PULP PROPERTIES AND FIBER CHARACTERISTICS OF XYLANASE-TREATED ASPEN APMP

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It is important to further improve the strength properties of alkaline peroxide mechanical pulp (APMP) in order to extend its applications in more paper grades. In this work, aspen APMP was pretreated by xylanase, and its effect on the improvement of paper strength properties was investigated. The results showed that, for xylanase-pretreated pulp, the tensile, tear and burst indexes were respectively about 14%, 23%, and 18% greater than those of untreated pulp. Meanwhile, the fines content and kink index decreased to some extent with the enzyme treatment. The total carboxyl content, crystallinity index, and water retention value of the pulp was increased significantly, and a higher porosity was observed on the fiber surface. Further investigation revealed that the lignin coverage of the fiber surface decreased from 59.2% to 55.2% after enzyme treatment, and the *C*1/*C*2 ratio decreased from 24.4 to 14.4. The improvement of strength properties can be ascribed to the increase in carboxyl groups and crystallinity, and a decrease in fines content and kink index, as well as to the removal of a portion of xylan and lignins from the fiber surfaces.

Keywords: Xylanase; Alkaline peroxide mechanical pulp; Fiber; Pulp strength

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INTRODUCTION

The pulp and paper industry in Asia has been rapidly growing in the past decade. Effectively utilizing wood raw materials has become crucial for the protection of the environment and sustainable development of the papermaking industry. High-yield pulps are attractive due to their efficient utilization of lignocellulosic materials (Tang *et al*. 2009). As a fast-growing hardwood species, aspen is one of the major papermaking raw materials, especially for producing chemi-mechanical pulps, due to its attributes of relatively high brightness and cellulose content, as well as low lignin content. Alkaline peroxide mechanical pulping (APMP) and pre-conditioning refiner chemical alkaline peroxide mechanical pulping (P-RC APMP) processes are suitable technologies for producing pulps with yields of 90% from aspen woodchips. These pulps are widely used in a variety of paper grades such as paperboard, culture paper, toilet paper, and some value-added paper products. The major advantages of aspen APMP are its low costs, high bulk, low pollution, and high opacity. In China, several APMP lines were put into production recently, due to the advantages of producing pulp at a low cost and with a high paper bulk and a high opacity (Liu *et al*. 2011); however, a major problem for APMP is its lower strength properties.

Application of enzymes offers an environmentally friendly means of improving the strength properties of pulp. The pretreatment of pulp with cellulase prior to refining can achieve more fibrillation of fibers, which enhances the inter-fiber bonding and increases the tensile strength of bleached softwood kraft pulp (Lecourt *et al*. 2011). It should be noted, however, that cellulase treatment causes loss of fiber intrinsic strength and negatively affects the tear strength (Gil *et al*. 2009).

In recent years, efforts have been paid to the treatment of fibers by laccase with the combination of other compounds, *e.g.* phenolic compounds (Aracri *et al*. 2011; Widsten *et al.* 2009) and amino acids (Witayakran and Ragauskas 2009; Chen *et al.* 2010), for enhancing inter-fiber bonding. Moreover, laccase pretreatment can allow the pulp to achieve better tensile and tear strengths with a lower mechanical energy cost with the presence of a synthetic mediator such as 1-hydroxybenzotriazole (HBT), compared to the conventional pulp (Cadena *et al*. 2010). However, the phenolic compounds can cause a loss of pulp brightness, and the synthetic mediator or the amino acid will have higher cost.

