

## EFFECT OF XYLANASE PRETREATMENT OF WOOD CHIPS ON FIBER SEPARATION IN THE CTMP REFINING PROCESS

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The effect of xylanase treatment of eucalyptus wood chips on chip refining and fiber properties was investigated. The fiber separation region and fiber surface structure were observed with SEM, TEM, and AFM. The fiber length and fines were analyzed with a Bauer-McNett classifier and optical image analysis of flowing suspensions (FQA). The results showed that xylanase degraded and hydrolyzed some xylan in the fiber wall, thus loosening the fiber wall structure. Therefore, in the subsequent refining process, fiber separation occurred in the secondary wall. This resulted in fibers with less lignin and extractives on the surface, which will benefit the interfiber bonding.

*Keywords:* Xylanase; CTMP; Fiber separation; SEM; TEM; AFM

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### INTRODUCTION

Application of biotechnology offers viable and novel means to reduce the environmental impact of the pulp and paper industry. Xylanases may be used in a wide range of applications, including debarking (Ratto et al. 1993), enhanced bleaching of kraft pulp (Paice et al. 1988; Clark et al. 1991; Senior et al. 1992; Suurnäkki et al. 1994; Li et al. 1996; Madlala et al. 2001), pulp drainage improvement (Bobu et al. 2003; Chen et al. 2006), enzymatic deinking (Morkbak et al. 1998), upgrading recycled pulp (Drnovsek 1997), production of dissolving pulp, removal of shives, and retting of flax fibers (Bajpai 1999). The most important application of xylanase is in the pretreatment stage of Kraft pulp bleaching. Xylanases have been found to increase final brightness value, to reduce the consumption of chlorine and chlorine dioxide, and to decrease the AOX content in the bleaching effluent (Paice et al. 1988; Clark et al. 1991; Senior et al. 1992; Ratto et al. 1993; Suurnäkki et al. 1994; Li et al. 1996; Morkbak et al. 1998; Madlala et al. 2001; Bobu et al. 2003; Chen et al. 2006). The proposed mechanisms of xylanase action in the bleaching of kraft pulp are as follows: (1) Lignin-carbohydrate bonds may be partially interrupted as a result of a xylan chain broken by hydrolysis action with the xylanase as a catalyzer. This improves the accessibility of the subsequent bleaching chemicals to the pulps and facilitates the removal of lignin. (2) The reprecip-

itated and reabsorbed alkali-resistant xylan, which forms a physical barrier against the extraction of residual lignin molecules from the fibers, may be removed from the fiber surface by xylanase treatment (Daneault et al. 1994). In addition, the removal of hexenuronic acid also contributes to the improvement of bleaching susceptibility (Suurnäkki et al. 1997). Therefore, the fiber surface becomes more permeable, and the chemical penetration into the fiber wall in the subsequent bleaching stage happens easily (Kantelinen et al. 1993).

However, there has been little emphasis on the application of xylanase in the mechanical pulping. Bio-mechanical pulping has been carried out mostly with ligninolytic enzymes, including lignin peroxidase, manganese peroxide, and laccase produced from white-rot fungi. The use of white-rot fungi for the treatment of wood chips prior to mechanical is usually referred to as 'biopulping'. This biopulping has many beneficial effects on pulping processes; however, the precise role of these enzymes remains unknown. For mechanical pulp, lignin-targeting enzymes are applied to degrade part of lignin in the raw materials for the purpose of facilitating fiber separation, improving strength property as well as reducing refining energy. However, the drawback of this process is that pulp yield and brightness have decreased in many cases (Prasad et al. 1996).

Compared with fungi as the main degraders of lignocellulose materials, xylanase has several advantages over them. The enzyme reactions are direct and rapid with the substrate, and they can be performed within 1 or 2 hours, whereas the corresponding fungal treatments require several days, even several weeks to degrade lignin. Although fungal hyphae penetrate wood very rapidly, up to 1mm per hour, the fungus attacks wood by means of enzymes secreted from their hypha (Eriksson 1990). Commercialized enzymes usually have high purity and activity, and they can easily fit into the existing process design. Furthermore, xylanase does not bring about lignin reduction. Xylanase is an enzyme that acts on the xylan in wood to hydrolyse it and lower its degree of polymerization (Petit-Conil et al. 2005). Xylanase treatment of wood chips can also reduce refining energy consumption (Petit-Conil et al. 2005).

