

## EFFECTS OF ENZYME PRETREATMENT ON THE BEATABILITY OF FAST-GROWING POPLAR APMP PULP

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Effects of enzyme pretreatment on the properties of fast-growing poplar APMP pulp were evaluated. Compared with the untreated pulp, the beatabilities of the pulp that had been pretreated by enzymes were improved significantly, such as a decrease of Canadian Standard Freeness (CSF) in the range of 25 mL to 55 mL, a decrease of PFI mill revolutions from 1000r to 5500r, and a decrease of beating energy consumption from 12.5% to 22.0%. The values of brightness, breaking length, tearing index, bursting index, and folding number of the pulp pretreated by cellulase were improved by 1.2%ISO, 23.7%, 14.8%, 14.6%, and 50% respectively, while that of the pulp pretreated by xylanase were respectively improved by 2.1%ISO, 16.8%, 8.8%, 8.9%, and 25%. The optimal enzyme dosages were 25 IU·g<sup>-1</sup> and 25IU·g<sup>-1</sup> for cellulase and xylanase, respectively. Fibre quality analysis results showed that the fibre length of pretreated pulp increased partly, fibre width and fines content decreased, fibres torsion increased, and fibre bonding got stronger. X-ray diffractometer analysis indicated that the degree of crystallinity of fibres increased after the enzyme pretreatment.

*Keywords:* Fast-growing poplar; Enzyme; APMP pulp; Beatability

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### INTRODUCTION

Fast-growing poplar is a new species of hardwood, which has been cultivated by researchers in the poplar laboratory of Beijing Forestry University through much intensive labor. The quantity and quality of chromosome of this kind of wood were improved to a large degree because researchers took out the original worse chromosomes by way of chromosome substitution. Compared with other hardwood species, the fast-growing poplar possesses many advantages such as faster growth, better adaptability, easier breeding, good quality, disease resistant ability, high economic efficiency, and broad application (Pang and Chen 2004; Kong and Chen 2003; Chen et al. 2000). Past investigations have shown that it was very suitable to serve as raw material for pulp and paper making, because its mean fibre length is longer, fibre length distribution is more uniform, and the ratio of length/width is higher (Kong and Chen 2003; Yao and Pu 1998). In recent years, the fast-growing poplar species has been widely planted in northern China, and it has become an important raw material for pulping within the pulp and paper industry of China.

Fast-growing poplar gives high yields when processed to make alkaline peroxide mechanical pulp (APMP) or preconditioning alkaline peroxide mechanical pulp (P-RC APMP). In recent years, APMP and P-RC APMP pulps have been widely used due to their high yield and low pollution (Zhan et al. 2009). However, the energy consumption in the pulping process is expensive. The pulp and paper making industry is considered an energy-intensive process industry, and energy consumption accounts for about one quarter of the manufacturing cost. With strict requirements on the properties of paper, energy consumption during the refining and beating processes are about 15% to 18% of the total electrical energy cost for producing paper from wood. The consumption of electrical energy has increased rapidly with the pace of development. As a result, energy conservation has become necessary in the paper making industry. Reducing energy consumption is playing an important role in pulp and papermaking industry, and is also one of the most efficient methods to decrease production costs and thereby improve market competitiveness.

Utilization of biotechnology in pulp and papermaking industry has grown dramatically since the mid-1980s (Wang et al. 2008; Vicuña et al. 1997; Zhao et al. 2001; Sun et al. 1983; Eigner et al. 1985; Xie 2003). The utilization of enzymes for drainage enhancement, deinking of secondary fibres, bleaching enhancement, and modifications of fiber characteristics has been intensively pursued (Li et al. 2008; Zhang et al. 2005; Pommier et al. 1990; Dwivedi et al. 2010; Oltus et al. 1987). The application of cellulase and hemicellulase in softwood kraft pulp (Bhat et al. 1991), bleached wheat straw pulp (Chen et al. 1997), old corrugated container (Pommier et al. 1990), old newspaper (Wu et al. 2000), and poplar SGW (Guan et al. 1999) can improve the drainability and forming properties, and can also enhance the paper qualities. Xylanase can help facilitate the removal of lignin on the fiber with the concomitant degradation of hemicelluloses. Then the fiber can become looser and softer, and the fibrillation extent of the treated fibre is better than that of untreated fibres. Part of the fine fibre can be degraded so that the treated pulp can more easily be beaten or refined, and the same measures can promote fibre drainage and improved pulp physical strength (Guan et al. 2000; Yang et al. 2009). Enzyme treatment can enhance swelling and absorption capability of fibres, improve the refining properties of pulp, and reduce refining energy (Darcfa et al. 2002; Shen et al. 2002; Zhang and Li 2005; Zhang and Xu 2009; Chen et al. 2010; Zhang et al. 2009; Jiang et al. 2007).

