INVESTIGATION OF THE EFFECT OF SUPERCRITICAL CARBON DIOXIDE PRETREATMENT ON REDUCING SUGAR YIELD OF LIGNOCELLULOSE HYDROLYSIS

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Supercritical carbon dioxide ($scCO_2$) was used to pretreat corncob, cornstalk and rice straw at the temperature of 80-160 °C and pressure of 5-20 MPa, with the raw materials moisture content of 0-75% for the durations of 15-60 min. It was found that the reducing sugar yield of all the three materials showed a maximum with the increase of the moisture content and pretreatment temperature. The optimum experiment result was obtained at 100 °C, 15 MPa with the duration of 30 min and moisture contents of 50%. The reducing sugar yield was 39.6%, 27.4% and 36.6% for corncob, cornstalk and rice straw, respectively, while for untreated materials it was 26.2%, 22.5% and 30.9%. High Performance Liquid Chromatography (HPLC) analysis for hydrolysates of corncob and rice straw indicated that $scCO_2$ pretreatment had a positive effect on the hydrolysis of both cellulose and hemicelluloses. Among the three materials, the reducing sugar yield of corncob was improved the most after $scCO_2$ pretreatment, which could be demonstrated by the X-ray diffraction (XRD) and Scanning Electron Microscope (SEM) analyses.

Keywords: lignocellulose, supercritical carbon dioxide, pretreatment, reducing sugars

INTRODUCTION

Lignocellulose biomass is a renewable resource and has great potential for the production of economical fuel ethanol, because it is inexpensive and available in large quantities. It includes the agricultural residue, forestry residue, yard waste, wood products, animal and human wastes etc., and stores energy from sunlight in its chemical bonds.[1] Lignocellulose biomass mainly consists of cellulose (32-47%), hemicelluloses (19-27%) and lignin (5-24%).[2] The former two components can be hydrolyzed to reducing sugars, which can be fermented into ethanol. However, a quite low sugar yield, less than 20% of the theoretical maximum, is usually obtained from enzymatic hydrolysis of lignocellulosic materials without pretreatment, due to the protective shield of lignin and the hemicelluloses around cellulose.

Besides the most widely used methods, such as steam explosion, diluted-acid pretreatment, alkali pretreatment and so on,[3-8] $scCO_2$ pretreatment has also been of interest in recent years, because it is environmentally benign and has a low demand of post-treatment processes. $ScCO_2$ is characterized by "gas-like" mass transfer and "liquid-like" solvating power,[2] which make it probably easier to permeate into the micropores of lignocellulose. Moreover, CO_2 molecules will rush out in an explosive fashion after the pretreatment, which may cause the lignocellulose structure to rupture, resulting in higher availability of cellulose to the enzymes in the subsequent hydrolysis step. The $scCO_2$ pretreatment has been proved to have a positive effect on improving the reducing sugar yield by several researchers.[9-11] It was found that the pretreatment temperature, moisture contents of raw materials, pretreatment time and pretreatment pressure would influence the reducing sugar yield.[2,9,10,12-19] However, research on $scCO_2$ pretreatment has been limited to a few lignocellulosic materials and the underlying mechanisms are not clear yet. Also, the enzyme hydrolysis is expensive and time-consuming. So we engaged to use diluted-acid hydrolysis as an economical and efficient method to evaluate the pretreatment. In this way, the optimal operation conditions and mechanisms of $scCO_2$

pretreatment were explored. Three kinds of agricultural residues – corncob, cornstalk and rice straw – were pretreated in $scCO_2$ at various temperatures (80-160 °C), pressures (5-20 MPa), durations (15-60 min) and raw material moisture content (0-75%). Diluted-acid hydrolysis was conducted in 1 wt% H_2SO_4 at 160 °C for 40 min for all treated samples. SEM and XRD characteristics were performed to investigate the microstructure change of the raw materials. In addition, HPLC analysis was used to determine the composition of the hydrolysate and finally the role of water in $scCO_2$ pretreatment was also discussed.

EXPERIMENTAL

Materials

Corncob, cornstalk and rice straw were collected from a local farm in Dalian, Liaoning Province. The three biomass materials were air-dried and milled into particles, then sieved using the Tyler screen (0.417 mm). Then the raw materials were stored in jars until further use. To identify the contents of cellulose, hemicelluloses and lignin, the three raw materials were characterized by the method proposed by Van Soest.[9,16] The components content of the raw materials are listed in Table 1. H_2SO_4 (98%) was purchased from Dalian Haiyun Co. Ltd., deionized water was supplied by Dalian University of Technology and CO_2 was purchased from Dalian Guangming gas Inc.

