Wet Oxidation Pretreatment of Poplar Waste for Enhancing Enzymatic Hydrolysis Efficiency

GuiGan Fang[1,2,3], ShanShan Liu[4], KuiZhong Shen[1,2], Yan Lin[1]

1. *Institute of Chemical Industry of Forestry Products, CAF; National Engineering Lab for Biomass Chemical Utilization; Key Lab of Forest Chemical Engineering, SFA; Key Lab of Biomass Energy and Material of Jiangsu, Nanjing, Jiangsu Province*, 210042, *China*

2. *Institute of New Technology of Forestry, CAF, Beijing*, 100091, *China*

3. *Collaboration Innovation Center for High Efficient Processing and Utilization of Forestry Resources, Nanjing Forestry University, Nanjing, Jiangsu Province*, 210037, *China*

4. *Qilu University of Technology, Ji'nan, Shandong Province*, 250353, *China*

Abstract: In this paper, we described the optimization of the wet oxidation pretreatment conditions to enhance enzymatic hydrolysis efficiency, using poplar waste from the stock section of a paper mill as the raw material. We showed that the optimal conditions of the pretreatment for poplar waste were an initial pH value of 10, a temperature of 195℃, a holding time of 15 min, and an oxygen pressure of 1.2 MPa. In this case, the yield of the obtained solid material produced by the process was 51.7% and the reducing sugar yield was 46.8%. The solid part obtained from the pretreatment process was hydrolyzed by cellulase L-10. The optimal enzymatic conditions were a temperature of 49℃, a duration time of 56 h, an enzyme dosage of 38 FPU/g at a pH value of 4.8, and a solid-to-liquor ratio of 1∶50. The resulting cellulose conversion rate reached 96.4% in terms of the pretreated substances. In addition, a chemical composition analysis of the poplar waste and pretreated material indicated that about 92% of the hemicelluloses and 43% of the lignin in the raw material were degraded and dissolved. In addition, the crystallization decreased from 57.5% to 54.8%. An obvious fibrillation of the fiber pretreated by the wet oxidization process was observed by SEM. Moreover, high-performance liquid chromatography (HPLC) results showed a high xylose content and monosaccharide degradation products in the pretreatment solution. In conclusion, the wet oxidation pretreatment process could efficiently degrade or remove the lignin and hemicellulose, as well as reduce the crystallinity of the lignocellulosic material, which resulted in an

GuiGan Fang, PhD, professor; research interests: clean production of pulping and papermaking, pulp wastewater treatment and biomass utilization;
E-mail: fangguigan@icifp.cn

Received: 19 January; accepted: 6 February.

improvement of the enzymatic ability and an increase in the cellulose conversion rate.

Keywords: poplar waste; wet oxidation pretreatment; enzymatic hydrolysis efficiency; cellulase hydrolysis

1 Introduction

The production of bio-liquefied fuel oil or bio-ethanol from lignocellulosic biomass is currently the most common form of biomass utilization around the world. The pretreatment of the lignocellulosic biomass is necessary to break down the dense crystalline texture of the cellulose and destroy the encapsulation of the cellulose by lignin and hemicelluloses. The relative separation of the cellulose, hemicellulose, and lignin, can improve the catalytic efficiency of the subsequent liquefaction and the accessibility of the enzymatic agent to the cellulose, which is conducive to the conversion of the carbohydrates to fermented sugar. There have, however, been difficulties with the development of economical and efficient lignocellulosic biomass pretreatment technology.

Generally, commonly used biomass pretreatment techniques can be classified into physical, chemical, biological, physical-chemical combination, and other combination methods. The wet oxidation pretreatment technology discussed in this paper can be classified as a physical-chemical pretreatment technology. In this process, fiber materials are treated by the application of heat, high pressure, an alkaline environment, water, and oxygen. As a result, the cellulose expands, and the hemicellulose and lignin degrade, which is helpful in improving the hydrolysis efficiency and increasing the biomass liquefaction adaptability[1-2]. Weakly alkaline chemicals like sodium carbonate are usually used to adjust the pH of the wet oxidation pretreatment separation solution and make it alkaline. In this environment, degradation of the cellulose can be prevented and the formation of by-products such as furfural can be inhibited. Research has shown that, with a pretreatment temperature of 195℃, a pretreatment time of 15 min, a sodium carbonate content of 2 g/L, and an oxygen pressure of 1.2 MPa for 60 g/L wheat straw pretreatment, the enzymatic hydrolysis rate of the cellulose could be greatly increased[3]. Compared with other pretreatment methods, less furfural, hydroxymethyl furfural, and acetic acid were produced during the wet oxidation pretreatment process, which was beneficial to the subsequent biochemical treatment[4]. However, the need for high temperatures and pressures incurs a higher level of investment and cost for the wet oxidation pretreatment process. This study focused on the optimization of the pretreatment conditions for the wet oxidation of poplar waste. The research results can be used as a reference for the development of bio-ethanol or biomass liquefaction oil production technology.