The effects of enzyme-assisted beating or refining on the properties of fast-growing poplar APMP pulp were studied in the present work. The morphology and properties of the fibre with or without pretreatment by enzyme were also studied.

## **EXPERIMENTAL**

### **Materials**

Fast-growing poplar wood chips were from Zhong Mao Sheng Yuan pulp company in Shandong province. The chips size were length 15 mm to 25 mm, thickness 3 mm to 5 mm and width 10 mm to 20 mm. The chemical composition (% dry weight, w/w) was as follows: cold water extractives 2.31%, 1% NaOH extractives 17.72%,

benzene-alcohol extractives 3.69%, holocellulose 80.41%, Klason lignin 17.57%, acid-insoluble lignin 1.89 %, and pentosan 24.95 %.

The cellulase was purchased from Sukehan Co. The characteristics of cellulase were as follows: solid, enzyme activity  $14400 \text{ IU}\cdot\text{g}^{-1}$ , optimal pH value 5.0 to 6.0, and optimal temperature  $50^{\circ}\text{C}$  to  $55^{\circ}\text{C}$ . The xylanase 51024 was purchased from Novozymes Biologicals Inc. The characteristics of xylanase 51024 were as follows: liquid, enzyme activity  $17,000 \text{ IU}\cdot\text{mL}^{-1}$  (Note: The activity unit of xylanase was  $\text{IU}\cdot\text{mL}^{-1}$  since it was liquid. But the xylanase dosage used in the paper was  $\text{IU}\cdot\text{g}^{-1}$ , which meant that the activity units of xylanase per grams pulp (dry) were used for pulp treatment.), optimal pH value 6.5 to 7.0, and optimal temperature  $45^{\circ}\text{C}$  to  $55^{\circ}\text{C}$ .

The APMP was produced in the pulping and papermaking laboratory of Shandong Polytechnic University, according to the following process: wood chips → screening → washing with  $60^{\circ}\text{C}$  to  $70^{\circ}\text{C}$  water → steeping with  $95^{\circ}\text{C}$  water for 20 min → the first stage extrusion with JS10 extruder (China) → the first stage chemical pretreatment (NaOH 3.3%(w/w),  $\text{H}_2\text{O}_2$  3.0% (w/w),  $\text{Na}_2\text{SiO}_3$  1.0% (w/w),  $\text{MgSO}_4$  0.2 % (w/w), EDTA 0.2% (w/w), liquid ration 1:4,  $75^{\circ}\text{C}$ , 50min) → the second stage extrusion with JS10 extruder → the second stage chemical pretreatment (NaOH 3.0% (w/w),  $\text{H}_2\text{O}_2$  3.0%(w/w),  $\text{Na}_2\text{SiO}_3$  2.0% (w/w),  $\text{MgSO}_4$  0.3% (w/w), EDTA 0.3% (w/w), liquid ration 1:4,  $70^{\circ}\text{C}$ , 60min) → refining with KRK refiner (Japan) (three-stage, consistency 20%(w/v), with a refining gap 0.5 mm, 0.30 mm, and 0.15 mm ) → latency removal with  $70^{\circ}\text{C}$  to  $80^{\circ}\text{C}$  water for 30 min → ending.

The brightness of the original pulp was 75.1 %ISO, and the Canadian standard Freeness (CSF) was 575 mL.

### Enzyme Pretreatment

Enzyme pretreatment conditions were selected based on our previously determined optimal experimental results with the triploid of *Populus tomentosa*. Cellulase pretreatment conditions were as follows: pulp consistency 10%, cellulase dosage  $20\text{IU}\cdot\text{g}^{-1}$ ,  $25\text{IU}\cdot\text{g}^{-1}$ , and  $30\text{IU}\cdot\text{g}^{-1}$  (based on bone dry pulp), pretreatmental pH value 6.0, pretreatment temperature  $55^{\circ}\text{C}$ , and pretreatment time 90 min. Xylanase pretreatment conditions were as follows: the pulp consistency 10%, xylanase dosage  $20\text{IU}\cdot\text{g}^{-1}$ ,  $25\text{IU}\cdot\text{g}^{-1}$ , and  $30\text{IU}\cdot\text{g}^{-1}$  (based on bone dry pulp), pretreatment pH value 6.5, pretreatment temperature  $50^{\circ}\text{C}$ , and pretreatment time 90 min.