Large amounts of hydrophobic material are present on the surfaces of CTMP fibers, which leads to reduced interfiber bonding. During the traditional CTMP refining process, the rupture of the fiber wall takes place preferentially in the primary layer (P) and in the middle lamella (ML) (Koljonen et al. 2003). Less S2 layers are exposed on the surfaces of CTMP or CMP fibers, because in CTMP or CMP processing, lignin in the CML were softened by the pre-steaming, preheating, or chemical treatment, which leads to fiber separation at the ML zone (Franzén et al. 1986; Cisneros et al. 1995; Salmén et al. 1999). When white fungal pretreatment is used in bio-pulping of CTMP, the loosened fiber structure contributes to savings in refining energy; however, a large amount of extractives and lignin are still present on the fiber surface (Cisneros et al. 1995; Koljonen et al. 2003; Fardim et al. 2005; Shao et al. 2006). Lignin that is present on the surface has been observed to have the form of patches or globules. Lignin and extractives are hydrophobic by nature. The presence of them is expected to negatively affect interfiber bonding (Shao et al. 2006).

During the TMP and PGW processes, fiber separation occurs between the primary and secondary walls, and preferably between the S1 and S2 layers (Shao et al. 2006). A

large amount of fibrils are present on the fiber surface, which can lead to high interfiber bonding strength.

In this study it was hypothesized that if xylanase pretreatment of wood chip is used prior to the CTMP process, some xylan in the fiber secondary wall will be removed, which may loosen the fiber structure in the secondary wall. Therefore, in the subsequent refining process, the fiber separation will occur more in the secondary wall, which will generate fines with less lignin present on the surface, thus, improving the interfiber bonding ability. In this study Pulpzyme HC was used on eucalyptus wood chip for producing Bio-CTMP. SEM, TEM, and AFM were used to characterize the fiber separation region.

## **EXPERIMENTAL**

### **Materials**

Eucalyptus chips from 4 to 5 year old trees were provided by Leizhou Forestry Enterprise, Zhanjiang, China. Chips were put in a plastic bag to balance moisture content.

### **Enzyme**

Pulpzyme HC, a stabilized liquid xylanase preparation formulated and standardized for use in pulp bleaching, was kindly provided by Novo-Nordisk Corporation, Tianjin, China. This commercial enzyme is derived from a selected strain of bacterial origin, produced by submerged fermentation of a selected strain of *Bacillus*, and has a specificity of catalyzing the hydrolysis of deacetylated xylan substrates. It contains endo-1, 4- $\beta$ -D xylanase activity (EC 3.2.1.8) and is virtually free of cellulase activity. Pulpzyme HC has an activity of 1000 AXU/g (xylanase units). One xylanase unit (AXU) is defined as the amount of enzyme in standard conditions (pH 9.0, 50 °C, 30 min incubation), which releases a defined amount of dye from dyed RRB xylan. The enzyme solution is stored within the temperature range 0-5 °C, and the recommended working conditions are pH 6.5-9.5 and 40-65 °C (Zhan et al. 2001).

### **Enzyme Pretreatment**

All xylanase treatments were carried out at 40-65 °C and at liquor to wood ratio of 6:1 (w:w). The pH was initially adjusted with a 1N H<sub>2</sub>SO<sub>4</sub> solution. The control CTMP was treated in the same way, but in the absence of xylanase. The Pulpzyme HC dosage ranged from 0 to 50 AXU/g of oven dry chip weight, and treatment time varied from 0-4 h. Detailed steps of the experiment were as follows:

The xylanase was diluted to the given concentration, mixed with chips and distilled water, and then heated to the desired temperature. Then the pH was adjusted to the target. After adding xylanase solution to the polyethylene bags, the chips and enzyme were mixed thoroughly to ensure a uniform xylanase distribution with the chips. The bags were then kept in a water-bath for the required time and at the appropriate temperature. After pretreatment, chips were separated by filtration with a bag-type strainer, and then the filtrate was analyzed for its reducing sugar content.

Some eucalyptus chips were used for SEM observation. Before the chips were treated by xylanase, cross sections of smooth surfaces were obtained by microtoming while in a frozen condition.

### **Chemical Treatment and Chip Refining**

The chemical treatment of CTMP was performed with washed eucalyptus chips, using a liquor-to-wood ratio of 4:1 in a rotary digester with capacity of 1.5 L, and a charge of 2 % NaOH and 10 % Na<sub>2</sub>SO<sub>3</sub>. The heating time was 1h from 24 °C to 120 °C, and the cooking time at the maximum temperature was 30min. After that, three-stage refining was performed in a refiner (ZSP-300) under the following conditions: 20-25 % consistency, and bar clearance of 0.50 mm, 0.30 mm, and 0.15 mm, respectively.