2 Materials and methods

2.1 Reagents

Poplar waste was acquired from Shandong Chenming Paper's P-RC APMP pulping production line, including wood chips, wood knots, and wood needles, without bark. This acquired waste was dried in air and sealed. The chemical composition of the raw materials was as follows: the nitric acid-ethanol cellulose content was 44.8%, the pentosan was 18.7%, the Klason lignin was 23.6%, the ash content was 2.63%, and the benzene-alcohol extractive was 1.10%. The cellulase L-10 was purchased from Qingdao Kangdien Group. The used H_2SO_4 solution was prepared with a mass fraction of 36.5%. Dinitrosalicylic acid, sodium potassium tartrate tetrahydrate, anhydrous sodium sulfite, and other agents were all of analytical grade and used without further purification.

2.2 Wet oxidation pretreatment of poplar waste

The wet oxidation pretreatment was carried out in

a 6-tank air bath digester (Model 6x3L, AG Brown Machinery, Australia). Then, 60 g of dry raw material was added to each tank and mixed with 1 L of distilled water. The initial pH value was adjusted with solid Na_2CO_3 or 36.5% (w/w) H_2SO_4 solution. After mixing, they were transferred into cans and oxygen was input through the top. After sealing, the cans were heated in the digester. After the completion of the reaction, the steel cans were taken out and rapidly cooled to room temperature under running water. The cans were then opened, and the contents were filtered to separate the solid from the liquid. The obtained liquid was reserved for measurement. The solid material was washed and put into a polyethylene bag, which was sealed and then equilibrated. Then, the moisture content was measured.

2.3 Cellulase hydrolysis of solid material after wet oxidation pretreatment

The solid part obtained by the wet oxidation pretreatment was hydrolyzed with cellulase. We took 2 g (dry mass) of pretreated material and put it into a 250-mL conical flask, followed by a cellulase solution. The cellulase content was 35 FPU/g, the solid-to-liquid ratio was 1∶50, the pH value was adjusted to 4.8 with 1 mol/L sodium acetate-sodium acetate buffer solution, and then enzymolysis was carried out in a constant-temperature culture oscillator (ZH WY-200B, Shanghai-City Analysis Instrument Co., Ltd.) for 72 h. After the completion of the reaction, the enzymolysis solution was filtered and the residue was collected for subsequent testing. A reduction in the sugar content was detected and the reducing sugar yield (*RSY*) was calculated using Eq.(1):

$$RSY = \frac{m}{m_0} \times 100\% = \frac{ncV}{m_0} \times 100\% \quad (1)$$

where

RSY — reducing sugar yield, %;

m_0 — dry mass of raw material, g

m — reducing sugar mass, g

V — total volume of hydrolysate, L

n — dilution multiple of hydrolysate

c — reducing sugar concentration, g/L

2.4 Optimization of enzymatic hydrolysis by response surface design

The process conditions of the enzymatic hydrolysis were optimized by the response surface method. The factors and levels were as follows: the enzyme hydrolysis temperature was 45℃, 50℃, and 55℃, the enzyme hydrolysis time was 24 h, 48 h, and 72 h, and the enzyme dosage was 25 FPU/g, 35 FPU/g, and 45 FPU/g, respectively. The experimental results were analyzed using Design Expert 7.1. In the test, the reducing sugar content was determined by the DNS method[5], involving a DNS configuration, glucose standard solution preparation, and color test.