The 30g (bone dry pulp) of original pulp and enzyme was placed into a polythene plastic bag according to the enzyme pretreatment conditions. Subsequently, the bags were put into a thermostatic water bath at constant temperature after mixing. During the process of reaction, the bags were taken out and the pulp was mixed every 10 min until the setting time. Subsequently, the bags were taken out and put into boiling water for 10 min to make the enzyme inactive and thus terminate the reaction. Finally, the pretreated pulp was washed thoroughly and was then ready for beating.

### Beating, Handsheet Forming and Testing Methods

The pulp pretreated by the enzyme was beaten in a PFI mill. Beating conditions were as follows: pulp consistency 10%, beating gap 0.25 mm, specific beating pressure  $3.33 \text{ N}\cdot\text{mm}^{-1}$ , and freeness (CSF) 250 mL.

Handsheets were formed with a PTI rapid handsheet former. The forming conditions were as follows: grammage  $60\text{g}\cdot\text{m}^{-2}$ , drying temperature  $95\text{ }^\circ\text{C}$ , drying time 7 min, drying vacuum 0.6 MPa, and conditioning treatment for 24 h in an atmosphere of relative humidity 50% and temperature  $23\text{ }^\circ\text{C}$ .

Pulp and paper properties were measured according to the following standard methods. Pulp was beaten according to ISO5264-2. Handsheets were made according to ISO5269-2. Canadian standard freeness (CSF), ISO5267-2; brightness, ISO2470; opacity, ISO2471; tensile index, ISO1924-1; tearing index, ISO1974; bursting index, ISO2578; and folding number, ISO5626 were according to the indicated ISO methods.

### Pulp Fibre Analysis with Fibre Quality Analysis (FQA)

Analysis of pulp fibre characteristics was done according to ISO16065. To prepare the sample, 0.1g of the pretreated pulp was initially dispersed in 1000 mL of water, and 100 mL of the fibre suspension was obtained. Fibre characteristics were analyzed with an FQA device (OpTest, Canada), model LDA-02. The fibre length, width, and fines of the APMP pulp were subsequently analyzed (Han 2007).

### Observation with Scanning Electronic Microscopy(SEM)

The fracture surface of the poplar APMP unpretreated and treated by enzyme were observed with SEM. First the samples were dehydrated with 30%, 50%, 70%, and 100% ethanol. Subsequently, the dehydrated pulp samples were frozen and vacuum dried for 48 h. Then the specimens were gold coated with gold-palladium in a Sputtergerät SCD 005 sputter coater (England). A sputter current of 60 mA, sputter time 90 s, and film thickness 20 nm to 25 nm were chosen as the coating conditions. The fibre surfaces of the samples were observed with a QUANTA 200 SEM (Holland).

### X-ray Diffractometer (XRD) Measurement

The unpretreated and treated pulp samples were first dehydrated (Huang et al. 2003), then frozen and vacuum dried for 48h. Subsequently, the dried samples were placed in the sample carrier, and the degree of crystallinity of the samples were analyzed with x-ray diffractometer (XRD, D8 ADVANCE, Germany). The important scanning parameters were as follows: x-ray tube Cu, tube voltage 40 kv, tube electricity 40 mA, scan speed  $0.03^\circ/\text{step}$  and  $0.1\text{s}/\text{step}$ , and scan range  $10^\circ\text{--}50^\circ$ . The degree of crystallinity of the samples can be obtained as follows,

$$X_C = \frac{F_K}{F_K + F_A} \times 100\%, \quad (1)$$

where  $X_C$  is degree of crystallinity,  $F_K$  is crystalline area, and  $F_A$  is amorphous area.

## RESULTS AND DISCUSSION

The effects of enzyme pretreatment on beatability, refining energy, physical strength, and optical properties of APMP pulp were studied. Refining energy based on PFI revolutions was related to freeness, meaning that the changes of freeness and PFI refining revolutions can indirectly indicate the refining energy consumption.

### Effects of Cellulase Pretreatment on Freeness and Beating Energy of Fast-growing Poplar APMP Pulp

The effects of cellulase pretreatment on the freeness and energy consumption of APMP pulp of fast-growing poplar are shown in Figs. 1 and 2.