In the past ten years, most of the research on xylanase applications in the paper industry have focused on boosting the pulp bleachability (Nair *et al*. 2010; Valls and Roncero 2009; Allison *et al*. 1996; Siles *et al*. 1995; Roncero *et al*. 1996; Jeffries *et al*. 1996; Pham *et al*. 1995), which results in a savings of bleaching chemicals. Various explanations have been proposed as to how the xylanase works. It may selectively hydrolyze the reprecipitated and reabsorbed alkali-resistant xylan on fiber surfaces, thereby facilitating the reagent penetration into fibers and leading to the release of lignin in the subsequent bleaching process (Torres *et al*. 2000). On the other hand, xylanase pretreatment of pulps can break down the lignin-carbohydrate bonds, facilitating the extractability of solubilized lignin (Li e*t al*. 1996; Allison *et al*. 1997). Finally, the removal of hexenuronic acid on the initial pulp also contributes to the improvement of bleaching susceptibility (Aracri and Vidal 2011; Fillat *et al*. 2011).

In addition, the effects of xylanase pretreatment on the strength properties of unbleached eucalyptus kraft pulps (MaÂximo *et al*. 1998), bleached eucalyptus kraft pulps (Batalha *et al.* 2011), and unbleached soft kraft pulps (Dickson *et al*. 2000) have been studied. Unfortunately, the results show that the strength properties of the resulting paper have little change, although the treatment can reduce the refining energy consumption of pulp.

However, there has been little emphasis on the application of xylanase in mechanical pulping (Lei *et al*. 2008). No xylanase pretreatment has seemingly been used for improving the physical strength of aspen APMP.

This paper presents a state-of the-art study where the potential of xylanase in enhancing the strength properties of APMP was assessed. The characteristics of the enzyme-treated pulp were analyzed and examined by using X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), environment scanning electron microscopy (ESEM), scanning probe microscopy (SPM), and a fiber quality analyzer (FQA). The improvement mechanisms of pulp strengths were considered for the application of xylanase to aspen APMP.

EXPERIMENTAL

Materials

The aspen APMP was sampled after the first-stage refining from a commercial pulp mill in Shandong, China, and with a Kappa number of 27. The xylanase (Pulpzyme® HC) was acquired by a submerged fermentation of genetically modified *Bacillus licheniformis*, and with the standardized concentration of 1000 AXU/g (active xylanase units per gramma), and the optimal pH value of 7.5.

Pulp Sample and Enzyme Treatment

The pulp suspension, with a consistency of 4%, was prepared with 0.1 M acetate buffer (pH of 7.5), and stirred at 50°C in a water bath. The pulp was then treated with the addition of 0.6 AXU/g of xylanase (relative to oven dry pulp) for 1 hour. Then the pulp was refined to about 45°SR by using a PFI mill, at 10% of consistency and with a clearance of 0.10 mm and revolutions of 6,000. The other pulp sample represented the control and was treated under the same conditions but without the addition of xylanase.

Handsheet Making and Property Determination

Handsheets with a grammage of 60±2 g/m^2 were prepared according to the TAPPI Standard Method T205. The tensile, tear, and burst strengths of the handsheets were determined according to TAPPI Standard Methods T494, T414, and T403, respectively. The water retention value (WRV) of the fibers was measured as described in the literature (Rom *et al*. 2007). The total acid group content was determined by conductivity titration according to the TAPPI Standard Method T237, and the pulp samples were washed until a neutral pH was reached with de-ionized water before testing was performed. Fiber morphology was examined with a Hi-Res Fiber Quality Analyzer (OpTest Equipment Inc, Canada).

Analysis of XRD

X-ray diffraction patterns for the handsheets were acquired with a Bruker D8 Diffractometer (Germany) using a Ni-filtered Cu Kα radiation (λ = 0.1542 nm) generated at 40 kV and 20 mA. The scanned range was from 2θ = 5° to 50°. The crystallinity of fibers (CrI) was calculated based on the empirical method (Segal *et al.* 1959),

$$\text{CrI (\%)} = (I_{200} - I_{\text{am}})/ I_{200} \quad (1)$$

where *CrI* is the degree of crystallinity, I_{200} is the peak intensity corresponding to cellulose *I*, and I_{am} is the peak intensity of the amorphous fraction.