The liquid was diluted a certain number of times. Then 0.5 mL of the diluted solution and 0.5 mL of distilled water (as blank control group) were put into two 25-mL tubes with standard plug, respectively. To each tube, we added 0.5 mL of distilled water and 3.0 mL of DNS reagent. Then, the tubes were heated in a boiling water bath until a color reaction was observed. After cooling, distilled water was added to bring the volume of the solution to 25 mL. The absorbance was measured at a wavelength of 550 nm using an ultraviolet spectrophotometer. At the same time with the 0.5 mL distilled water as blank. The reducing sugar concentration of the target liquid was calculated using Eq.(2):

$$c = \frac{A+0.0757}{0.9744} \times n \quad (2)$$

where

c — reducing sugar concentration, g/L

A — absorbance of liquid

n — dilution multiple of liquid

0.0757 and 0.9744 — intercept and slope of glucose standard curve, respectively

The cellulose conversion rate was used as the evaluation index and calculated using Eq.(3).

$$Y_c = \frac{m_{rs}/Y_T}{m_c} \times 100\% \quad (3)$$

where

m_{rs} — reducing sugar mass in pretreatment solution, g

Y_T — theoretical yield of reducing sugar, %. For

glucose obtained by cellulose hydrolysis, Y_T = 111.1%; for xylose obtained by hemicelluose hydrolysis, Y_T = 113.6%. Y_T was evaluated as the ratio of the cellulose and hemicelluloses in the raw material and hydrolysate.

m_c — mass of cellulose and hemicelluloses in raw material, g

Y_c — cellulose conversion yield, %

2.5 Chemical composition analysis

The cellulose, hemicellulose, Klason lignin, ash, and benzene-alcohol extractive content in the raw material and pretreated materials were analyzed according to the corresponding national standards.

2.6 Determination of cellulase activity

The cellulase activity was tested using the method developed by Ghose[6]. In this study, the cellulase activity was found to be 149.73 FPU/mL.

2.7 Determination of sugars and degradation products

The liquid was diluted to a specific multiple and then subjected to high-performance liquid chromatography (HPLC). This was done using an Agilent 1100 chromatographic column and Aminex HPX-87H column, combined with a refractive index detector. The column temperature was 63℃, the flow rate was 0.6 mL/min, the injection volume was 10 μL, and 4 mmol/L H_2SO_4 was used as the mobile phase. The concentrations of the monosaccharides and monosaccharide degradation products in the liquid were calculated from the corresponding peak areas in the HPLC chromatogram.

2.8 X-ray diffraction (XRD) analysis

The solid part obtained as a result of the wet oxidation pretreatment was subjected to step dehydration in gradually increasing concentrations of ethanol (30%, 50%, 70%, 80%, and 90%), and then 100% ethanol. This procedure was done twice, with a dehydration time of 15 min for each step. The solid was then vacuum-dried at a low temperature and then ground into powder. The crystallinity of the obtained sample was measured by a peak-dividing method using an X-ray diffractometer (D8-FOCUS, Brock, Germany). The moving speed of the goniometer was 2°/min under the conditions of Cu Kα radiation (λ=0.154 nm), a voltage of 10 kV, and a fixed time of 3 s. The crystallinity was calculated by dividing the diffraction intensity produced by the 002 crystal plane on the XRD pattern by the scattering intensity of the non-crystalline region background, as shown in Eq.(4). On the XRD curve, the maximum peak at 2θ of 22° was the diffraction intensity of the 002 crystal plane. A peak and valley appeared at 2θ of 18°, corresponding to the scattering intensity of the amorphous background.

$$I_{cr} = \frac{I_{002} - I_{am}}{I_{002}} \times 100\% \quad (4)$$

where

I_{cr} — crystallinity, %

I_{002} — maximum diffraction intensity on 002 crystal plane

I_{am} — scattering intensity of amorphous background

2.9 Scanning electron microscope (SEM) analysis

The surface morphology of the materials was observed by a scanning electron microscope (S-3400, Toshiba, Japan) at a voltage of 15.2 kV.

3 Results and discussion

3.1 Optimization of pretreatment conditions for wet oxidation

3.1.1 Initial pH value

The initial pH value of the wet oxidation pretreatment was optimized within a range of 3 to 10. The other experimental conditions were as follows: the temperature was 195℃, the holding time was 15 min, and the oxygen pressure was 1.2 MPa. The experimental results are shown in Fig.1. This showed that the yield under the neutral pretreatment condition was much higher than that under acidic and basic conditions, while the highest yield was 52.0% at an initial pH value of 7. This was because the wet oxidation pretreatment reaction of the feedstock was more intense under acidic or alkaline conditions, leading to greater dissolution

of the material. Meanwhile, very little dissolution material was generated under near-neutral conditions. On the other hand, the yield of enzymatic-reduced sugars from the solid part after pretreatment was higher under acidic or alkaline conditions. When the initial pH value was 3 or 10, the yield of the reducing sugar was 42.0% or 43.6%, respectively. The yield of the reducing sugar decreased to only 38.4% when the initial pH value was 7. One possible reason for this was that the fiber structure was more effectively broken down in an acidic or alkaline environment, thus enhancing the accessibility of the cellulose, and improving the enzymatic hydrolysis efficiency and the yield of the reducing sugar. The neutral pretreatment condition had very little effect on the accessibility of the cellulose.

