1% yield. In the second stage, cellulose-rich materials in solid residues were further hydrolyzed in the ionic liquid at pH 2–3 and 90°C to produce glucose with 26.9% yield, and pure lignin was also obtained. Structures of the hydrolysates were identified by ^{13}C NMR and IR spectrum analysis, and the ionic liquid was recycled throughout the process. It is expected that the information provided here would be useful for the development of new methods to selectively produce monosaccharides from catalytic hydrolysis processes of biomass. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 129: 472–479, 2013

KEYWORDS: cellulose and other wood products; hydrophilic polymers; ionic liquids

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INTRODUCTION

The worldwide energy crisis has led to extraordinary efforts on the exploration of the alternative energy sources. In this respect, biomass-based fuels have attracted much interest due to their plentiful supply as a renewable energy source. However, the conversion of lignocellulose materials into biofuels needs to overcome some technological barriers and is even more difficult than sugars transformation.[1] Acid hydrolysis is one of the traditional pretreatment methods for lignocelluloses. This is usually performed by reaction with concentrated acids, including H_2SO_4 and HCl under the conditions of 160–230°C and ~10 atm.[2,3] Though the concentrated acids are powerful catalysts for lignocelluloses hydrolysis, they are energy-intensive and not eco-friendly. During the acid hydrolysis, the formed degradation products, including hydroxymethyl furfural (HMF) and furfural, may inhibit downstream fermentation of monosaccharides.[4] Conversion of lignocellulosic materials to sugars can be processed under relatively mild conditions but with much lower efficiency. It was reported that a yield of 16.6 g xylose per 100 g substrate could be obtained by a dilute sulfuric acid ($w = 2\%$) pretreatment of corn stover at 120°C, whereas the yield of glucose was only 4.1 g per 100 g substrate in this process.[5]

Ionic liquids (ILs), as novel greener solvents with unique properties, have been found to be capable of decomposing fiber structure of lignocelluloses and dissolving cellulose and hemicellulose.[6–8] It was reported that 1-*n*-butyl-3-methylimidazolium chloride [C_4mim]Cl could efficiently dissolve polysaccharides, lignin, lignocelluloses, chitin, and other biopolymers.[8–13] Under microwave heating, [C_4mim]Cl can dissolve up to 25 wt% cellulose,[9] and non-hydrated chloride ions in [C_4mim]Cl has a strong ability to disrupt inter- and intra-molecular hydrogen bonds as well as van der Waals interactions of the carbohydrates.[14]

Due to the extremely low volatility, high thermal stability, and fluidity at ambient temperature,[15] ILs can be utilized as effective solvents for hydrolysis of lignocelluloses. Li et al.[16] reported the high yield of total reducing sugars and glucose (77% and 43%) from the hydrolysate of cellulose in the H_2SO_4/[C_4mim]Cl system. The combination of ionic liquid with acid has been tested for the acid catalyzed hydrolysis of lignocelluloses.[17] Using hydrochloric acid as catalyst in [C_4mim]Cl, the shortest reaction time for corn stalk was observed at pH 1.0 and 100°C, and the yield of total reducing sugars was 66 wt% of the polysaccharides contained in the substrates. It thus demonstrated that acid-

catalyzed hydrolysis in ILs provide a potential route for the conversion of lignocelluloses to further biorefinery. In the presence of trifluoroacetic acid in [C_4mim]Cl, 97% carbohydrate fraction in pine wood can be converted into water-soluble compounds, including monosaccharide, oligosaccharides, furfural, and 5-hydroxymethylfurfural. Most of the lignin fraction in pine wood was recovered as the solid residue.[6] Recently, it was claimed by Li and co-workers[17] that a remarkable efficient hydrolysis of hemicellulose, cellulose, and lignin was achieved with the HCl pretreatment of three wood species in 1-allyl-3-methylimidazolium chloride [Amim]Cl. Although yield of reducing sugars was high in this process, these hydrolysates contain a complex mixture of pentose, hexoses, and other monosugar components, and it would be difficult to separate these components for further application. Zhang et al.[18] reported that cellulose could be converted into water-soluble reducing sugars in the absence of acid with a yield of 58% in the [C_4mim]Cl-H_2O (1:4 wt ratio) mixture at 120°C. This indicates that ILs display catalytic action in the hydrolysis of cellulose while mixing with water. However, there is no ILs recovered during this process, and it is thus not eco-friendly. Also, selective hydrolysis of lignocelluloses has not been reported previously.

Based on the current advances in biofuel technologies, the selectively hydrolysis of lignocelluloses and separation of cellulose and hemicelluloses from corn stalk is prior considered in the biofuel study. In this work, a selective two-stage hydrolysis and efficient isolation process of lignocelluloses from corn stalk was studied in [C_4mim]Cl by the modulation of pH values. It provides an effective method for the selective hydrolysis and separation of lignocelluloses components.