Analysis of XPS

X-ray photoelectron spectra were acquired for handsheet surfaces, with a physical Electronics ESCALAB 250 XPS (Thermo Fisher Scientific, USA). The detector was set at an angle of 90° to the sample surface. The analysis area was 0.8 mm^2. A Gaussian curve fitting program was used to deconvolute the *C*1 carbon (functional groups of *C-C*, *C-H*, and *C=C*) signal at 285 eV as an internal standard. The chemical shifts relative to

*C*1 used in the deconvolution were 1.7±0.2 eV for *C*-*O* (*C*2), 3.1±0.2 for *C*=*O* or *O*-*C*-*O* (*C*3), and 4.3±0.2 eV for *O*-*C*=*O* (*C*4) groups (Dorris and Gray 1978).

The handsheet samples for the XPS analysis were subjected to a Soxhlet extraction with acetone, which was followed with deionized water. Four hours of treatment time was used for each solvent. The samples were then placed on a clean glass slide, pressed slightly, and then dried in an oven at 60 ºC. The glass-smooth side of the sample was used for the XPS examination. The surface coverage of lignin was calculated according to the following equation developed by Ström and Carlsson (1992). Theoretical *O*/*C* ratios of 0.83 and 0.33 were used for cellulose and lignin, respectively (Gray 1978).

$$\text{Surface coverage of lignin} = (O/C_{\text{after extraction}} - O/C_{\text{carbohydrates}}) / (O/C_{\text{lignin}} - O/C_{\text{carbohydrates}}) \quad (2)$$

Analyses of ESEM and SPM

Fibers were examined with a FEI Quanta 200 ESEM. The nanoscale structure of the fiber surfaces was also imaged with a NanoScope IIIa Multimode SPM (Veeco, Santa Barbara, USA), using a tapping mode under atmospheric pressure and room temperature. Real time scanning was performed at scan rates of 1.0 Hz, scanning angle of 0°, and tapping frequencies ranging from 250 to 300 kHz. Before the SPM analysis, a drop of fiber suspension (about 0.1 g of pulp in 100 mL of Milli-Q water) was transferred onto a purged silicon wafer to disperse a single fiber on the surface, and then dried in a vacuum desiccator for 24 hours. SPM observations were conducted at two to four locations on each characterized fiber.

RESULTS AND DISCUSSION

Xylanase Dosage and Pulp Strengths

The effects of xylanase dosage on the tensile, tear, and burst strengths of APMP are shown in Fig. 1 and Fig. 2.