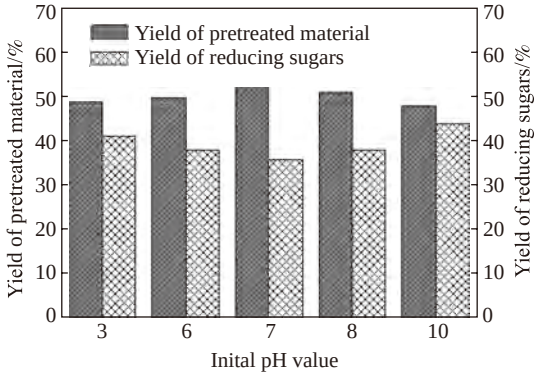

Fig.1 Effects of initial pH value on wet oxidation pretreatment

3.1.2 Holding time

The holding time is an important factor influencing the wet oxidation pretreatment, and has different effects on the pretreatment yield and reducing sugar yield. Therefore, there is an optimum holding time for the process. The holding time was optimized under the conditions of a fixed initial pH value of 10, a temperature of 195℃, an oxygen pressure of 1.2 MPa, and a holding time ranging from 5 to 30 min. The experimental results are shown in Fig.2.

Fig.2 showed that the yield of the pretreated material decreased gradually as the holding time increased, especially within a range of 5 min to 15 min. The yield of the material was 52.1% for a holding time of 15 min, while the yield was reduced to 49.8% at 30 min. In addition, the yield of the reducing sugars increased rapidly between 5 min and 15 min, then it increased slowly as the holding time was prolonged. The yield of the reducing sugars at 5 min was 33.6%, and it increased to 39.0% when the holding time was extended to 15 min. Provided the holding time was kept at 30 min, the yield of the reducing sugar was 40.3%, which was only 1.3 percentage points higher than that at 15 min. Considering the yields of the pretreated material and reducing sugars, a suitable holding time for the wet oxidation pretreatment was 15 min. At this time, nearly half of the material was dissolved out, and the dense structure of the fiber was destroyed, resulting in a high enzymatic hydrolysis rate.

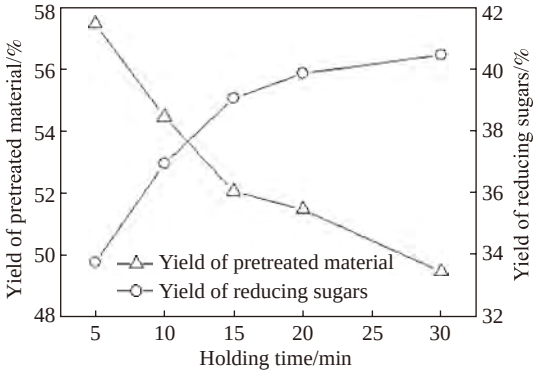

Fig.2 Effects of holding time on wet oxidation pretreatment

3.1.3 Oxygen pressure

Oxygen is found to have a great effect on delignification under alkaline conditions. While under the conditions of alkalinity and high temperature, hemicellulose degrades, as does cellulose. When the initial pH value was 10, the temperature was 195℃ and the holding time was 15 min, the wet oxidation pretreatment of the poplar waste was studied with an oxygen pressure ranging from 0.4 MPa to 1.2 MPa in this paper. The experimental results are shown in Fig.3.

As can be seen in Fig. 3, it was found that the yield of the pretreated material decreased almost linearly as the oxygen pressure increased within a range of 0.4 MPa to 1.2 MPa. When the oxygen pressure was 0.4 MPa, the yield of the pretreated material was 57.0%, which fell to 55.1% at an oxygen pressure of 0.8 MPa; the yield further decreased to 49.4% when the pressure