EXPERIMTENTAL

Materials and Apparatus

Air-dried corn stalk (*Zeal Mays* L.), originally grown in Henna, China, and harvested in September, 2010, was obtained from local farms and used as the raw material. After the corn stalk was milled, the particles were separated by using a 40-mesh sieve and then dried overnight in an oven at 105°C. The ionic liquid [C_4mim]Cl (purity > 99%) was obtained from J & K Chem (Beijing, China). Microcrystalline cellulose (Advice®, DP 270) and Lignin was purchased from Sigma-Aldrich (Shanghai, China). Deionized water was obtained freshly by a deionized water equipment from HEKEDA Ultrasonic Equipment Co. (Shenzhen, China). Hydrochloric acid, ethanol, and other chemicals were supplied by Tannin Kernel Chem Reagent Co. (Zhengzhou, China). Chromatography reagents were freshly distilled before use. The granular active carbon packed into the chromatographic column (CC, Φ150 × 6 cm) was purchased from J & K Chem (Beijing, China).

Infrared analysis was performed by using a Nicollet 6700 Fournier transform infrared spectrometer from Thermo Scientific, USA. The carbon-13 nuclear magnetic resonance (^{13}CNMR) spectra were determined with a Bruker Avance 100 MHz spectrometer using tetramethylsilane (TMS) as an internal standard. Scanning electron microscope (SEM) images were taken by using a JEOL JSM-5610LV equipment (Japan) with an energy dispersive spectrometer. Solution pH values were measured by a pHS-25 pH-meter from Nanjing Everich Medicare, China. Ultraviolet–visible spectroscopy (UV-Vs) was monitored with a 722G spectrophotometer from Shanghai Modern Science Instrument Co.

Acid-Catalyzed Hydrolysis of Corn Stalk in the IL

Twenty-three grams of [C_4mim] Cl was melted in a sealed beaker placed in a heated oil bath. The ground corn stalk (1.625 g) was dispersed in the liquid [C_4mim]Cl and then stirred at 90°C. The pH value of the mixture was adjusted to 4.5 by adding an appropriate amount of aqueous HCI with a concentration of 10% (v/v). Thin-layer chromatography was applied for tracking and monitoring the progress of hydrolysis in the IL. After 7 h, the resulting viscous homogeneous mixture was cooled and then diluted with deionized water at the ratio of 1 : 1 (v/v), leading to the precipitation in the static settlement. The solid residue I was removed through vacuum filtration, washed with distilled water, and then dried overnight at 105°C. In the second stage of hydrolysis, the dried solid residues I were sequentially hydrolyzed at 90°C and pH 2–3 in the IL. A homogenous emulsion was obtained after 7 h and diluted with deionized water at the ratio of 1 : 1 (v/v). After static settlement, unhydrolyzed solid residue II was removed by filtration, washed three times with water, and then dried at 105°C. Hydrolysates and the ionic liquid in the liquid phases were separated from both two stages by the column chromatography.

Chromatographic Separation and NMR Analysis

Hydrolysates of both two stages were loaded onto the active charcoal column, followed by chromatography purification with a gradient elution from H_2O, 10% EtOH-H_2O and 30% EtOH-H_2O, respectively, to afford 12 fractions, of which pure compounds were identified by ^{13}C NMR analysis, and TMS was utilized as an internal standard.

Analysis of Total Reducing Sugars

Total reducing sugars (TRS) in the hydrolysates of corn stalk were determined by using the dinitrosalicylate (DNS) method as described in literatures.[16,17] The color intensity of the mixture was measured at 540 nm by a 722G UV-Vis spectrophotometer. The concentration of total reducing sugars was calculated from the standard curve established in the present work. The yield of total reducing sugars was calculated as follows:

$$\text{Yield}\ (\%) = \frac{\text{TRS concentration (mg/ml)} \times V_1\ \text{(ml)} \times (M_0/M_1)}{M_S\ \text{(mg)}} \times 100\%$$

where M_s is the mass of the original substrate, V_1 is the volume of the sample, M_0 is the total mass of the reaction solution, and M_1 is the mass of the sample. The average error of TRS concentrations was estimated to be about 2.0%.

Characterization of the Solid Residues

Solid residues I and II, obtained as described above, were characterized by an IR spectrometer. The IR spectra of the microcrystalline cellulose and lignin standards were also determined by the same method for the purpose of comparison. At the same time, the morphology of the residues was investigated by SEM.

