CELLULOSE CHEMISTRY AND TECHNOLOGY

ROLE OF PRETREATMENT WITH A COMPOSITE MICROEMULSION IN SACCHARIFICATION OF BAMBOO

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The highly dense biostructure and complex coating of biomass, which can decrease saccharifying efficiency and increase production cost, has been the bottleneck of biomass utilization. In this study, a self-designed composite microemulsion was used for lignocellulose (bamboo) pretreatment. The improvement of physicochemical properties and saccharifying efficiency after pretreatment was studied. The results showed that a stable composite microemulsion was obtained with an optimal mass ratio of 1:2:1:3:3, corresponding to cetyltrimethyl ammonium bromide/n-butyl alcohol/BminCl/ammonia. Lignocelluloses yielded a reducing sugar content of 57.8% via pretreatment with the composite microemulsion under the following material conditions: particle size between 0.25 mm to 0.38 mm, temperature of 70 °C, and reacting time of 16 h. The relative crystallinity of lignocellulose increased marginally and thermal stability improved obviously, while cellulose was protected well after pretreatment.

Keywords: composite microemulsion, ionic liquids, pretreatment, enzymatic hydrolysis

INTRODUCTION

Biomass energy is the most abundant renewable resource on earth. Thus, several studies have focused on transforming agricultural and forestry wastes into biomass energy and chemicals.[1] The main components of lignocelluloses are cellulose (28% to 50%), hemicelluloses (25% to 38%), and lignin (12% to 25%). The amount of polysaccharide hydrogen bond and the protective effect of lignin on cellulose are the bottleneck of efficient lignocellulose utilization. Therefore, pretreatment is the key step during the process of lignocelluloses transformation.[2] Recent studies have utilized several methods for lignocelluloses pretreatment, such as physical, chemical, physical-chemical, biological, and some synthesis methods. However, all these methods have disadvantages, including high cost, heavy pollution, hard chemical recovery, and insignificant results.[3-4] Ionic liquids (ILs), a kind of green solvent produced after supercritical fluid and aqueous two-phase system, have shown unique advantages in biomass pretreatment. ILs are associated with a devisable structure, stable physicochemical properties, low vapor pressure, they are colorless and odorless, recyclable, and can be used as direct solvents of cellulose. ILs can break the hydrogen bond inside and between cellulose in the pretreatment process to create a loose and multihole structure of biomass, which is helpful for the subsequent permeability and reaction of chemicals.[5-6] Recently, the use of ionic liquids has provided significant scope as a cellulosic biomass pretreatment process. ILs have proven to be highly effective in removing lignin and hemicelluloses and reducing cellulose crystallinity, which is helpful in the biorefinery process.[7-8] ILs can also be applied to dissolve a series of lignocellulosic biomasses, such as hardwood, softwood and herbaceous materials.[9]

In this study, we have prepared a composite microemulsion with higher permeability, which can be used as carrier. This system can strengthen the delignification process during pretreatment and enhance the effect of enzymatic hydrolysis after the addition of hydrogen bond breaker – ammonia water and ILs. The physicochemical properties of lignocelluloses before and after pretreatment were also studied. Chemicals can penetrate easily into capillaries by controlling the mass ratio of the component to ensure that the emulsion belongs to the microemulsion area, and by using the ultra-strong permeability on wood materials of microemulsion.[10] Several studies have indicated that using ILs and ammonia water for lignocelluloses pretreatment can significantly increase the effect of enzymatic hydrolysis.[11-13] The degradation products of lignocelluloses, which are separated from the pretreatment solution, can be reused after extraction by dichloromethane. Moreover, the different boiling points of the components in the microemulsion can be useful for their recycling.

EXPERIMENTAL

Materials

A bamboo, containing 45.40% cellulose, 27.68% hemicelluloses, 22.87% lignin, and 7.2% ash, served as raw material in this study. Unpurified 1-butyl-3-methylimidazolium chloride could be used directly. Aqueous ammonia, n-butyl alcohol, cyclohexane, and cetyl trimethyl ammonium bromide were all analytically pure. Distilled water was prepared in our laboratory. Cellulase type C-1184 was used; treatment method: freeze drying; enzyme activity: 118 U/mg. Composite microemulsion (solid content: 10%) was also synthesized in our laboratory.[15]

Instruments

X-ray diffractometer (XRD, D8 ADVANCE, Bruker Company, Germany), Cu target, 40 KV, 40 mA, step width: 0.02 degree, scanning speed: 17.7 s/step; TG (TG company, USA; type: Q500TGA, 20 °C to 500 °C, 10 °C/min, rate of nitrogen flow: 30 mL/min); Fourier transform-infrared spectroscope (FT-IR, VECTOR33, Bruker Company, Germany).