After the xylanase pretreatment, the eucalyptus chips were separated by filtration, and filtrate was collected for the analysis of reducing sugars liberated from the substrate eucalyptus xylan with the DNS method (Miller 1959). The xylose standard curve was made with a series of xylose concentration (birch wood xylose purchased from Sigma-Aldrich Inc.) ranging from 40 to 240 µg/ml.

### **Scanning Electronic Microscope Analysis**

Paper samples (about 40 g/m<sup>2</sup>) from control CTMP and Bio-CTMP were formed in a Buchner funnel. Control chips, xylanase-pretreated chips, and handsheets were all air-dried and observed with SEM (XL-30, Phillips Corp., Dutch).

### **Transmission Electron Microscopy**

Transverse, ultra-thin sections (100nm thickness) of fiber after the first-stage refining were prepared using an ultra microtome and a diamond knife. Samples were examined with TEM. Fixation, dehydration, and infiltration were performed according to the method reported by Petit-Conil (Petit-Conil et al. 2005). Ultra-thin sections (~70 nm) were cut with a diamond knife onto distilled water, and then they were collected onto uncoated, copper grids. In order to improve the contrast of the images, the grids were post-stained with 5 % aqueous uranyl acetate (10 minutes) and lead citrate (10 mins). The sections were examined in a JEOL 2011 (Scanning) Transmission Electron Microscopy operated at 200 kV. The images were taken with a Gatan digital camera.

### **AFM Observation**

AFM tests were performed with a Nanoscope IIIa scanning probe microscope, combined with a MultiMode AFM head and J type scanner. Dual height and phase images were captured with AFM tapping mode in an air environment using a commercial Si tetrahedral tip mounted on a rectangle-shaped cantilever (cantilever length: 160 µm, lever width: 50 µm, spring constant: 42 N/m, resonance frequency of about 300 kHz, tip radius of curvature: under 10 nm).

A suspension of pulp fibers (consistency under 1 %) was prepared in deionized water from CTMP and Bio-CTMP, respectively, then critical point drying (CPD) was performed for both samples. Prior to the CPD these samples were dehydrated with 30 %, 50 %, 70 %, 80 %, 90 %, and 100 % ethanol in sequence. After CPD at least fifteen fibers

on the glass slide with double-side adhesive tape were scanned for each sample, and the representative images were selected for analysis. The main axis of the fiber was parallel to the slow scan axis of the AFM. Scan size varied between 2  $\mu\text{m}$  and 5  $\mu\text{m}$  with a resolution of 512pix $\times$ 512pix. Free amplitude (A0) of about 25 nm and moderate tapping with a set-point amplitude ratio (rsp) between 0.4-0.6 were used, and the AFM was operated in air.

### Fiber Classification and FQA Analysis

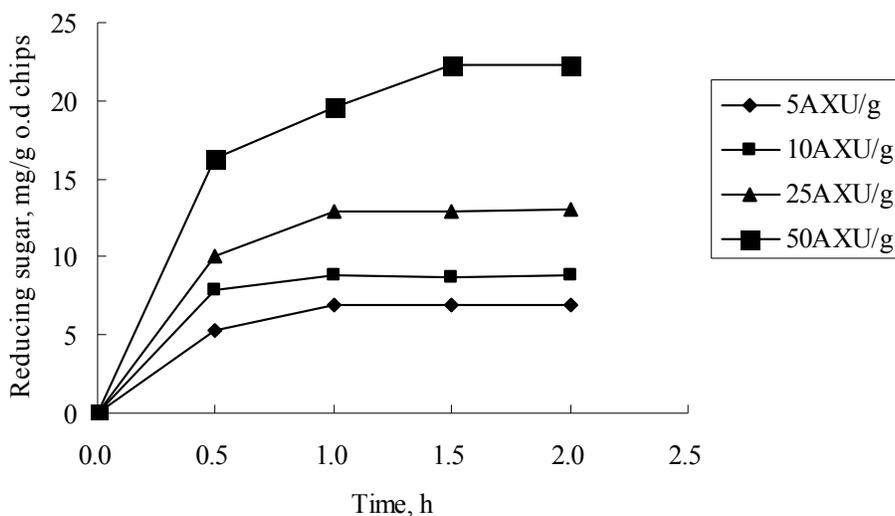
Suspensions prepared from CTMP and Bio-CTMP pulp were classified according to TAPPI Standard (T223) in order to obtain the percentage of each fraction.

Fiber length and fines content were measured by an FQA device from OpTest Equipment Inc.