Methods

To conduct the $scCO_2$ pretreatment, firstly 1.5 g biomass and a certain amount of deionized water (0.5-4.5 g) were introduced into the reactor (material 316 L; volume 30 mL). Secondly, the reactor was sealed and preheated by hot water (80-100 °C) for about 5 min, then CO_2 was charged into the reactor to reach an appropriate pressure (5-20 MPa). Thirdly, the reactor was transferred to an oil bath (80-160 °C) and the pretreatment was kept for about 15-60 min. After that, CO_2 was quickly released within 15 seconds. Finally, the pretreated biomass was dried in an oven at 50 °C for 24 h, and stored in ziplock bags for further analysis.

Diluted-acid hydrolysis was conducted to evaluate the effects of $scCO_2$ pretreatment. Firstly, 0.4 g of pretreated biomass and 20 mL of 1 wt% diluted sulfuric acid were loaded in the reactor, and then the reactor was sealed and put into an oil bath at 160 °C for 40 min. The reactor was then put into an ice-water bath to quench the hydrolysis reaction. At last, the hydrolysate accumulated in the reactor was filtered and stored in a glass bottle at 4 °C for further tests.

Determination of reducing sugars

The 3,5-dinitrosalicylic acid (DNS) method was used to determine the reducing sugar yield. Each sample of 0.5 mL filtered hydrolysate was diluted with 2.0 mL deionized water and reacted with 2.0 mL DNS reagents for a while. All reacted samples were instantly placed into a hot water bath (100 °C) for 10 min and then immersed in a cold water bath for 10 min. After that, deionized water was added to dilute each sample to 10 mL. The intensity of the developed color was determined by a UV-Vis spectrophotometer with the wavelength of 540 nm.

A linear regression line was used to model the relationship between two variables of absorbance and sugar mass based on a standard glucose solution. Then, Eq. 1 was used to calculate the reducing sugar yields.

$$\text{Reducing Sugar Yield} = \frac{\text{mass of reducing sugar}}{\text{mass of biomass}} \times 100\% = \frac{Y \times V_2}{M \times V_1} \times 100\% \quad \text{(Eq. 1)}$$

where Y is the mass of reducing sugars in analysis, which is calculated from absorbance according to a standard curve; V_1 is the volume of the hydrolysate used in analysis and V_2 is the volume of liquid added to the reactor in hydrolysis; M is the mass of biomass used in hydrolysis.

The components of the reducing sugars including glucose, xylose etc. were tested on Agilent 1200 HPLC in order to verify the increase of glucose yield after $scCO_2$ pretreatment. The samples were analyzed with a Shodex SC1011 sugar column (300 × 8.0 mm, 6 μm particle size), using water as mobile phase with a flow rate of 0.6 mL/min, analysis temperature of 35 °C, column temperature of 75 °C and analysis time of 50 min.

Table 1
Composition of raw materials

Raw materials	Soluble components,* %	Hemicelluloses, %	Cellulose, %	Lignin, %	Ash, %
Corncob	22.1	37.6	35.4	4.7	0.2
Rice straw	26.1	25.3	39.1	9.2	0.3
Cornstalk	36.3	20.7	34.4	3.3	5.3

*Soluble components consist of protein, lipid, starch and so on

Scanning electron microscopic (SEM) analysis of residues was performed with a KYKY-2800B scanning electron microscope (KYKY CO., LTD., Beijing, China). The micrographs were obtained with an accelerating potential of 15 kV under low vacuum.

Monochromatic Cu Ka radiation (wavelength = 1.54056 Å) was produced by an X-Pert Pro X-ray diffractometer (PANalytical, Netherlands). The powdery samples were packed tightly into a rectangular aluminium cell prior to exposure to the X-ray beam. The scanning regions of the diffraction angle, 2θ, were 10-90° and radiation was detected with a proportional detector.