Ammonia and composite microemulsion pretreatment of lignocelluloses

The bamboo powder and solution were mixed in a solid-to-liquid ratio of 1:25. After sealing, the mixed solution was maintained in a bath with constant heating temperature operated at a rate of 150 rpm to allow reaction. The same volume of ethanol was added after the reaction. The mixture was stirred vigorously for 10 min, then filtrated and washed for three times. The solids were then collected, dried and subjected to enzymatic hydrolysis. The efficiency of enzymolysis is appreciated by the yield of reducing sugar.

Enzymatic hydrolysis and measurement of reducing sugar

Craft of enzymatic hydrolysis: the dosage of cellulase (C-1184, Sigma) was 50 FPU/g (relative to absolutely dry raw materials), 50 °C, pH=4.8, liquid-solid ratio of 30:1, rotating speed of 150 rpm for 48 h.

After enzymatic hydrolysis, the sample was diluted ten times and the content of reducing sugar was measured with the dinitrosalicylic acid method. The experiment was repeated three times and the average value was calculated.

Yield of reducing sugar (%) = M_2 (quantity of reducing sugar after enzymolysis) / M_1 (quantity of sample before pretreatment) × 100%

RESULTS AND DISCUSSION

The phase action of composite microemulsion with ammonia

The phase action of the composite microemulsion, which can reflect the proportion of each component in the microemulsion system, is shown in Fig. 1. The emulsion phase involves the gathering of the surfactant (CTAB) and cosurfactant (n-butyl alcohol), with a mass ratio of 1:1. The hydrophobic phase involves cyclohexane. The hydrophilic phase involves ILs and ammonia, with a mass ratio of 1:1. The phase diagram is composed of two areas. The turbid area, also known as the lactescence, is muddy and milky, whereas the microemulsion area is transparent, stable, and isotropous, and contains an ultra-low interfacial tension and a nano-particle size. Aside from the proportion of constituents, the temperature, pH, salinity, and adulterants in the system also serve as influencing factors on the formation of microemulsion.[16]

This study proved that the microemulsion had a stronger permeability on lignocelluloses, compared with a general water solution and lactescence.[15,17] As shown in Fig. 1, the mass ratio of the component in the subsequent pretreatment was CTAB:n-butyl alcohol:cyclohexane:BmimCl:ammonia = 1:2:1:3:3 (as the point P in Fig. 1). The hydrophilic phase is the continuous phase that increases the effective components, which can react with lignocelluloses.

Influencing factors on enzymatic hydrolysis

We investigated the influencing factors on the yield of lignocelluloses and reducing sugar, such as particle size, temperature and time. The comparative results are shown in Figs. 2, 3 and 4.

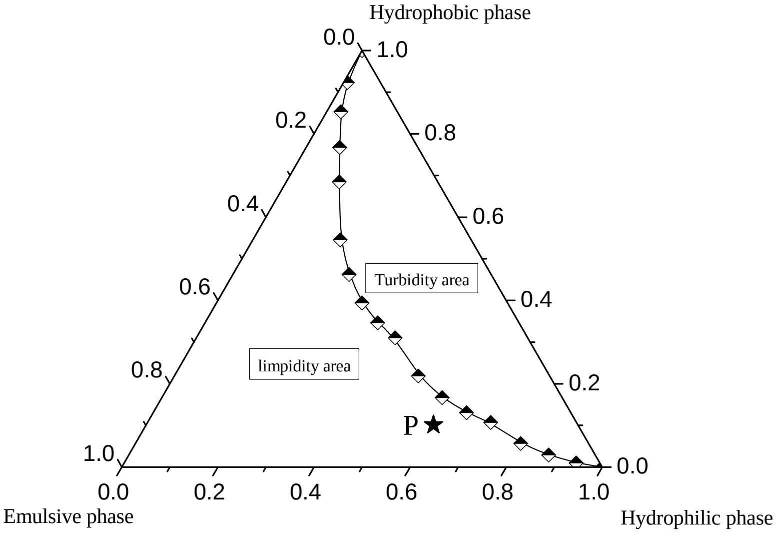

Figure 1: Three-phase diagram of the emulsion system (70 °C)