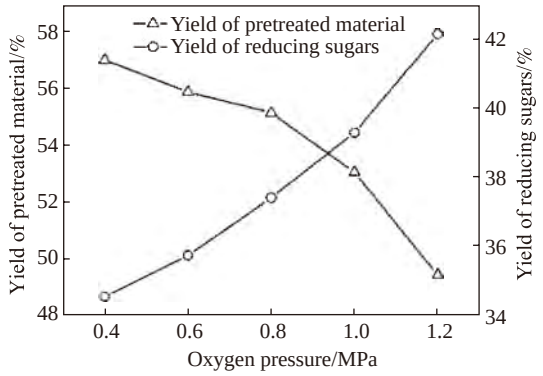

Fig.3 Effects of oxygen pressure on wet oxidation pretreatment

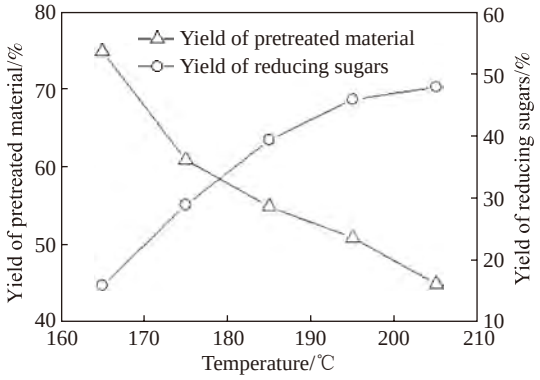

Fig.4 Effects of temperature on wet oxidation pretreatment

was 1.2 MPa. As the oxygen pressure increased, the effect of the wet oxidation pretreatment gradually increased, as did the dissolution of the raw materials. In terms of the enzymatic reduction of sugars, the yield of the reducing sugars increased almost linearly with the oxygen pressure. When the oxygen pressure was 0.4 MPa, the yield of the reducing sugars was 34.5%. The yield of the reducing sugars was 37.4% when the oxygen pressure increased to 0.8 MPa, and the yield further increased to 42.1% (an increase of 7.6 percentage points) when the pressure was increased to 1.2 MPa. These results show that, more lignin dissolved and hemicellulose degraded with the increase of the oxygen pressure. As a result, the fiber structure of the raw material becomes loose. This also increases the availability of the cellulase to the hydrolysis substrate, and thus improves the efficiency of the enzymatic hydrolysis, thus enhancing the yield of the reducing sugars.

3.1.4 Temperature

The effects of different pretreatment temperatures in the wet oxidation process were studied. The initial pH value was set to 10, the holding time was 15 min, the oxygen pressure was 1.2 MPa, and the temperature was varied from 165℃ to 205℃. The results are shown in Fig.4. As can be seen from Fig.4, as the pretreatment temperature rose, the yield of the pretreated material decreased rapidly. The pretreated material yield was 74.9% at 165℃. As the temperature increased from 185℃ to 205℃, the pretreated material yield fell from 54.9% to 42.7%. The pretreatment temperature had an important effect on the yield of the wet oxidation pretreatment process. The amount of the dissolved material increased with the pretreatment temperature. In addition, the yield of reducing sugars increased with the temperature. The reducing sugar yield was 15.9% at 165℃, 39.1% at 185℃, and 46.8% at 195℃. This clearly indicates how higher temperatures enabled the dissolution of the lignin and hemicellulose components. Therefore, the enzymolysis efficiency of the cellulose increased greatly. Thus, the optimum wet oxidation pretreatment temperature was found to be 195℃.

According to the results of above single-factor experiments, the optimum conditions for wet oxidation pretreatment were determined to be as follows: an initial pH value of 10, a holding time of 15 min, an oxygen pressure of 1.2 MPa, and a temperature of 195℃. Under these conditions, the pretreated material yield was 51.7% while the yield of reducing sugars was 46.8%.

3.2 Analysis of wet oxidation pretreated materials

The poplar raw materials were pretreated by the wet oxidation process according to the optimized conditions. The chemical composition of the pretreated materials was analyzed in addition to that of the raw material. Furthermore, the crystallinity of the materials before and after the pretreatment was compared by XRD analysis.

3.2.1 Component analysis

The chemical compositions of the poplar waste and the pretreated material are listed in Table 1. We can

Table 1 Chemical composition of poplar wastes before and after wet oxidation pretreatment

Index	Raw material /%	Material after pretreatment/%	
		Percentage of material after pretreatment	Percentage of raw material
Yield	—	51.7	—
Cellulose(nitric acid-ethanol)	44.3	63.3	32.7
Pentosan	18.7	2.77	1.43
Klason lignin	23.6	26.1	13.5
Ash	2.63	0.15	0.08
Benzene-alcohol	1.10	0.76	0.39