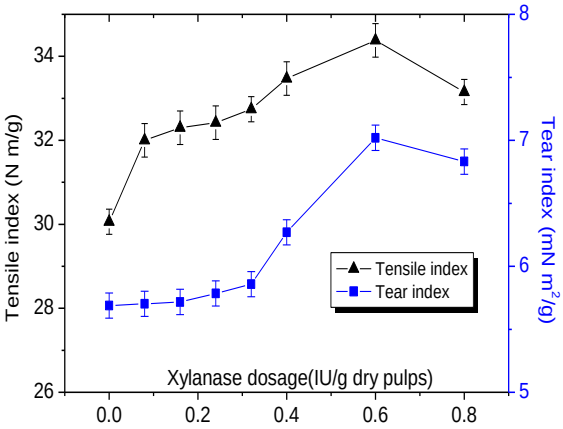

Fig. 1. Xylanase dosage *vs*. tensile and tear indexes of pulp

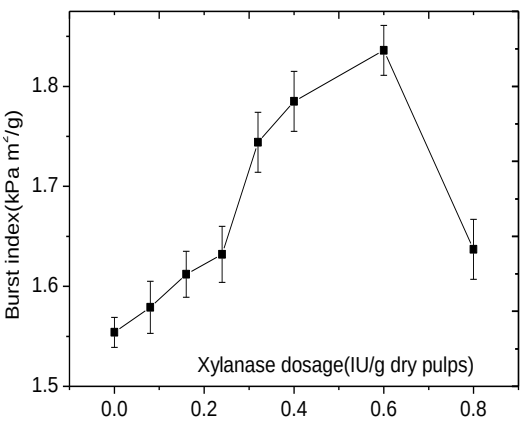

Fig. 2. Xylanase dosage *vs*. burst index of pulp

The results showed that the pulp strengths were apparently enhanced with the increase of xylanase dosage, especially at 0.6 AXU/g (o.d. pulp) dosage. Compared with the control sample, the tensile, tear, and burst indexes were greater by about 14.4%, 23.4%, and 18.1%, respectively, for the xylanase-treated pulps. The result from Lei *et al*. (2008) showed that more reducing sugars were produced when the eucalyptus chips were treated with increasing dosage xylanase. Therefore, the hydrolysis of xylan helps to improve the pulp strengths.

Interestingly, this positive effect on pulp strengths by means of xylanase treatment was not observed when using the kraft pulps. The tensile index and tear index of bleached eucalyptus kraft pulp decreased after xylanase treatment (Batalha *et al*. 2011). For the unbleached eucalyptus kraft pulp, the xylanase pretreatment also reduced the tensile strength and tear strength, and retained the burst strength although the refining energy was saved (MaÂximo *et al*. 1998). The reasonable explanation is that the mechanical pulp contained a large quantity of fines that easily adsorb xylanase due to their high specific surface area. The hydrolysis of xylan led to the dissolution and removal of a majority of fines, which helped to enhance the strength properties.

Figures 1 and 2 also show that adding more dosage of xylanase negatively influenced the pulp strengths. The reason is that the presence of suitable dosage of xylan is beneficial to the swelling of fibers, which can avoid the shortening of fibers during the subsequent refining of pulp. Therefore, the optimal dosage of xylanase was found to be 0.6 AXU/g under the experimental conditions used in this study, and the subsequent treatment was conducted at a xylanase dosage 0.6 AXU/g.

Effect of Xylanase Treatment on Pulp Properties

The total carboxyl content, water retention value (WRV), and crystallinity of pulp samples were examined to explain how the improvement in strength properties was achieved, as shown in Table 1. It can be seen that the total content of carboxyl groups was increased by about 13% for the xylanase-treated pulp, which will definitely benefit in increasing hydrogen bonding between fibers. Obviously, the increase in carboxyl groups facilitated swelling of the pulp fibers, and this was confirmed by the increase of water retention value from 187.4% to 208.7%. In addition, the hydrolysis of xylan led to a higher porosity, which improved the water absorption and swelling of the pulps.

The pulp crystallinity can be used to estimate the elimination of lignins and hemicelluloses (amorphous material) (Roncero *et al.* 2005). The removal of hemicellulose and lignin materials leaves a high proportion of crystal cellulose, which leads to an increase in pulp crystallinity. As shown in Table 1, the crystallinity increased by about 13% for the xylanase-treated pulp. This increase in crystallinity by means of xylanase treatment is consistent with the results obtained by Roncero *et al.* (2005), who found that the crystallinity of unbleached *Eucalyptus globulus* kraft pulp increased from 78.89% to 81.8% after hydrolysis by xylanase. It was understandable that the hydrolysis of xylan resulted in a relatively increased cellulose content. Furthermore, the dissolving of xylan introduced more pores on the fiber structure, which made it is easier to develop the internal fibrillation of fibers during the refining process. It can also be seen in Table 1 that the beating degree of the pulp was decreased. It should be caused by the elimination of xylans that promote the swelling of fibers.

Table 1. Total Carboxyl Content, WRV, and Crystallinity of Control Fibers *vs*. Xylanase-Treated Fibers

Pulp Sample	Total Carboxyl ($mmol \cdot Kg^{-1}$)	Water Retention Value (%)	Crystallinity (%)	Beating Degree (°SR)
Control	210.8±0.6	187.4±2	54.3±1	43.5±1
Xylanase-treated	238.5±0.7	208.7±2	61.5±1	41.5±1

Fiber Quality Analysis

Due to most of the lignin being retained in the fibers, and also the generation of more fines during refining, mechanical pulp was stiffer than the other pulp grades, which greatly impacted the pulp strengths.