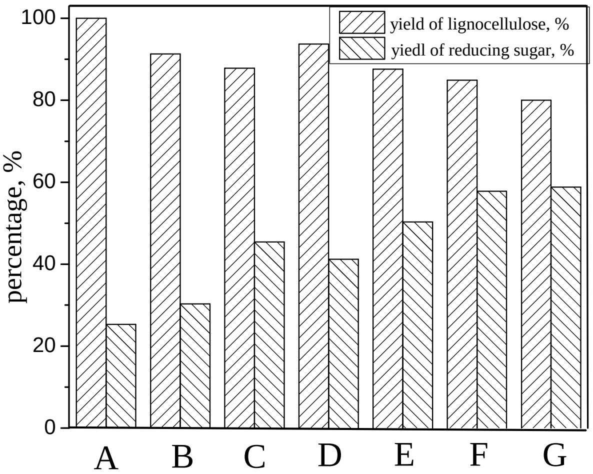

Figure 2: Effects of grain size and solution type on the yield of lignocellulose and reducing sugar at 70 °C for 16 h (the material particle size of A, B, C and D are all 0.25 mm to 0.38 mm; A: without pretreatment; B: hot water pretreatment; C: aqueous ammonia 20%; D: without aqueous ammonia; E: particle size >0.38 mm; F: particle size from 0.25 mm to 0.38 mm; G: particle size <0.25 mm)

Ammonia, a kind of hydrogen bond breaker, can strengthen the delignification process farther and break the bond between carbohydrate and lignin during the pretreatment process. Hemicelluloses can also be partly removed, while cellulose is well protected.[13] Hence, the change of lignocellulose yield can signify the effectiveness of the pretreatment. The reducing sugar yield of the comparative sample without any microemulsion pretreatment was of only 25.3%, whereas the yields of samples B-D were much higher. Meanwhile, the lignocellulose yield of the sample with 20% aqueous ammonia (C) was lower than that of the sample without aqueous ammonia; however, the yield of reducing sugar, which can reach up to 57.8%, was higher than the latter.

The microemulsion, with a high penetrating power, can take ammonia and ILs into the micron and nanometer hole of lignocellulose.[12] Ammonia opens the channels of lignocellulose and impedes the various actions of lignin and hemicelluloses on cellulose, increasing the contact area between the enzyme solution and cellulose and enhancing the yield of reducing sugar. The particle size of the raw material is also a factor for pretreatment. The smaller is the size, the more sufficient is the contact and the better is the pretreatment efficiency. However, the yield of reducing sugar cannot increase obviously when the particle size is reduced under 0.25 mm.

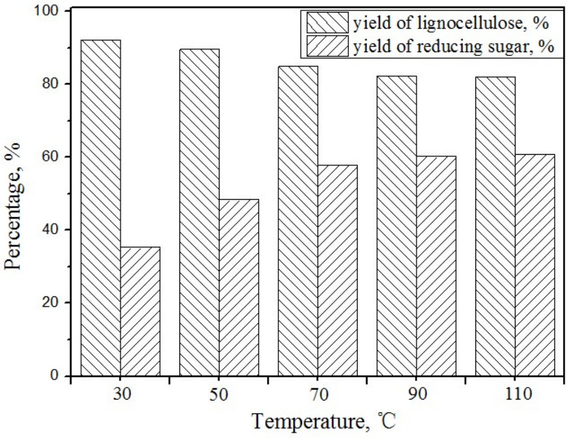

Figure 3: Effects of temperature on the yield of lignocellulose and reducing sugar (0.25 mm to 0.38 mm, 16 h)