see that the proportions of the chemical constituents of the material changed significantly after the wet oxidation pretreatment. The most significant change was the change of the cellulose content. As a result of the pretreatment, the cellulose content increased from 44.3% to 63.3% with the proportion increasing by 19 percentage points, which was equivalent to 32.7% of the raw materials. This percentage change indicated that the cellulose in the raw material degraded to some extent. In addition, the pentosan content also decreased from 18.7% to 2.77% (equivalent to 1.43% of the raw material). About 92% of the hemicellulose degraded and dissolved, which was consistent with the findings of reference[7]. Furthermore, given the lignin content listed in Table 1, we can see that the lignin content in the pretreated material was 13.5% as the base of the raw material, implying that nearly 43% of the lignin dissolved, with a considerable part of the lignin remaining in the pretreated material. Moreover, after the wet oxidation pretreatment, the ash content and benzene-alcohol extractive content also decreased. Thus, during the wet oxidation pretreatment, a large amount of the hemicellulose and some of the lignin dissolved. These changes greatly reduced the physical space barrier effects between the fiber material and the enzymes, and were thus conducive to the cellulose hydrolysis reaction.

3.2.2 XRD analysis

To study the changes in the crystallization area of the fiber, the raw materials and materials after wet oxidation pretreatment were analyzed by XRD. The results are shown in Fig.5. For this figure, 2θ is set as the abscissa and the XRD intensity is set as the ordinate. The XRD pattern of the raw material exhibited the typical structure of cellulose. By applying Eq.(4), the crystallinity of the raw materials was found to be 57.5%, while the crystallinity of the pretreated material was 54.8%. The 2.7 percentage points difference in the crystallinity indicated structural changes in the material pretreated by wet oxidation. Given the above results of the enzymolysis, these changes in the crystallinity were beneficial to the reaction of cellulose and lignin while enhancing the efficiency of the enzymatic reaction.

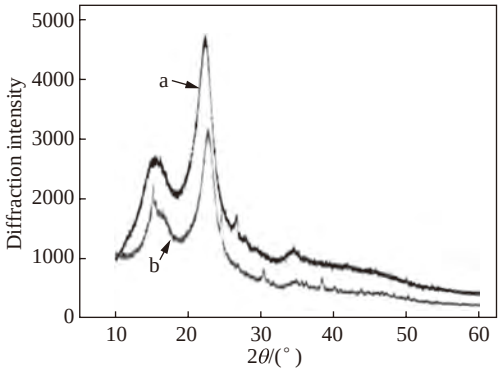

Fig.5 XRD patterns of poplar waste (a) and material after wet oxidation pretreatment (b)

3.3 Characteristics of wet oxidation pretreatment solution

The sugars and degradation products in the separated pretreatment solution were analyzed by HPLC. The results are listed in Table 2.

Table 2 shows that the xylose content of the sugar fraction was highest in the separated wet oxidation

Table 2 Sugars and degradation products in pretreatment solution as detected by HPLC

Sugar	Concentration /g·L^{-1}	Degradation products	Concentration /g·L^{-1}
Cellobiose	0	Formic acid	1.67
Glucose	0.72	Acetic acid	2.14
Xylose	3.08	Levulinic acid	0
Arabinose	0	Hydroxymethyl furfural	0.34
Xylitol	1.26	Furfural	1.25

pretreatment solution, in which the concentration was 3.08 g/L. The second was xylitol with a concentration of 1.26 g/L. Both were obtained from the pentose component and their total concentration was 6.05 times greater than that of the glucose content. In addition, the HPLC revealed that cellobiose and arabinose were barely detectable in the separated pretreatment solution. On the other hand, some degradation products were detected in the pretreatment solution. Among these, acetic acid and furfural were mainly derived from the degradation of xylose, from the pentose component in the feed. Formic acid and hydroxymethyl furfural mainly resulted from the degradation of glucose, which was basically a cellulose component of the raw material. These results showed that the degradation of hemicellulose was much more intense than that of cellulose in the wet oxidation pretreatment process, which was consistent with the chemical composition changes listed in Table 1.