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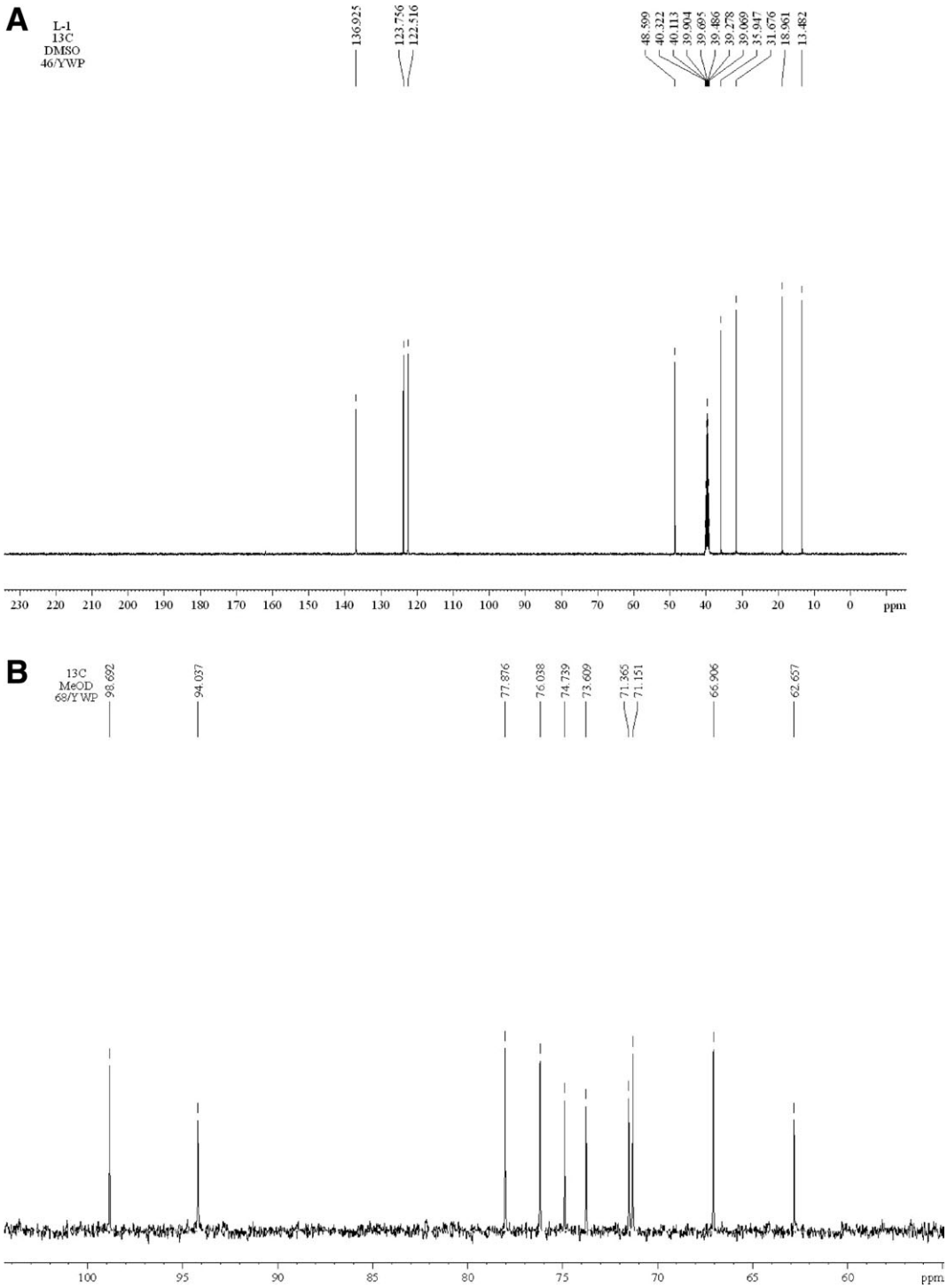

Figure 1. ^{13}C NMR spectra of the components in the aqueous solution after the first-stage hydrolysis. (A) The ^{13}CNMR spectrum of the regenerated [C_4mim]Cl from the aqueous solution. (B) The ^{13}CNMR spectrum of xylose produced in the first-stage hydrolysis.

RESULTS AND DISCUSSION

Compared to previous reports,[18,19] the work described here is a selective and controllable hydrolysis process because the selective hydrolysis of cellulose and hemicellulose as well as the precipitation of lignin can be controlled by pH values. All the hydrolysates have higher yields, and temperature and pH value are the two most important factors for the hydrolysis process.