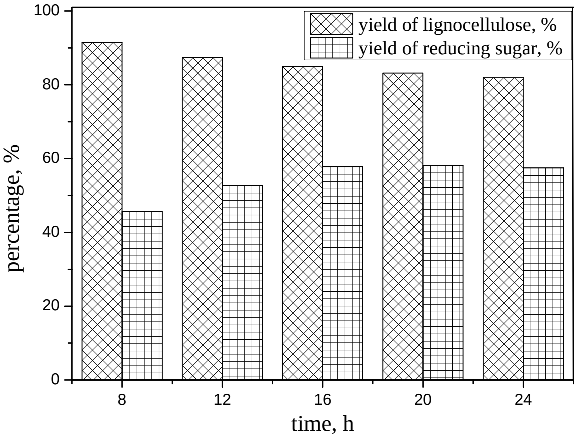

Figure 4: Effects of time on the yield of lignocellulose and reducing sugar (0.25 mm to 0.38 mm, 70 °C)

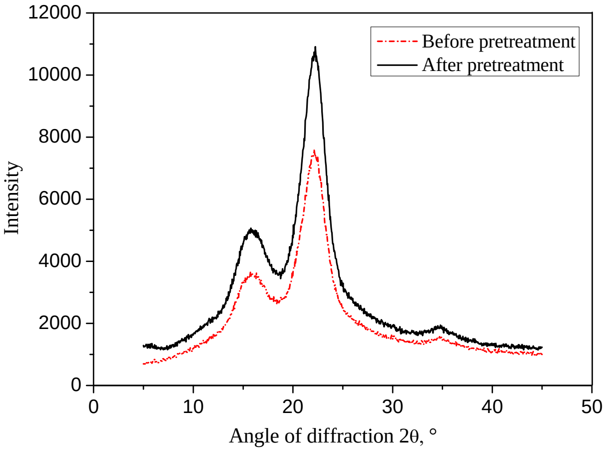

Figure 5: XRD pattern of lignocellulose before and after pretreatment (0.25 mm to 0.38 mm, 70 °C, 16 h)

The applied temperature is one of the most important influencing factors for pretreatment. The degradation of lignin and hemicelluloses is more sufficient with the increase in temperature. Thus, the yield of lignocellulose showed a decreasing trend, whereas the yield of reducing sugar showed an increasing trend. A higher temperature could not enhance however the effect of pretreatment further, as shown in Fig. 3. High temperature conditions can break the stability of the microemulsion, especially at or exceeding the boiling point of n-butyl alcohol, the destructiveness would be more intense.[15] Moreover, the degradation of partly cellulose can also decrease the effect of pretreatment.

The reaction time has the same influence as that of reaction temperature on the effect of pretreatment, as shown in Fig. 4. Aqueous ammonia and ILs showed insufficient reaction with lignocellulose in the prophase of reaction. Thus, the degree of delignification and yield of reducing sugar were restricted. The yield of lignocellulose can reach up to 84.91% and the yield of reducing sugar can reach up to 57.8% at 70 °C for 16 h. The decreasing trend of lignocellulose yield slows down, whereas that of the yield of reducing sugar becomes slightly slower. This result is probably attributed to the partial degradation of cellulose during a long time reaction.

Analysis of physicochemical properties after pretreatment

Fig. 5 shows the XRD diagram of lignocelluloses before and after pretreatment. Lignocellulose showed an obvious diffraction peak (strong absorption) when the diffraction angle was 22.5° after microemulsion pretreatment. A part of the crystal has changed its structure in the process of crystallization redirection.[18] The relative crystallinity (CrI) was calculated using the Segal method, CrI = $100*[(I_{002}-I_{am})/I_{002}]$, where I_{002} is the crystalline region strength, which is reflected by the absorption peak at 22.5°, and I_{am} is the non-crystalline region strength, which is reflected by the absorption peak at 16.2°. The CrI value before and after pretreatment were 50.1% and 50.8%, respectively. A part of the lignin and hemicelluloses were degraded and removed during pretreatment; thus the relative crystallinity increased slightly.[20]