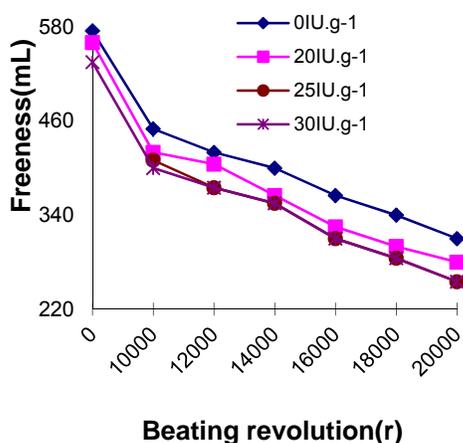


Fig. 1. Effects of cellulase treatment on freeness

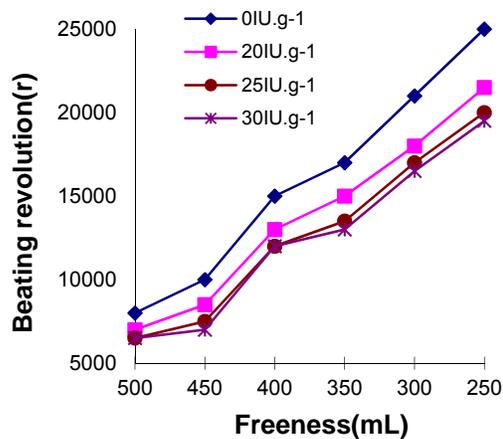
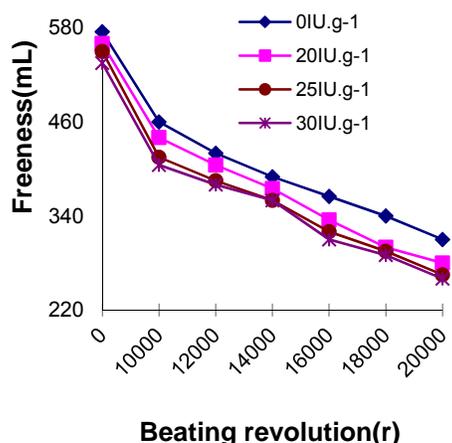


Fig. 2. Effects of cellulase treatment on beating revolution required to achieve a given freeness

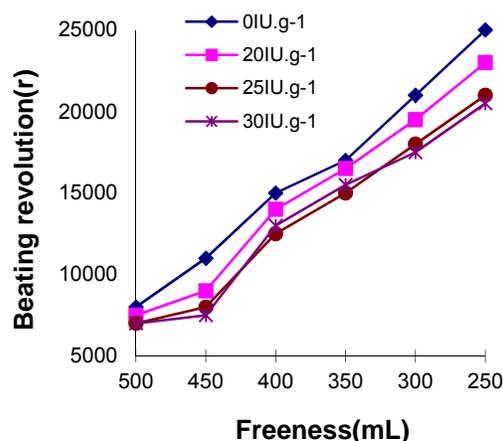
From the results in Figs. 1 and 2, it can be seen that the pretreated pulp showed a decrease of freeness (CSF) in the range of 30 mL to 55 mL at the same number of revolutions, and a decrease of PFI mill revolutions from 1000r to 5500r when reaching the same freeness, compared with the untreated pulp. This demonstrates that a decrease of beating energy consumption occurred in the range of 12.5% to 22%. Cellulose may hydrolyze micro-fibre on the fibre surfaces and make fibre structure less compact, softer, and more bulky. Beneficial effects of these changes include easier beating or refining of the pulp, resulting in a reduced refining (beating) energy. The effectiveness of cellulase-assisted refining was different at cellulase dosages of 20 IU·g<sup>-1</sup>, 25 IU·g<sup>-1</sup>, and 30 IU·g<sup>-1</sup>. The effect of cellulase-assisted refining got better when the cellulase dosages increased. Considering the little difference of the effects of cellulase-assisted refining in 25IU·g<sup>-1</sup> and 30IU·g<sup>-1</sup>, and taking the cellulase treatment cost into account, the optimal dosage of cellulase was selected to be 25 IU·g<sup>-1</sup>.

### Effects of Xylanase Pretreatment on Freeness and Beating Energy of Fast-growing Poplar APMP Pulp

Figures 3 and 4 show the effects of xylanase pretreatment on the freeness and energy consumption of APMP pulp of fast-growing poplar.