Separation of Hydrolysates by Activated Charcoal Column Chromatography

Owing to the fact that [C_4mim]Cl and reducing sugars have different molecular size and polarity in the aqueous solution,

Table I. Comparison of ^{13}C NMR Spectral Data (100 MHz, MeOD) of the Hydrolysates from the First-Stage Hydrolysis and D-Xylopyranose[22]

	δ_C (ppm)			
No.	Hydrolysates	α-D-Xylopyranose	Hydrolysates	β-D-Xylopyranose
C1	94.0	93.3	98.6	97.7
C2	73.6	72.3	76.0	75.1
C3	74.7	73.9	77.9	76.9
C4	71.4	70.5	71.2	70.3
C5	62.7	62.1	66.9	66.2

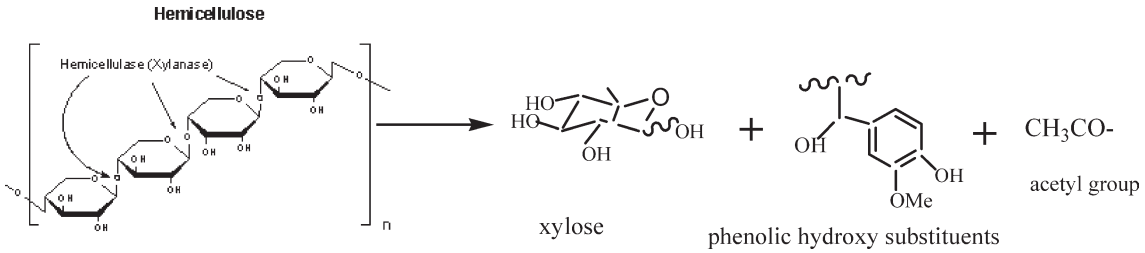

Figure 2. Graphic illustration of the possible hydrolytic mechanism of hemicellulose from corn stalk.

satisfactory separation of hydrolysates of corn stalk from the ionic liquid was achieved using an active charcoal column (Φ150 × 6 cm) with a stepwise gradient elution. According to the ^{13}C NMR results, fractions 1–3 were aqueous [C_4mim]Cl, fractions 4–6 were pure IL, fractions 7–8 were the mixtures of the phenolic substituents (HMF and furfural), and fractions 9–12 were mainly composed of monosaccharides. The order of elution from the column chromatography was aqueous IL (ready to be regenerated by vacuum distillation), mixture of phenolic substituents, HMF, furfural, and monosaccharide mixtures.

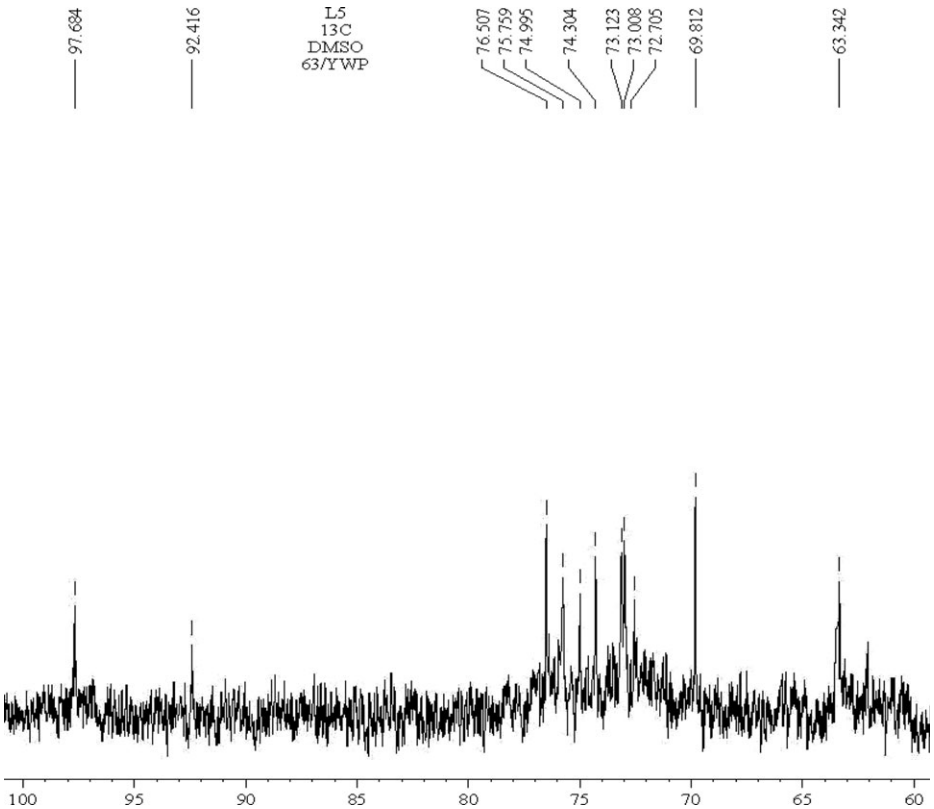

Figure 3. The ^{13}CNMR spectrum of the water-soluble hydrolysates produced in the second-stage hydrolysis.