Table 2. Effect of Xylanase Treatment on Fiber Morphology

Pulp Sample	Fines (%)	$\overline{Ln}$ (mm)	Coarseness ($mg \cdot m^{-1}$)	Kink Index	Curl Index
Control	12.8±0.2	0.556±0.03	0.147±0.02	1.41±0.05	0.049±0.008
Xylanase-treated	10.7±0.2	0.591±0.02	0.189±0.03	0.90±0.04	0.062±0.009

As shown in Table 2, the fines content decreased from 12.8% to 10.7% with xylanase pretreatment. Since fines had much higher specific surfaces than fibers, it was easier for them to become hydrolyzed by xylanase, which led to a lower fines content. The reduction of fine content also explained the decrease of beating degree. The numeric average fiber length ($\overline{Ln}$) and fiber coarseness were therefore greater for the xylanase-treated pulp, which will contribute to improved paper tear strength.

The tensile strength of the paper will be negatively affected by the kink of fibers. It was shown that the kink index decreased from 1.41 to 0.90 for xylanase-pretreated fibers, which will have a positive effect on the pulp strengths. Similarly, the curl of fiber affects mostly the tensile strength and the bonding ability between fibers (Page *et al.* 1985). In this study, the curl index of the fibers increased from 0.049 to 0.062 after xylanase pretreatment. This is the same trend as reported by Dickson *et al.* (2000). It was suggested that the fibers became more flexible and less cut during the subsequent refining, producing fibers of greater length; therefore, the moderate curl of fibers can help in increasing the interweaving chance of fibers and enhance the bonding between mechanical pulp fibers, which contributes to improving paper strength properties.

Chemical Structure of Fiber Surface

Fiber morphology and topochemistry were strongly related to the bonding ability of fibers during the handsheet forming process. XPS has been shown to be a useful tool for exploring the chemical components on fiber surfaces. The curves of the C_{1s} XPS spectra of the handsheets are shown in Fig. 3. The surface lignin content and fiber composition information were respectively summarized in Table 3 and Table 4.

It was noted that the total *O*/*C* ratio increased from 53.40% to 55.42%, and the surface lignin coverage decreased from 59.20% to 55.16% with the xylanase pretreatment. It was suggested that the hydrolysis of xylan in lignin-carbohydrate compounds (LCC) reduced the size of LCC and facilitated the dissolution of lignin.

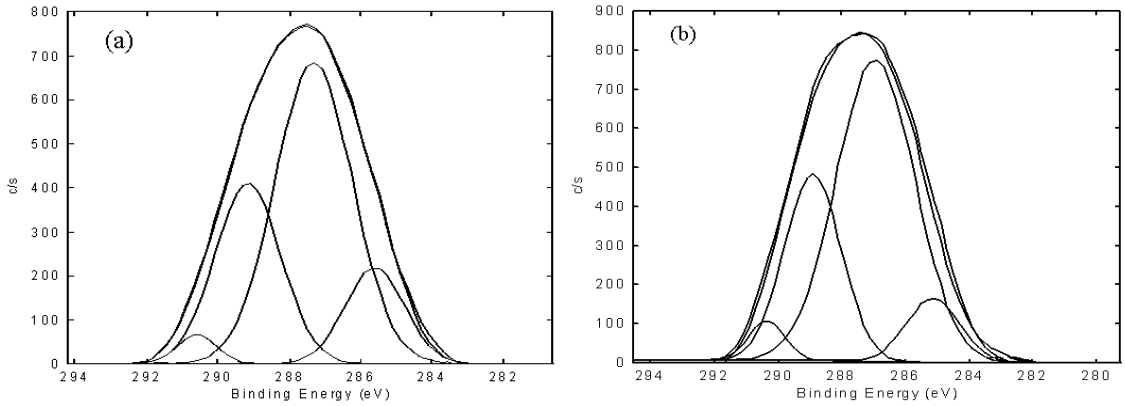

Fig. 3. Curves of the C_{1S} XPS spectra of (a) control fibers and (b) xylanase-treated fibers