3.4 Enzymatic hydrolysis of materials after wet oxidation pretreatment

3.4.1 Optimization of enzymatic hydrolysis process

After the wet oxidation process, the pretreated material was hydrolyzed by cellulase L-10. In this case, the enzymatic hydrolysis process was optimized by the response surface design method. The results are listed in Table 3. A regression analysis was conducted with the three factors and the cellulase conversion rate Y_c using the Design Expert software. The regression equation was: Y_c=–258.68569+12.41336X_1+0.51555X_2+1.32002X_3–0.00313X_1X_2+0.00800X_1X_3+0.00104X_2X_3–0.12551X_1^2–0.00275X_2^2–0.02113X_3^2 (where, X_1=hydrolysis temperature, ℃; X_2: enzymolysis time, h; X_3: enzyme dosage, FPU/g). To obtain the optimal hydrolysis conditions, the first-order partial derivative of the regression equation was obtained and was set to zero. The result of the partial derivative equation was such that: X_1=49.00, X_2=56.00, X_3=38.33. This corresponded to the highest point of the corresponding surface. Therefore, the optimum conditions were obtained as follows: the hydrolysis temperature was 49℃, the enzymolysis time was 56 h, and the enzyme dosage was 38 FPU/g. The cellulose conversion rate, as calculated with the regression equation, was 96.7%. To confirm this, an experiment was conducted under the obtained hydrolysis conditions and the corresponding cellulose conversion rate was 96.4%. This was in good agreement with the predicted results. Therefore, the optimized enzymatic hydrolysis conditions are reliable. Lv et al studied the enzymatic hydrolysis of cellulase on bleached softwood pulp and reported that the soluble sugar conversion rate (with a similar enzymatic conversion rate) was 81.5%[8]. The results of this study also indicated that the wet oxidation pretreatment process was favorable to the subsequent enzymatic hydrolysis of the residue.

Table 3 Design and results of enzymatic hydrolysis experimental by the response surface method

Temperature /℃	Time /h	Enzyme dosage /(FPU · g^{-1})	Cellulose conversion rate/%
45	24	25	84.4
55	24	25	83.3
45	72	25	90.1
55	72	25	87.9
45	24	45	87.0
55	24	45	87.9
45	72	45	94.1
55	72	45	93.1
41.6	48	35	85.3
58.4	48	35	87.0
50	7.6	35	84.4
50	88.4	35	96.7
50	48	18.2	82.1
50	48	51.8	96.0
50	48	35	95.2

3.4.2 Characteristics of enzymatic hydrolysate of material after wet oxidation pretreatment

The hydrolysate solution obtained by optimized enzymatic hydrolysis was analyzed by HPLC. The components and contents of the monosaccharide and monosaccharide degradation products were determined. The results are listed in Table 4.

Table 4 shows that there were some glucose and xylose components in the hydrolysate solution, while

Table 4 Composition of cellulose hydrolysate detected by HPLC

Sugar	Concentration /g • L^{-1}	Degradation products	Concentration /g • L^{-1}
Cellobiose	0	Formic acid	0
Glucose	13.44	Acetic acid	5.18
Xylose	0.34	Levulinic acid	0
Arabinose	0	Hydroxymethyl furfural	0
Xylitol	0	Furfural	0

no cellobiose, arabinose, nor xylitol were detected in the solution. The concentration of the glucose was 13.44 g/L, accounting for 97.54% of the total sugars; the xylose content was low, having a concentration of 0.34 g/L. This indicated that the cellulase L-10 had high selectivity for cellulose hydrolysis. In addition, acetic acid was detected only at a concentration of 5.18 g/L. This concentration inferred that the detected acetic acid was that added to adjust the pH value of the hydrolysate solution, rather than being a degradation product of cellulose. In addition, neither furfural nor hydroxymethyl furfural was detected in the hydrolysate solution.

3.4.3 Morphological observation

The surface morphology of the residue before and after the enzymatic hydrolysis reaction was observed by a scanning electron microscope (SEM), as shown in Fig.6. Figure 6a shows that the degree of fine fibrosis of the fiber was clearly increased by the wet oxidation pretreatment process of the raw fiber material, and there were a lot of depressions and cracks on the surface of the single fiber or fiber bundle. These changes increased the specific surface ratio of the material and facilitated the contact of the cellulase and material, thereby enhancing the efficiency of the enzymatic hydrolysis. Upon comparing the SEM images of the wet oxidation pretreated materials (a) with those of the enzymolysis residue (b), it was found that the fiber structure changed after the cellulase hydrolysis. Many holes and cracks appeared in the surface and interior of the hydrolysis residue fiber, indicating that more carbohydrates degraded and dissolved during the hydrolysis.