Table II. Comparison of ^{13}C NMR Spectral Data (100 MHz, MeOD) of the Hydrolysates from the Second-Stage Hydrolysis and D-Glucopyranose[26]

	δ_C (ppm)			
No.	Hydrolysates	α-D-Glucopyranose	Hydrolysates	β-D-Glucopyranose
C1	92.4	92.1	97.9	96.8
C2	74.3	72.3	75.0	74.8
C3	73.1	73.0	75.8	76.6
C4	72.7	70.6	73.0	70.3
C5	69.8	71.8	76.5	76.7
C6	61.8	61.2	63.3	

IDENTIFICATION OF THE REGENERATED IL AND THE WATER-SOLUBLE HYDROLYSATES AFTER THE FIRST-STAGE HYDROLYSIS

^{13}C NMR spectra of the regenerated IL and the hydrolysates produced in the first stage were presented in Figure 1. The regenerated [C_4mim]Cl was examined by ^{13}CNMR in DMSO-d_6 solvent, and the result was shown in Figure 1(A). From the comparison of ^{13}C NMR spectra for the original and regenerated ILs, it can be seen that the regenerated IL had considerable high purity and was able to be reused in the next hydrolysis process. Signals of the released hydrolysates in the first stage were shown in Figure 1(B). The results showed that D-xylose and other phenolic hydroxyl substituents were released from the first-stage hydrolysis,[20] indicating breakdown of the lignin–hemicellulose matrix and the decomposition of hemicellulose under such circumstances. The ^{13}C NMR signals of D-xylose in Figure 1(B) and its ^{13}C NMR spectral data were assigned in Table I.[21,22] In the ^{13}C NMR spectra, D-xylose is the predominant components. Nevertheless, signals of other reducing sugars, such as arabinose, mannose, galactose, and among others, which should be released from hemicellulose simultaneously, were too weak to be detected in the ^{13}C NMR spectrum. Based on the above discussion, the possible mechanism for the first-stage hydrolysis was shown in Figure 2.

The very weak resonance signals in Figure 1(B) observed at δ_C173.8, 22.1, 177.8, and 23.2 were assigned to *O*-acetyl group, and the signals at δ_C115.9 (CH), 123.4 (C), 125.4 (C), 132.0 (C), 145.0 (C-OH), 149.5 (C-OH or C-OR), and 72.6 (-OCH_2-) were assigned as the aromatic characteristic compounds. This indicates the cleavage of aryl ether linkage in the lignin–hemicellulose matrix and the formation of the free phenolic hydroxyl groups.[8,23,24] Furthermore, TRS yield of the first-stage hydrolysis was determined to be 23.1%.

Identification of the Water-Soluble Hydrolysates after the Second-Stage Hydrolysis

The ^{13}C NMR spectrum of the hydrolysates released from the second-stage hydrolysis process was displayed in Figure 3. It is shown that glucose was the major component in the water-soluble hydrolysates and other monosaccharides were barely observed. The signals of glucose were assigned in Table II, which are in agreement with data reported in the spectral database and literatures.[25,26] This implied that cellulose of corn stalk was hydrolyzed in the second stage under lower pH value, and there was no hemicellulose remained in the solid residue I. The TRS yield of the second-stage hydrolysis was determined to be 26.9%.

Identification of the Solid Residues

The structure of the solid residues I and II were determined by infrared spectroscopy, and the results were displayed in Figures 4(A) and 5(A), respectively. For the sake of comparison, the spectrum data of microcrystalline cellulose and lignin standards were also determined and shown in Figures 4(B) and 5(B), respectively. It can be seen from Figure 4(A) and 4(B) that significantly characteristic peaks of cellulose in the IR spectrum were given at