Table 3. Effect of Xylanase Treatment on *O*/*C* Ratio and Fiber Surface Lignin

Pulp Sample	Total *O*/*C* Ratio (%)	Surface Coverage of Lignin (%)
Control	53.40±0.35	59.20±0.65
Xylanase-treated	55.42±0.40	55.16±0.52

Table 4. Effect of Xylanase Treatment on C_{1S} Peak Areas of Fibers

Pulp Sample	Relevant Peak Area (%)				C_1/C_2 (%)
	C_1 (*C-C, C-H*)	C_2 (*C-O*)	C_3 (*C=O, O-C-O*)	C_4 (*O-C=O*)	
Control	13.5±0.50	55.45±0.80	28.29±0.42	2.75±0.25	24.36±1.2
Xylanase-treated	8.73±0.62	60.64±0.68	27.04±0.50	3.58±0.30	14.40±1.3

As a rule of thumb, the *C*1 component can give information similar to the *O*/*C* ratio, as they both are directly affected by the amount of surface lignin (Gustafsson *et al.* 2003). The *C*1/*C*2 ratio shows the relative magnitude of lignin and cellulose on the fiber surface. Table 4 shows that the C_1 component of the fibers decreased from 13.51% to 8.73% after xylanase pretreatment. The C_2 component (*C-O*, from the carbohydrate hydroxyl) increased from 55.45% to 60.64%. The C_1/C_2 ratio sharply decreased from 24.36% to 14.40%. This indicated that the removal of lignin and the hydrolysis of xylan on the fiber surface caused the exposure of much more cellulose, which helped to enhance the hydrogen bonding between fibers. The C_3 component slightly decreased and the C_4 component (*O-C=O*, from the carboxyl group) increased from 2.75% to 3.58%, which resulted from the exposure of more carbohydrate.

Morphology of Fiber Surface

As shown in Fig. 4a, the untreated pulp fibers were stiffer and had a smoother surface than the treated fibers. The xylanase pretreatment resulted in higher porosity and peeling of pulp fibers (Fig. 4b), and the fibers became more flexible and released more fibrils. The hydrolysis of xylan and the removal of surface lignin made the refining easier, and a better fiber characteristic was achieved. The peeling of the fiber surface layer was also observed by other researchers (Torres *et al*. 2000; Roncero *et al*. 2005; Roncero *et al*. 2000), who used a xylanase treatment for bleaching eucalyptus kraft pulp.