RESULTS AND DISCUSSION

There are many factors influencing the pretreatment results according to literature.[9,10,15] The orthogonal experiment was designed as the $L_{16}4^5$ table with four factors (the pretreatment temperature, the moisture content of the raw materials, the pretreatment time and the pretreatment pressure) and four levels. Because of the paper length limitation, no details about the orthogonal experimental design are given here. According to the analysis of variance and significance testing about this design, the pretreatment temperature influenced the results most and the moisture content of the raw materials second. The pretreatment time and pressure were found to have secondary influences on the results. Based on the analysis above presented, the pretreatment experiments were conducted at the temperature of 50-160 °C, moisture of 0-75%, duration of 30 min and the pressure of 15 MPa.

Effect of pretreatment temperature

To explore the effect of temperature on the $scCO_2$ pretreatment, three raw materials, including corncob, cornstalk and rice straw, with moisture content of 50% were pretreated at 15 MPa, and various temperatures (50-160 °C) for 30 min and the results are shown in Fig. 1.

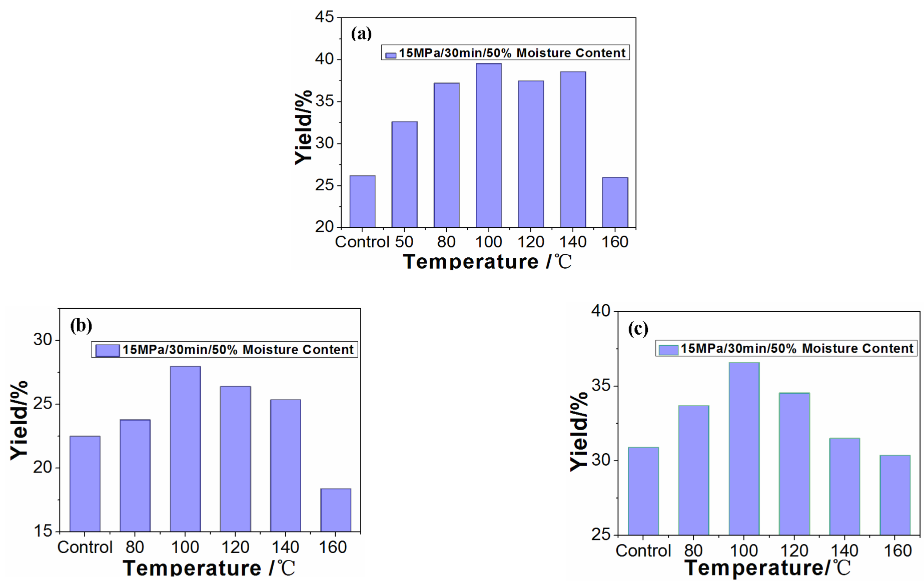
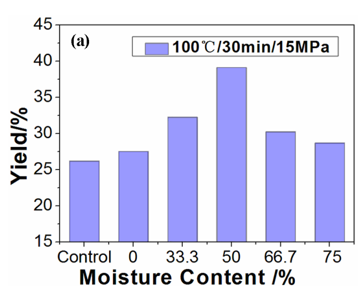


Figure 1: Effect of pretreatment temperature on the hydrolysis of lignocellulose pretreated with $scCO_2$ at 15 MPa and moisture content of 50% for 30 min; (a) corncob; (b) cornstalk and (c) rice straw



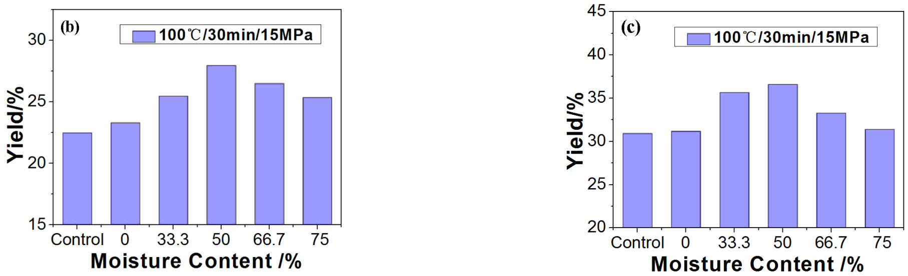


Figure 2: Effect of moisture content of lignocellulose on the hydrolysis of lignocellulose pretreated with $scCO_2$ at 15 MPa and 100 °C for 60 min; (a) corncob; (b) cornstalk and (c) rice straw