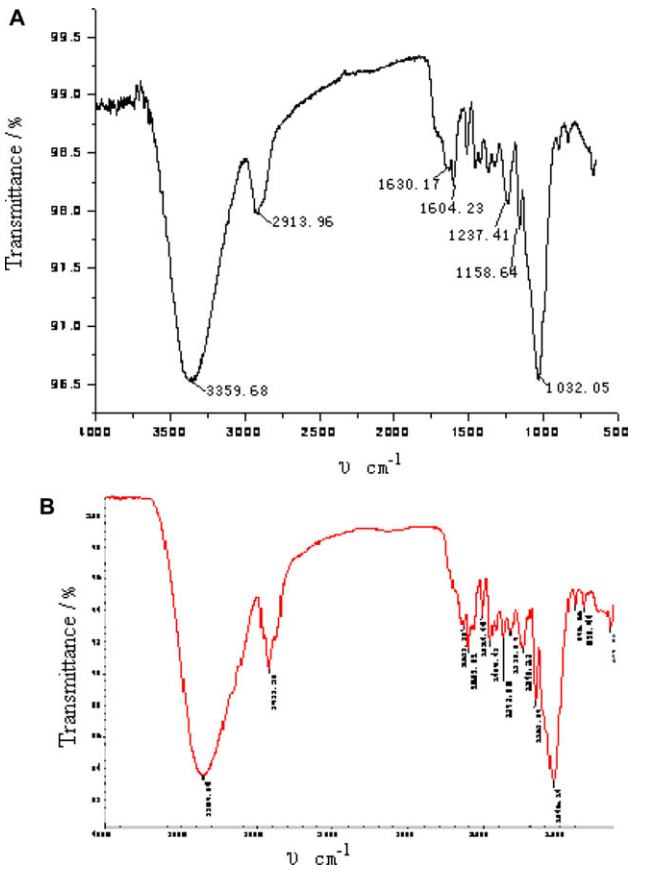

Figure 4. IR spectra of the cellulose-rich materials (A) and the microcrystalline cellulose (B). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

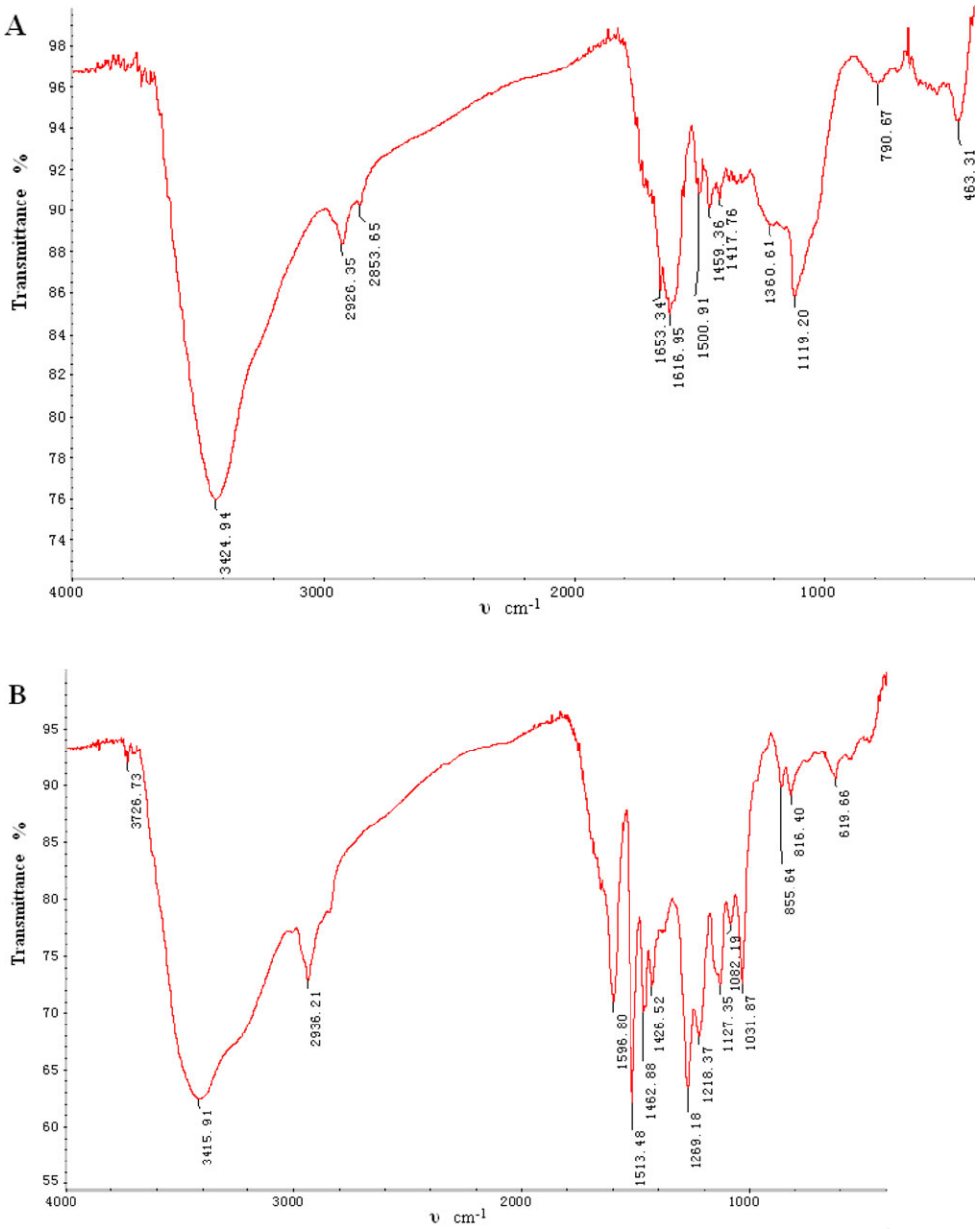

Figure 5. IR spectra of the lignin-rich materials (A) and the lignin standard sample (B). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