**Fig. 3.** Effects of xylanase pretreatment on freeness



**Fig. 4.** Effects of xylanase pretreatment on beating revolutions to reach a given freeness

Figures 3 and 4 show that, compared with the unpretreated APMP pulp, the pretreated pulp showed a decrease of freeness in range of 25 mL to 50 mL at the same PFI revolutions, and a decrease of PFI mill revolutions in the range of 1000r to 4500r when reaching the same freeness, which means a decrease of refining energy consumption in the range of 12.5% to 18%. It was concluded that xylanase pretreatment could reduce the refining energy. Xylanase may degrade xylan on the fibre surface and make the fibre structure more porous, which can be beneficial to easy beating or refining of the pulp. The effect of xylanase-boosted refining got better when the xylanase dosages increased. Considering the little difference of the effects of xylanase-boosted refining in  $25\text{IU}\cdot\text{g}^{-1}$  and  $30\text{IU}\cdot\text{g}^{-1}$ , and taking the xylanase treatment cost into account, the optimal xylanase dosage was determined to be  $25\text{IU}\cdot\text{g}^{-1}$ .

### Effects of Enzyme Pretreatment on the Properties of APMP Pulp of Fast-growing Poplar

The differences of properties between the unpretreated and pretreated APMP pulp of fast-growing poplar are shown in Table 5.

**Table 5.** Effects of Enzyme Treatment on Properties of Fast-growing Poplar APMP Pulp

Pulp properties	Unpretreated pulp	Cellulase pretreated pulp	Xylanase pretreated pulp
Brightness (% ISO)	75.1	76.3	77.2
Opacity (%)	81.4	82.0	81.5
Tensile index ( $\text{N}\cdot\text{m}\cdot\text{g}^{-1}$ )	21.9	27.1	25.6
Tearing index ( $\text{mN}\cdot\text{m}^2\cdot\text{g}^{-1}$ )	3.51	4.03	3.82
Bursting index ( $\text{KPa}\cdot\text{m}^2\cdot\text{g}^{-1}$ )	1.23	1.41	1.34
Folding number (time)	4	6	5
* Freeness, 250mL. Cellulase dosage, 25 $\text{IU}\cdot\text{g}^{-1}$ . Xylanase dosage, 25 $\text{IU}\cdot\text{g}^{-1}$ .			

As can be seen in Table 5, compared with the untreated pulp, the brightness, tensile index, tearing index, bursting index, and folding number of the pulp pretreated by cellulase were respectively improved by 1.2% ISO, 23.7%, 14.8%, 14.6%, and 50% at the same freeness. And the corresponding values for the pulp pretreated by xylanase were respectively improved by 2.1%ISO, 16.8%, 8.8%, 8.9%, and 25%. Cellulase pretreatment led to a slight increase of optical properties, and significant increase of physical strength properties for the APMP pulp of fast growing poplar. Xylanase pretreatment led to an increase of physical strength properties, and significant increase of brightness. The results indicated that cellulase pretreatment enhanced the physical strength properties of APMP pulp, and xylanase pretreatment improved the brightness of APMP pulp, respectively. Micro-fibrils on the fibre surface were hydrolyzed by cellulase, and the length and flexibility of the treated pulp were improved, which may contribute the physical strength properties of the treated pulp. Xylan on the fibre surface were degraded partly by xylanase, and structure of lignin-carbohydrate complex(LCC) were demolished and part lignin were dissolved and wiped out from pulp by washing, which may be the reason that xylanase pretreatment improved the brightness of APMP pulp.

### Analysis of Fibre Characteristics

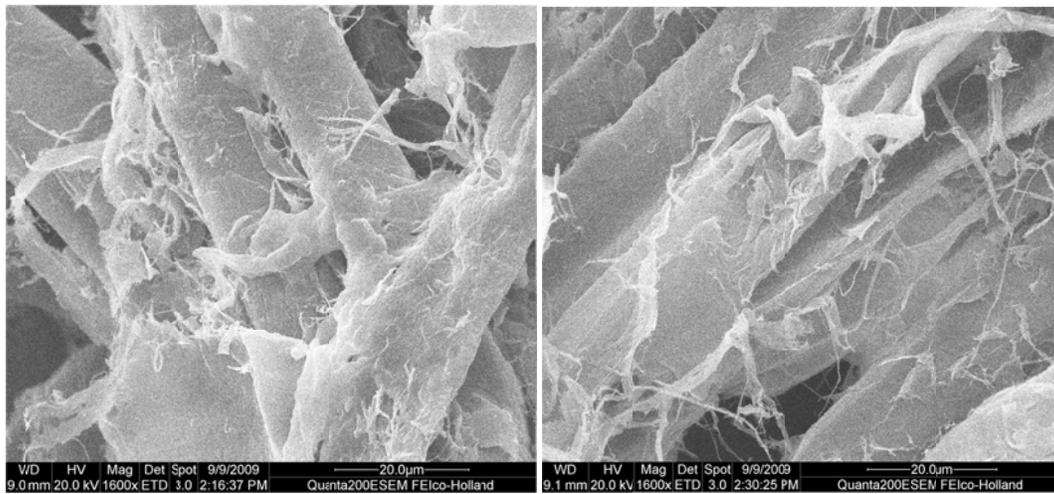
Table 6 shows the Fibre Quality Analysis (FQA) results of the untreated and treated pulp fibres. Compared with the untreated pulp fibre, the fibre length ( $L_n$ ,  $L_w$ , and  $L_{ww}$ ) of the pretreated pulp increased slightly, fibre width and fines decreased, and fibre torsion increased. Compared with the chemical pulp (such as kraft pulp and soda-AQ pulp), high yield pulps (such as APMP pulp and BCTMP pulp) have more fines content resulting from the refining process. Due to the fact that the specific surface area of fines is relatively larger, the enzyme can more easily find and degrade these fines, hence the average fibre length of the pretreated pulp samples were longer than that of the untreated pulp. One conclusion is that the enzyme pretreatment can easily split and torque fibres, making it possible to avoid cutting them during the subsequent refining or beating process, and improve strength properties.