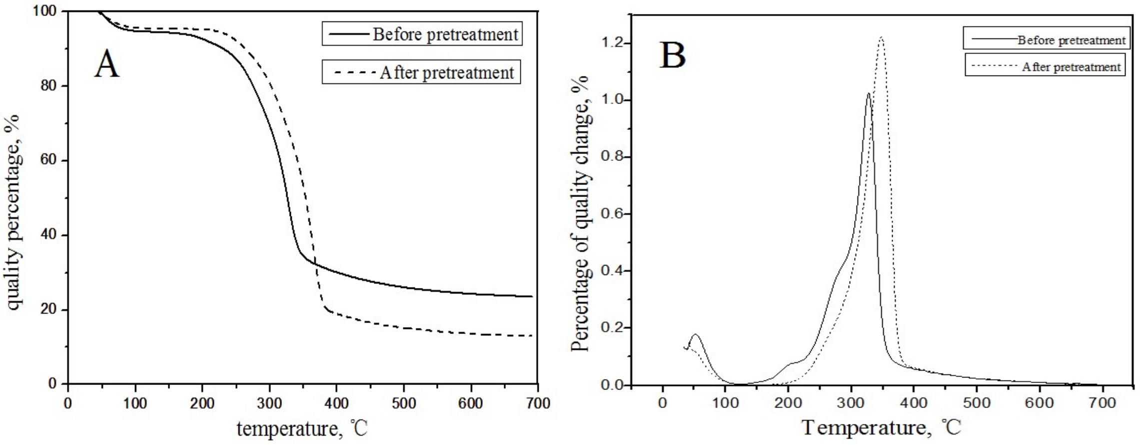

Figure 6: TG and TGA analysis of lignocellulose before and after pretreatment. A: TG; B: TGA (0.25 mm to 0.38 mm, 70 °C, 16 h)

The pyrolysis process of lignocelluloses is basically identical before and after pretreatment, as shown in Fig. 6. An obvious peak of thermal degradation appeared below 100 °C, which represents the water separation stage, including loss of free water, physical absorption water, and crystal water. The region between 150 °C and 450 °C is the major pyrolysis area of lignocellulose. In this area, the hemicelluloses, which consist of different sugar units, have a lower pyrolysis temperature range, from 190 °C and 335 °C. Cellulose is the straight-chain polymer, which is the polymerization product of glucose, which is connected by a 1,4-glycosidic bond. Cellulose has both a crystalline area and a non-crystalline area. Its compact structure provides a higher pyrolysis area from 260 °C to 395 °C. Although lignin is an amorphous aromatic polymer, which has a net structure, its pyrolysis area is extensive, which continues from 200 °C to 700 °C.[21] Two obvious peaks of thermal degradation (221 °C and 286 °C) were observed between 200 °C to 300 °C before the pretreatment. However, these two peaks weakened significantly after the pretreatment. Moreover, the maximum peak of thermal degradation of cellulose showed a rearward movement, which appeared between 327.1 °C and 339.7 °C, which is because a large part of hemicelluloses and lignin were degraded and removed after the pretreatment.[22-23]

FT-IR analysis revealed that several great changes of lignocelluloses' peaks appeared at 1732 cm^{-1} and 1247 cm^{-1} before and after the pretreatment, respectively. These peaks coincide with the results of our prophase research.[15] The peak at 1732^{-1} is the characteristic peak of hemicelluloses xylan,[20] it not only represents the stretching vibration band of C=O on carboxyl and ester group, but also stands for the absorption peak of ether linkage. The disappearance of this peak after pretreatment showed that a large part of hemicelluloses was removed. The peak at 1247 cm^{-1} is the common absorption peak of cellulose and hemicelluloses. Its strength is evidently weakened, whereas the absorption of characteristic peaks (1595 cm^{-1} and 1505 cm^{-1}) of lignin showed no obvious change. This result proves that the structure of lignin is destroyed, but not as obviously as that of hemicelluloses. Meanwhile, the strength of the peaks at 2900 cm^{-1} and 1425 cm^{-1} (characteristic of cellulose) did not change obviously, which indicated that cellulose was well protected by the microemulsion.

CONCLUSION

The experimental results showed that particle size, temperature and reaction time have important effects on pretreatment. The yields of lignocelluloses and reducing sugar were of 84.91% and 57.8%, respectively, under the optimum process conditions of 0.25 mm to 0.38 mm for 70 °C and 16 h, whereas the yield of reducing sugar was only 25.3% without any pretreatment. The relative crystallinity of lignocellulose increased from 50.1% to 50.8% after the pretreatment. The maximum peak of thermal degradation showed a rearward movement from 327.1 °C to 339.7 °C. The results indicated that the heat stability of lignocelluloses was enhanced, and cellulose was protected well during the pretreatment process.

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