As shown in the figures, temperature affected the pretreatment results of the three raw materials in a similar way. The reducing sugar yield showed maxima with the pretreatment temperature. When pretreatment temperature was lower than 100 °C, the reducing sugar yield increased with the increase of temperature; when it was higher than 100 °C, the reducing sugar yield decreased with the temperature. Compared to the control samples, the reducing sugar yields of the samples pretreated at 100 °C increased by 13.35%, 5.47%, and 5.68% for corncob, cornstalk and rice straw, respectively. The reducing sugar yields of the corncob control sample and sample pretreated at 100 °C were also analyzed by HPLC. It was found that the glucose yield of the pretreated sample increased from 6.59% to 8.78%, fructose yield increased from 6.65% to 8.06%, compared to the control sample. The glucose was derived from cellulose hydrolysis and fructose might have been formed as an isomerization product when glucose was decomposed to fragmented compounds (e.g., glycolaldehyde, erythrose, glyceraldehdye etc.) and dehydrated compounds (e.g., levoglucosan, 5-hydroxymethylfurfural).[20] Therefore, the reducing sugar yield hydrolyzed from cellulose increased from 13.24% to 16.84% after the $scCO_2$ pretreatment. In addition, pentose (which comes from the hemicelluloses hydrolysis) yield was found to increase from 12.28% to 16.01%. It can be concluded that $scCO_2$ pretreatment has some positive effect on the hydrolysis of both cellulose and hemicelluloses.

As far as the rice straw is concerned, the glucose yield of rice straw pretreated at 100 °C increased by 1.98% after the diluted-acid hydrolysis, according to the HPLC analysis. Gao *et al.*[2] pretreated rice straw using $scCO_2$ at 110 °C and 30 MPa for 30 min with the raw materials moisture content of 50%, and an increment of 5% of the glucose yield was obtained after enzyme hydrolysis. It can be concluded that diluted-acid hydrolysis was capable to evaluate the pretreatment.

The temperature contributes to the $scCO_2$ pretreatment in many ways. On the one hand, a higher temperature would be beneficial to mass transfer, softening of lignin and degradation of hemicelluloses, which would greatly enhance the final explosion.[21-23] On the other hand, temperature could also affect the properties of $scCO_2$, which is essential to $scCO_2$ pretreatment, including density, diffusion coefficient of CO_2, and solubility of water in $scCO_2$. For the constant pressure of 15 MPa and volume of 30 mL, higher temperature means lower density of $scCO_2$, which will lead to weaker explosion. Also, at a higher temperature, more hemicelluloses would degrade, which would decrease the reducing sugar yields. To sum up, there would be a best temperature for the $scCO_2$ pretreatment process, which in our experiments was 100 °C.

Effect of moisture content of raw materials

To investigate the effect of moisture contents on the $scCO_2$ pretreatment, some experiments were conducted at 100 °C and 15 MPa for 30 min with various moisture contents (0%-75%). As we could see in Fig. 2, moisture content affects the reducing sugar yield of corncob, cornstalk and rice straw pretreated by $scCO_2$ in a similar way. The reducing sugar yields increased as the moisture content increased, and the maximum reducing sugar yield was obtained at a moisture content of 50%, and then the reducing sugar yields decreased as the moisture contents increased. The highest reducing sugar yields after hydrolysis were of 39.15%, 27.97% and 36.60% for corncob, cornstalk and rice straw, respectively, pretreated at 100 °C, 15 MPa, 30 min and moisture content of 50%.

As can be seen in Fig. 2, when the raw

materials were completely dry (moisture content 0%), the reducing sugar yields from the $scCO_2$ pretreated samples did not show significant differences. As we know, there were large amounts of strong hydrogen bonds in lignocellulose, and pure $scCO_2$ as a non-polar solvent would have weak interaction with the hydrogen bonds especially in low temperature (below 200 °C). So $scCO_2$ may have the role of explosion without water. However, the explosion may be helpless to the strong hydrogen bonds breaking between cellulose. So, water acts as a polar molecule, which is responsible for breaking bonds under $scCO_2$ conditions.