3357.8, 1633.2, and 1046.1 cm^{-1}(OH), 2922.2cm^{-1} (-CH and -CH_2), 1373.5 and 1162.5cm^{-1}(-C-O-C), and stretching 1032.0 cm^{-1} (C-O/C-C).[27] This revealed that solid residue I is mainly composed of cellulose, namely it is a cellulose-rich material. Furthermore, it was observed that the IR spectrum of solid residue II [Figure 5(A)] was in agreement with that of the lignin standard [Figure 5(B)]. The characteristic absorption peaks of lignin[28] in the fingerprint region were 1588.6 and 1513.1 cm^{-1} for aromatic skeletal vibrations, 1482.8 and 1426.5 cm^{-1} for C-H deformation, 1269.1 cm^{-1} for syringyl ring plus guaiacyl ring, 1218.3 cm^{-1} for syringyl ring and C = O stretch, and 1127.3 cm^{-1} for aromatic skeletal vibration. This suggested that the solid residue II remained after the second-stage hydrolysis was lignin-rich materials. Further analysis of the solid residue II was conducted using the Congo red reagent. The negative result of staining indicated that no cellulose exists in this solid residue.

The comparison of the SEM images of the cellulose-rich material, microcrystalline cellulose, and the ground corn stalk were shown in Figure 6. The fiber structure of the cellulose-rich materials [Figure 6(A)] showed an obviously difference in morphology from that of microcrystalline cellulose [Figure 6(B)] and the ground corn stalk [Figure 6(C)]. The cellulose-rich materials displayed a much more disordered homogeneous microstructure in which the longitudinal tissues, spongy part, and the fairly long cellulose fibers were fused together.

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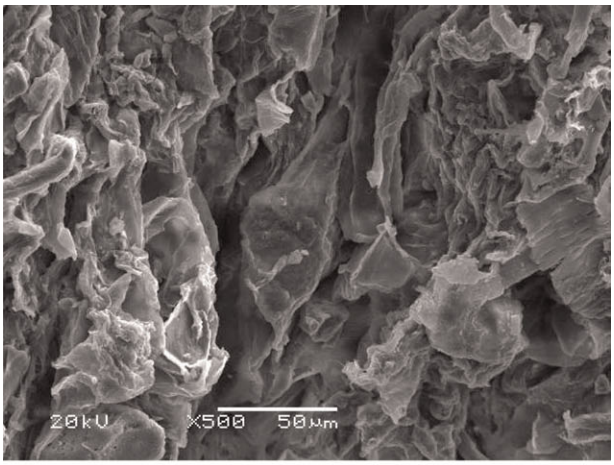
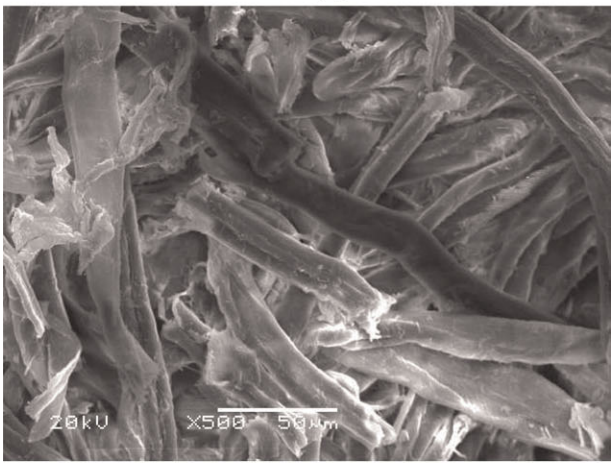
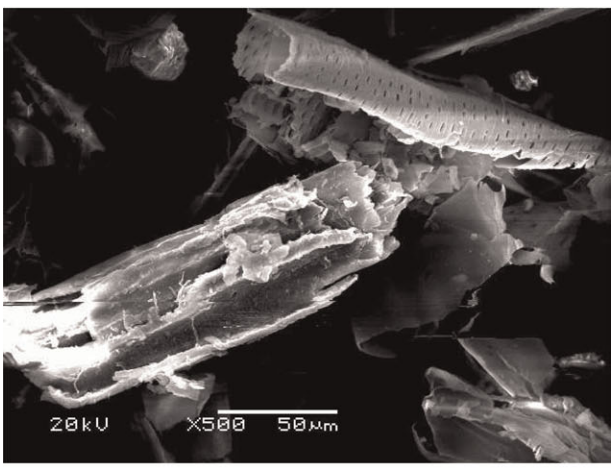


(A)



(B)



(C)

Figure 6. SEM images of cellulose composite materials. (A) the morphology of cellulose-rich materials; (B) the morphology of microcrystalline cellulose; (C) the morphology of the ground corn stalks.