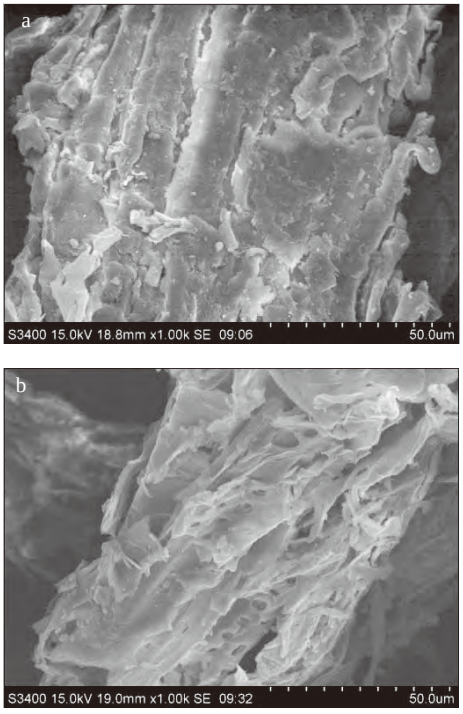

Fig.6 SEM images of wet oxidation pretreated material(a) and enzymatic hydrolysis residue (b)

4 Conclusions

The wet oxidation pretreatment process of poplar waste was addressed by this study. After the pretreatment, both hemicellulose and lignin dissolved and degraded to a considerable degree, thus improving the enzymolysis of the pretreated material and producing a higher yield of reducing sugar. The optimum conditions for the wet oxidation pretreatment of poplar waste were as follows: an initial pH value of 10, a holding time of 15 min, an oxygen pressure of 1.2 MPa, and a temperature of 195℃. Under these conditions, the material yield was 51.7% and the reducing sugar yield was 46.8%. Under the optimum conditions of the wet oxidation pretreatment process, the hemicellulose content of the substance was reduced from 18.7% to 1.43%, the Klason lignin was reduced from 23.6% to 13.5%, and the cellulose content in the pretreated material reached 63.3%. This meant that about 43% of the lignin and 92% of hemicellulose were degraded and dissolved. The crystallinity of the poplar waste, as a result of being pretreated by the wet oxidation process, decreased from 57.5% to 54.8%. Fibrillation of the fiber

pretreated by the wet oxidization process was obvious in the SEM observations. In addition, the HPLC results showed high xylose and monosaccharide degradation product contents in the pretreatment solution, such as acetic acid, formic acid, and furfural.

With raw material that had been treated by the wet oxidation process, the enzymatic hydrolysis process was optimized by application of the response surface method. The optimum conditions were as follows: a temperature of 49℃, a duration time of 56 h, an enzyme dosage 38 FPU/g, a pH value of 4.8, and a solid-to-liquor ratio of 1∶50. Under these conditions, the enzymatic hydrolysis rate reached 96.4%. Therefore, the wet oxidation pretreatment method offered obvious advantages in terms of improving the enzymolysis efficiency of materials and therefore showed promise for industrial applications. However, higher temperature and pressure are required for the wet oxidation pretreatment process, therefore incurring higher investment and usage costs. To overcome this disadvantage of the method, we will go on to explore possible solutions, such as the use of idle pulp mill heating equipment, to improve the engineering aspects and reduce the necessary investment.

Acknowledgment

The authors are grateful for the financial support of the Twelfth Five-Year National Science and Technology Support Program (2014BAD02B02).

References

[1] Homsen A. Pretreatment of Corn Stover Using Wet Oxidation to Enhance Enzymatic Digestibility[J]. Applied Biochemistry and Biotechnology, 2003, 104: 37-50.

[2] Mart C, Klinke H B, Belinda A. Wet oxidation as a pretreatment method for enhancing the enzymatic convertibility of sugarcane bagasse[J]. Enzyme and Microbial Technology, 2007, 40: 426-432.

[3] Schmidt A S, Thomsen A B. Optimization of wet oxidation pretreatment of wheat straw[J]. Bioresource Technology, 1998, 64(2): 139-151.

[4] Chang J, Cheng W, Yin Q, et al. Effect of steam explosion and microbial fermentation on cellulose and lignin degradation of corn stover[J]. Bioresource Technology, 2012, 104: 587-592.

[5] Miller G L. Use of dinitrophenyl acid reagent for the determination of reducing sugar[J]. Analytical Chemistry, 1959, 31: 420-428.

[6] Ghose T. Measurement of cellulase activities[J]. Pure and Applied Chemistry, 1987, 59(2): 257-268.

[7] Zhu W, Zhu J, Gleisner R, et al. On energy consumption for size-reduction and yields from subsequent enzymatic saccharification of pretreated lodgepole pine[J]. Bioresource Technology, 2010, 101(8): 2782-2792.

[8] Lv J, Zhan H Y, Fu S Y. Optimization of enzymatic hydrolysis of bleached pulp by response surface methodology[J]. Transactions of China Pulp and Paper, 2010, 25(2): 16-21. PBM