There were three possible effects of water on the pretreatment, including swelling effects, hydrolysis of hemicelluloses catalyzed by carbonic acid and the cooking effect of high temperature steam or water.[2,9-11] Liquid water had a swelling effect on lignocellulose biomass, which could enlarge the micropores for CO_2 molecules to penetrate deeper into the biomass. Also, when CO_2 was dissolved in water and carbonic acid was formed, the hemicelluloses might have been hydrolyzed with the help of it. However, this factor might not be dominant given the relatively low water content of the system. In addition, considering that the temperature, pressure and water content in the $scCO_2$ pretreatment are similar to those of steam explosion pretreatment, the water in CO_2 may present a high-temperature cooking effect. So, with the help of water, the reducing sugar yields increased at first. However, as the moisture contents increased, there would be more liquid phase water covering the surface of lignocellulose, which would make the mass transfer worse and block the penetration of CO_2 molecules into the biomass, so the number of CO_2 molecules released in the final explosion was decreased and the explosion was weakened. In summary, there is an optimum water content of the raw materials.

SEM and XRD analyses

The crystalline and the surface morphology of the untreated and $scCO_2$ treated samples (100 °C, 30 min, 15 MPa and 50% moisture content of corncob, cornstalk and rice straw) were analyzed by XRD and SEM, respectively.

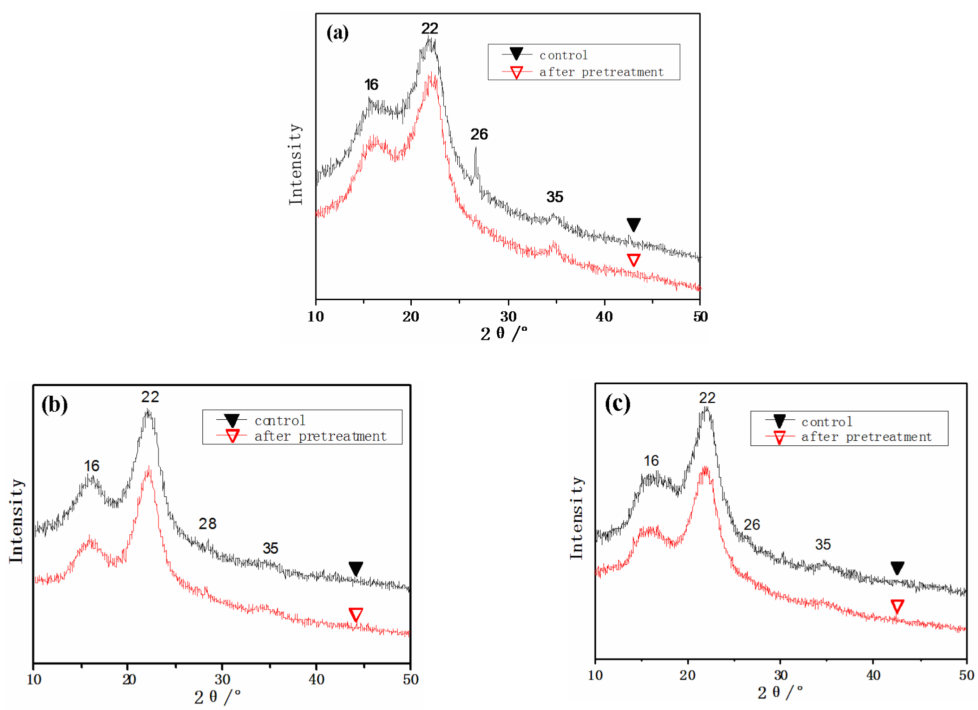


Figure 3: XRD patterns of control samples and samples pretreated at 15 MPa, 100 °C and 50% moisture content for 30 min; (a) corncob; (b) cornstalk; (c) rice straw