CONCLUSIONS

In this study, hydrochloride acid/[C_4mim]Cl have been used to selectively hydrolyze the lignocellulosic materials and to separately produce the hydrolysates of cellulose and hemicellulose. The results of NMR, IR, and SEM analysis showed that under the conditions of pH 4.5 and 90°C, the lignin–hemicellulose matrix in corn stalk was decomposed and hemicellulose was fully degraded into water-soluble monosaccharides in the IL. In the second-stage hydrolysis at pH 2–3, the cellulose-rich materials were sequentially hydrolyzed in the IL at 90°C. It was found that cellulose in the cellulose-rich materials was completely degraded into glucose. The IL was recovered and reused in the hydrolysis process. This is a simple and efficient method for the selective hydrolysis of lignocelluloses into monosaccharides. Further investigation on the improvement of the yield of hydrolysates and prediction of large-scale industrial application is underway in our laboratory.

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REFERENCES

1. Huber G. W.; Iborra S; Corma A. *Chem. Rev.* **2006**, *106*, 4044.
2. Ren Y; Wang J; Liu Z; Ren Y; Li. G. *Renew. Energy* **2009**, *34*, 2774.
3. Kumar P; Barrett D. M.; Delwiche M. J.; Stroeve P. *Ind. Eng. Chem. Res.* **2009**, *48*, 3713.
4. Lavarack B. P.; Giffin G. J.; Rodman D. *Biom. Bio.* **2002**, *23*, 367.
5. Lu X. B.; Zhang Y. M.; Yang J; Liang Y. *Chem. Eng. Technol.* **2007**, *30*, 938.
6. Sievers C.; Valenzuela-Olarte M. B.; Marzialetti T; Musin I; Agrawal P. K.; Jones C. W. *Ind. Eng. Chem. Res.* **2009**, *48*, 1277.
7. Fort D. A.; Remsing R. C.; Swatloski R. P.; Moyna P; Moyna G; Rogers R. D. *Green Chem.* **2007**, *9*, 63.
8. Sun N; Rahman M; Qin Y; Maxim M. L.; Rodríguez H; Rogers R. D. *Green Chem.* **2009**, *11*, 646.
9. Swatloski R. P; Spear S. K.; Holbrey J. D.; Rogers R. D. *J. Am. Chem. Soc.* **2002**, *124*, 4974.
10. Pu Y; Jiang N; Ragauskas A. J. *J. Wood ChemTechnol.* **2007**, *27*, 23.
11. Phillips D. M.; Drummy L. F.; Conrady D. G.; Fox D. M; Naik R. R.; Stone M. O.; Trulove P. C.; De Long H. C.; Mantz R. A. *J. Am. Chem. Soc.* **2004**, *126*, 14350.
12. Biswas A; Shogren R. L.; Stevenson D. G.; Willett J. L.; Bhowmik P. K. *Carbohydr. Polym.* **2006**, *66*, 546.
13. Xie H.; Zhang S.; Li S. *Green Chem.* **2006**, *8*, 630.
14. Remsing R. C.; Swatloski R. P.; Rogers R. D.; Moyna G. *Chem. Commun.* **2006**, *12*, 1271.
15. Seoud E. l.; Koschella O. A.; Fidale A.; Dorn L. C.; Heinze S.; *T. Biomacromolecules* **2007**, *8*, 2629.
16. Li C.; Zhao Z. K. *Adv. Synth. Catal.* **2007**, *349*, 1847.

17. Li C.; Wang Q.; Zhao Z. K. *Green Chem.* **2008**, *10*, 177.
18. Zhang Y.; Du H.; Qian X. E.; Chen Y. X. *Energy Fuels* **2010**, *24*, 2410.
19. Li B.; Filpponen I. *Ind. Eng. Chem. Res.* **2010**, *49*, 3126.
20. Krull L. H. *J. Agric. Food Chem.* **1980**, *28*, 917.
21. Spectral Database for Organic Compounds SDBS. SDBS No.10471 D-xylopyranose, CAS Registry No.7261–26-9.
22. Voelter W; Breitmaier E. *Org. Magn. Reson.* **1973**, *5*, 311.
23. Ikeda T.; Holtman K.; Kadla J. F.; Chang H. M.; Jameel H. *J. Agric. Food Chem.* **2002**, *50*, 129.
24. Lu F.; *Ralph J. Plant* **2003**, *35*, 535.
25. Spectral Database for Organic Compounds SDBS. SDBS No.2023, D-glucopyranose, CAS Registry No.2280–44-6.
26. Gorin P. A. J.; Mazurek M. *Can J. Chem.* **1975**, *53*, 1212.
27. John R.; Alvin L.; Santiago G.; Carlos O. *J. Adv. Pharm. Technol. Res.* **2011**, *2*, 144.
28. Zhou G. W.; Gail T.; Andrea P. *Plant Methods*, **2011**, *7*, 9.