**Table 6.** Fibre Characteristics of Untreated and Pretreated APMP Pulp

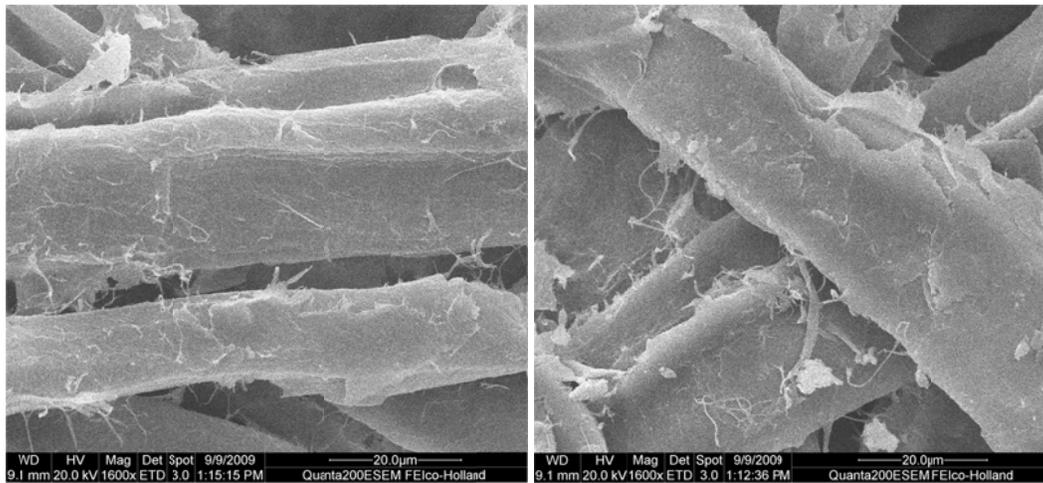
Fiber characteristics	Untreated pulp	Cellulase pretreated pulp	Xylanase pretreated pulp
Arithmetic length (mm)	0.517	0.566	0.554
Length weighted length (mm)	0.613	0.659	0.641
Weighted weighted length (mm)	0.706	0.785	0.774
Fiber width ( $\mu\text{m}$ )	20.9	20.1	20.6
Fibres torsion (mm)	0.51	0.58	0.55
Fines (%)	38.11	33.16	35.36
* Freeness, 250 mL. Cellulase dosage, 25 IU·g <sup>-1</sup> . Xylanase dosage, 25 IU·g <sup>-1</sup> .			

### Morphology of the Fibre Surface

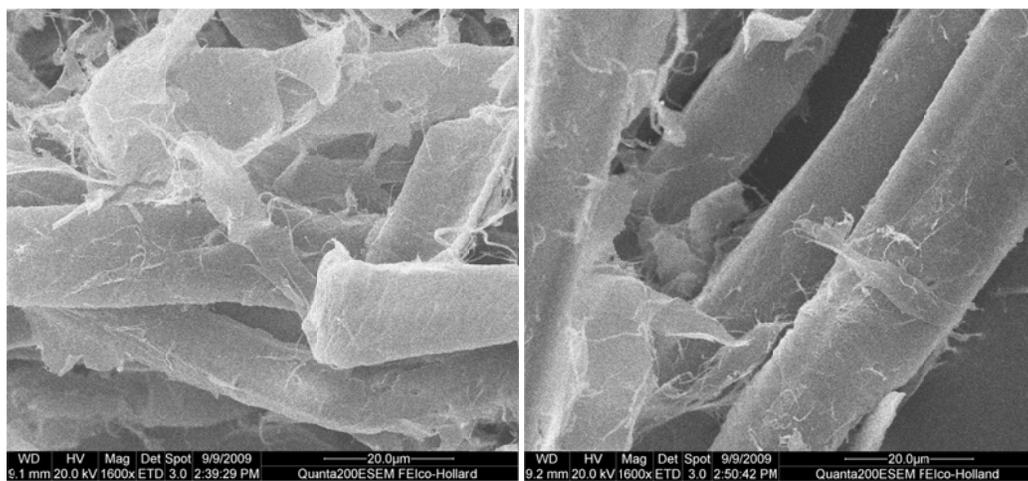
Figure 5 shows the SEM observations of the untreated and treated pulp fibres. The fibres were taken from the untreated and treated pulp refined for 250 CSF. The dosages of cellulase and xylanase were both 25 IU·g<sup>-1</sup> in the treating process of pulps.