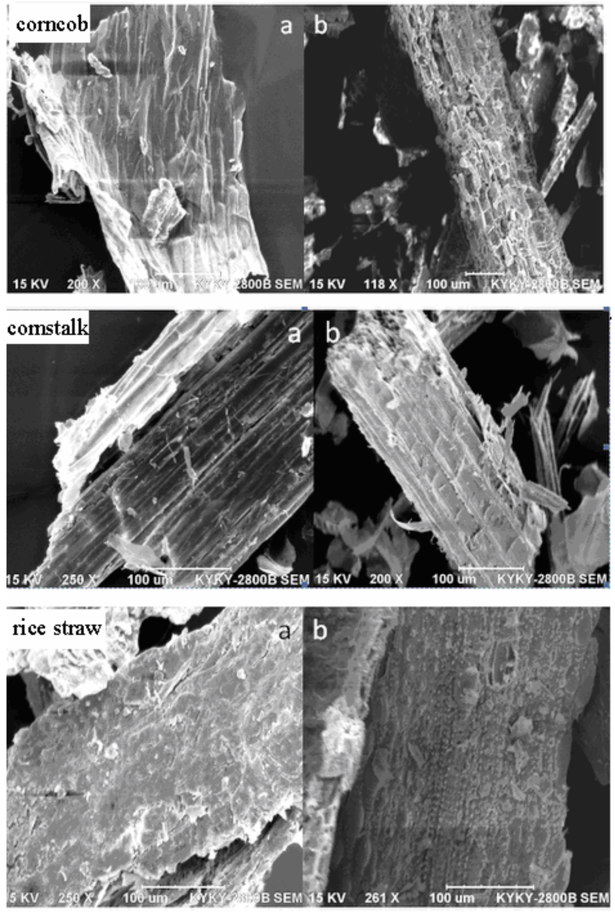


Figure 4: SEM micrographs of (a) untreated samples and (b) samples pretreated at 15 MPa, 100 °C and 50% moisture content for 30 min using $scCO_2$

As shown in Fig. 3a, the peak at 26° disappeared after pretreatment indicating an obvious change in the XRD pattern of corncob. However, there is little difference between the XRD patterns of untreated and pretreated cornstalk and rice straw, as can be seen in Fig. 3b and Fig. 3c. According to the equation put forward by Segal,[24] the crystallinities were of 67.5%, 63.2% and 60.7% for corncob, cornstalk and rice straw, respectively, for the untreated, and of 65% and 71.3% and 66.1%, for the treated samples. The decrease of crystallinity in corncob after pretreatment may explain why the corncob had a high reducing sugar yield. It can be concluded that the $scCO_2$ pretreatment had a slight effect on decreasing the crystallinity of cellulose. Anyway, the change in crystallinity is not the main factor influencing the hydrolysis of biomass, according to the literature.[10]

The SEM micrographs before and after pretreatment are shown in Fig. 4. It is observed that the surface morphology of the three raw materials presents significant changes after pretreatment. The surface of the untreated samples was smooth and compact, and it became loose, lamellar and porous after treatment, presenting signs of explosion. It is obvious that the specific area of biomass increased after $scCO_2$ pretreatment, which was beneficial to hydrolysis. From the SEM images, it can be seen that corncob and rice straw showed worse damages than cornstalk did after pretreatment, which might also cause different increments of reducing sugar yield.

As stated above, from all the three materials the maximum reducing sugar yield was obtained under the same pretreatment conditions (100 °C, 30 min, 15 MPa, moisture content 50%). However, there were some differences as far as the conversion rate of polysaccharides (cellulose and hemicelluloses) is concerned. The conversion rate of polysaccharides is defined as the mass ratio of the hydrolyzed polysaccharides and the total polysaccharides. The conversion rates for the control samples of corncob, corn stalk and rice straw were of 36.4%, 48.0% and 40.8%, respectively, and those for the pretreated samples (100 °C, 30 min, 15 MPa and 50% moisture content) were of 54.9%, 56.83% and 50.76%, respectively. So, the increments were of 18.5%, 9.9% and 8.8%, respectively. It can be concluded that the $scCO_2$ pretreatment had the greatest effect on corncob, of the three raw materials, which could also be demonstrated by XRD and SEM analyses.

CONCLUSION

In summary, the $scCO_2$ pretreatment had a positive effect on improving the reducing sugar yield of corncob, cornstalk and rice straw for hydrolysis. Diluted-acid hydrolysis was feasible to evaluate the pretreatment, although the reducing sugar yields gained using this method were not as high as those obtained by enzyme hydrolysis. The temperature and the moisture content of the raw materials were found to be the two most important pretreatment factors. The highest reducing sugar yield for the three materials was obtained for all treated samples at 100 °C, 30 min, 15 MPa with the moisture content of 50% and, under these conditions, the reducing sugar yields were of 39.6%, 27.4% and 36.6%, respectively, for corncob, cornstalk and rice straw. Compared to the control samples, the reducing sugar yields were increased by 13.35%, 5.47%, and 5.68% for corncob, cornstalk and rice straw. HPLC analysis indicated that the hydrolysis of both cellulose and hemicelluloses were enhanced after the pretreatment. In addition, the specific area of raw material surface, especially for corncob, was greatly increased after pretreatment, according to the SEM image. However, the role of water in the pretreatment and some underlying reasons of the experimental results are still not clear yet, further research is underway.

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