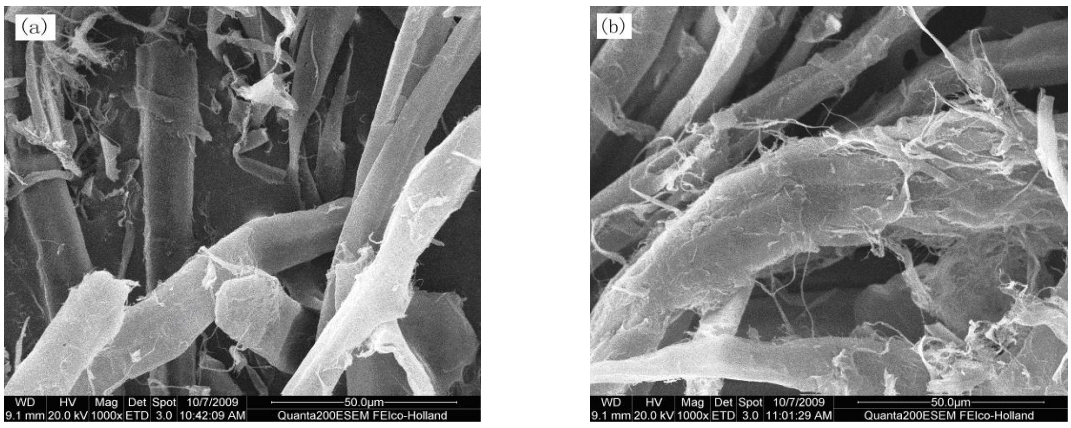

Fig. 4. ESEM images of (a) control fibers, and (b) xylanase-treated fibers

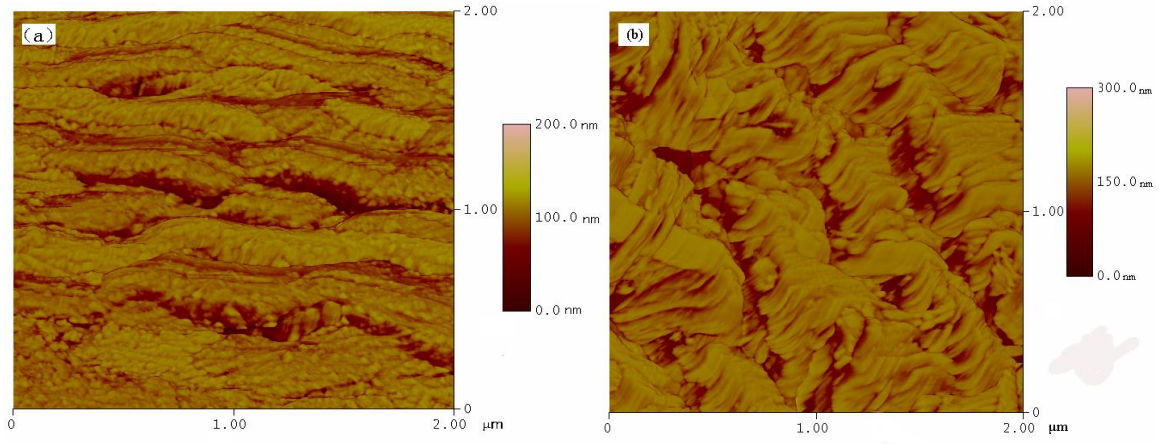

Fig. 5. SPM phase images of (a) control fibers, and (b) xylanase-treated fibers at scanning area of 2.0 µm × 2.0 µm

The nanoscale structure of the fiber surfaces was further studied with an SPM, as shown in Fig. 5. It can be seen that granular substances covered the surface of untreated fibers (Fig. 5a), featuring the remnants of lignin-rich middle lamella materials on the fiber surface.

According to the literature (Gustafsson *et al.* 2003, Li *et al.* 2006), the granular substances consist of lignin, hemicelluloses, extractives or their complex composites. For the xylanase-treated fiber, these granular substances were removed, and more micro-fiber structure appeared. This observation was in good agreement with the sharp decrease of the C_1/C_2 ratio on fiber surface by XPS analysis.

The hydrolysis of xylan and the removal of lignin loosened the fiber structure after the xylanase pretreatment, which promoted the removal of the primary cell wall during the PFI refining. The appearance of much more cellulose on the fiber surface enhanced the bonding force between fibers.

CONCLUSIONS

Pretreatment of pulp with xylanase before refining can improve the tensile, burst, and tear strengths of aspen alkaline peroxide mechanical pulp. The enhancement of pulp strengths results from the increases of carboxyl group content, crystallinity index, and water retention value of the fibers. Moreover, the decreases in fines content and kink index of the fibers help to increase the interlacement chance of fibers, and consequently improve pulp strengths. Finally, the hydrolysis of xylan from fiber walls and the removal of fiber surface lignins contribute to the exposure of more cellulose, which further enhances the bonding force between fibers.

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