a Unpretreated pulp



b Cellulase pretreated pulp



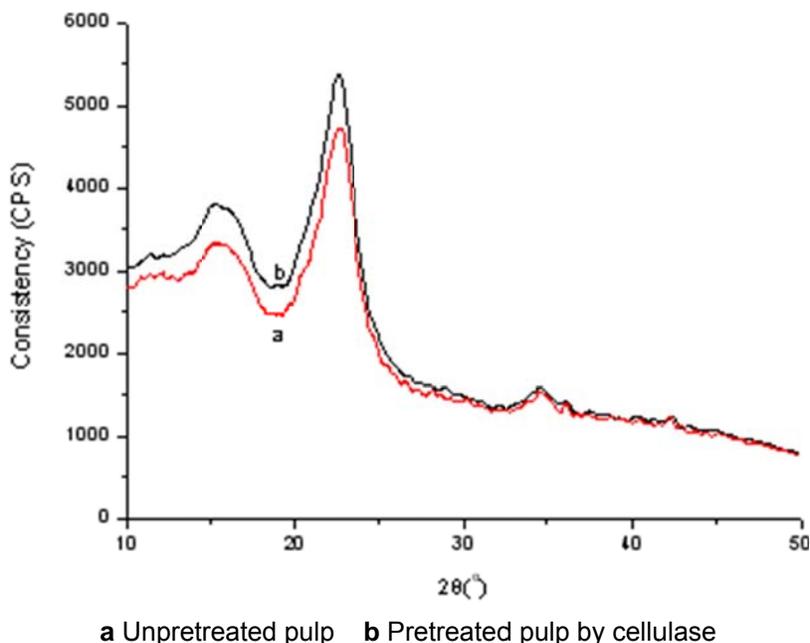
c Xylanase pretreated pulp

**Fig. 5.** SEM images of fibre of APMP pulp of fast-growing poplar

As shown in Fig. 5, the untreated pulp appeared stiffer, and had more fines and a lower fibrillation extent. In addition, the enzyme-pretreated fibres were softer and had a higher fibrillation extent; there were also less fines and more holes in the fiber surface. The pretreated fibres had higher fibre-bonding capability, which increased the pulp strength properties. Compared with the chemical pulp, the fibres of high-yield pulps (HYP) tend to be inflexible and short, which results in poor fibre-bonding capability. Enzymic pretreatment may modify the properties of HYP by hydrolyzing micro-fibre on the fibre, degrading xylans, and destroying LCC structure, making HYP fibre flexible and improving the fibre-bonding capability of HYP. The enzymic pretreatment loosens the fibre cell wall and softens the fibre so as to be more easily refined and beaten.

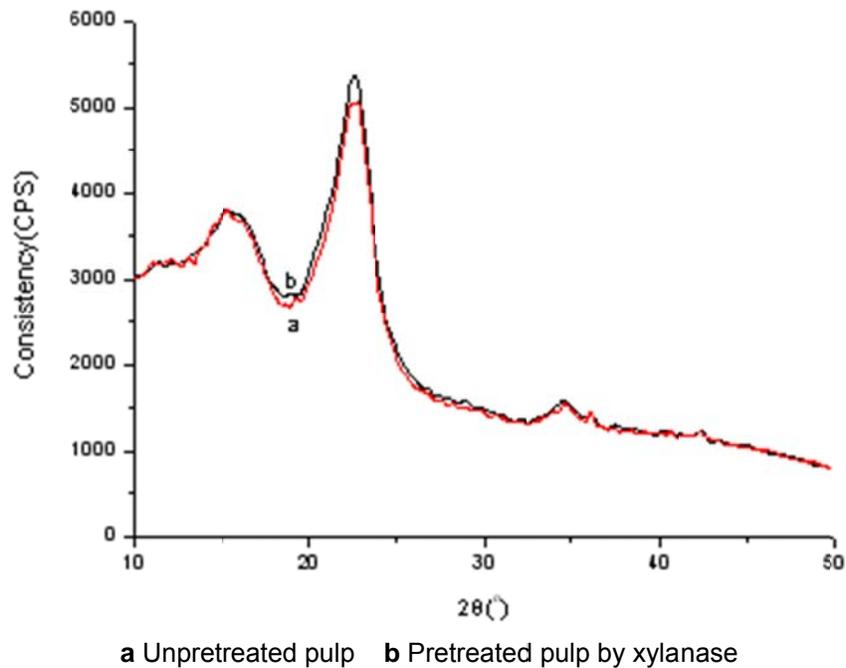
### XRD Analysis of Pulp

Figures 6 and 7 show the XRD analysis results of the untreated and pretreated pulp fibres. The fibres were taken from the untreated and treated pulp refined for 250 CSF. The dosages of cellulase and xylanase both were  $25 \text{ IU}\cdot\text{g}^{-1}$  for the treatment of the pulps.



**Fig. 6.** XRD patterns of the untreated and pretreated pulp fibres by cellulase

Figure 6 shows that the degree of crystallinity of the cellulose of APMP pulp fibres increased obviously after the cellulase pretreatment. The degree of crystallinity of the untreated pulp was 74.37%, and that of the APMP pulp pretreated by cellulase was 79.04%. Cellulase may reach easily to the amorphous area of pulp fibres to hydrolyze micro-fibres, but the crystalline area of pulp fibres may be difficult to be attacked by cellulase. The crystalline area of the pretreated pulp was wider than that of the untreated pulp. This may be the reason that the degree of crystallinity of the pretreated pulp was larger than that of the untreated pulp.



**Fig. 7.** XRD patterns of the unpretreated and pretreated pulp fibres by xylanase

Figure 7 indicates that the degree of crystallinity of the cellulose of APMP pulp fibres increased slightly after the xylanase pretreatment. The degree of crystallinity of unpretreated pulp was of 74.37%, and that of APMP pulp pretreated by xylanase was 75.91%. Xylanase may reach easily to the amorphous region of pulp fibres to degrade xylane on the fibre surface, thus reducing the relative amount of amorphous area of pulp fibres. The proportion of the crystalline area of the pretreated pulp was increased, and it may be the reason that the degree of crystallinity of the pretreated pulp was larger than that of the unpretreated pulp.

XRD analysis indicated that enzyme pretreatment was beneficial for improvement of the degree of crystallinity of cellulose. Increase of the crystallinity degree of the pretreated pulp may be contributed to the increase of the strength of the treated pulp fibres, while enhancing fibre flexibility and avoiding the excessive cutting of fibres, which was responsible for the improvement of the physical strength properties of the APMP pulp pretreated by enzyme.

## CONCLUSIONS

1. Compared with the unpretreated pulp, the freeness of the enzyme-pretreated pulp was decreased in the range of 25 mL to 55 mL, and refining energy consumption decreased from 12.5% to 22.0%. The beatability of poplar APMP pulp pretreated by cellulase or xylanase was improved.
2. Cellulase and xylanase pretreatment may improve the physical strength and the brightness of poplar APMP pulp. But the opacity was slightly changed. Cellulase

pretreatment was very good for improving the physical strength properties of APMP pulp and decreasing beating energy consumption. Xylanase also was very good in improving APMP pulp brightness. Effectiveness of cellulase pretreatment was greater than that of xylanase pretreatment. The optimal enzyme dosage was 25 IU·g<sup>-1</sup> for both cellulase and xylanase.

3. Fibre length of the enzyme pretreated pulp increased slightly, while fibre width and fines content decreased. The fibre torsion increased. Compared with the unpretreated fiber, the fibres pretreated by enzyme were softer and had higher fibrillation extent. Enzyme pretreatment increased the crystallinity of cellulose, which can enhance fiber flexibility and physical tensile properties of APMP pulp.

### ACKNOWLEDGEMENTS

The authors are grateful for the support of National Natural Science Foundation, Grant. No. 30972326, 31070525, Natural Science Foundation of Shandong Province, Grant. No. ZR2010CM065, and Taishan Scholar Program of Shandong Province, Grant. No. 2605.

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Article submitted: March 5, 2011; Peer review completed: April 17, 2011; Revised version received and accepted: May 11, 2011; Published: May 17, 2011.