## RESULTS AND DISCUSSION

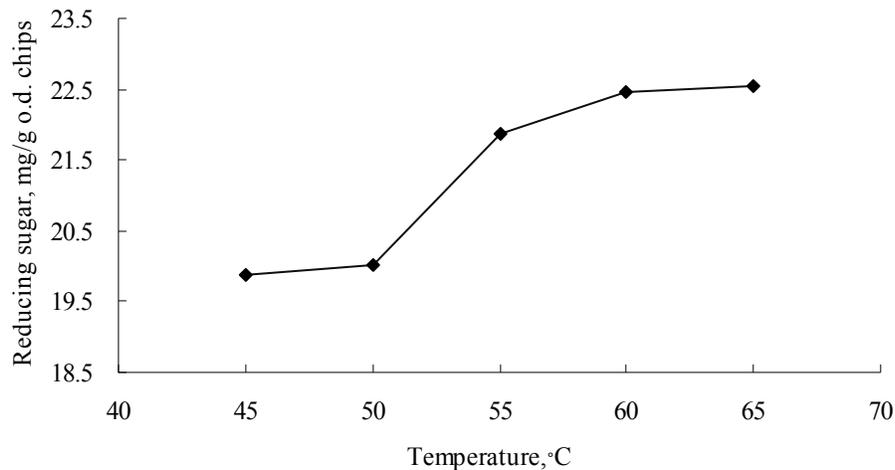
### Enzymatic Hydrolysis of Xylans in the Eucalyptus Chips

Figure 1 shows that xylanase pretreatment of wood chips produced reducing sugar, and with increasing xylanase dosage and treatment time, more reducing sugar was produced, which can be used to prove that xylanase catalysis has an effect on the wood chips. After 1.0-1.5 hrs the reducing sugar did not increase much with additional treatment time. This purified hemicellulase contains only endo-1, 4- $\beta$ -D xylanase activity and is free of cellulose activity. It can randomly attack the xylan polymer, and catalyzes the endohydrolysis of 1,4- $\beta$ -D-xylosidic linkages in xylans, yielding xylooligosaccharides. With the increase of enzyme dosage, solubilization of xylan increases, especially at 50 AXU/g dosage. Therefore, the subsequent wood chip treatment was conducted at an enzyme dosage 50 AXU/g for 1.5 hrs.

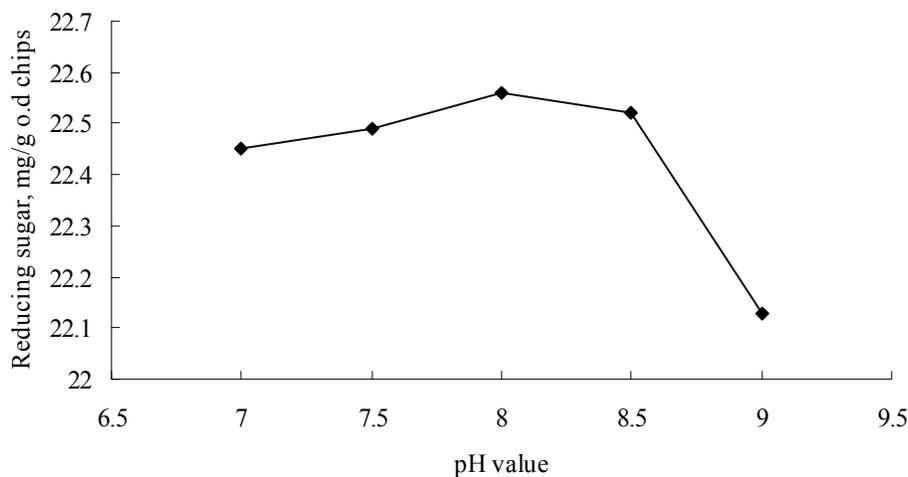


**Figure 1.** The effect of xylanase dosage and treatment time on the solubilization of xylan in the enzymatic pretreatment stage. pH 8, temperature 60 °C, eucalyptus wood chips

The influence of temperature on the reducing sugar was also investigated. As shown in Fig. 2, increasing hydrolysis temperature led to more reducing sugar produced; however, from 55 °C to 65 °C, the increase was slow. Therefore, 60 °C was chosen for the subsequent wood chip treatment. Figure 3 shows that the results were affected by pH value. This is attributed to the fact that the enzyme activity is dependent on the pH of the solution. According to the results, pH 8 was the optimum treatment condition. Therefore, the xylanase pretreatments for the following SEM, TEM, and AFM tests were conducted under the condition of enzyme dosage 50 AXU/g for 1.5 hrs at 60°C, pH 8.



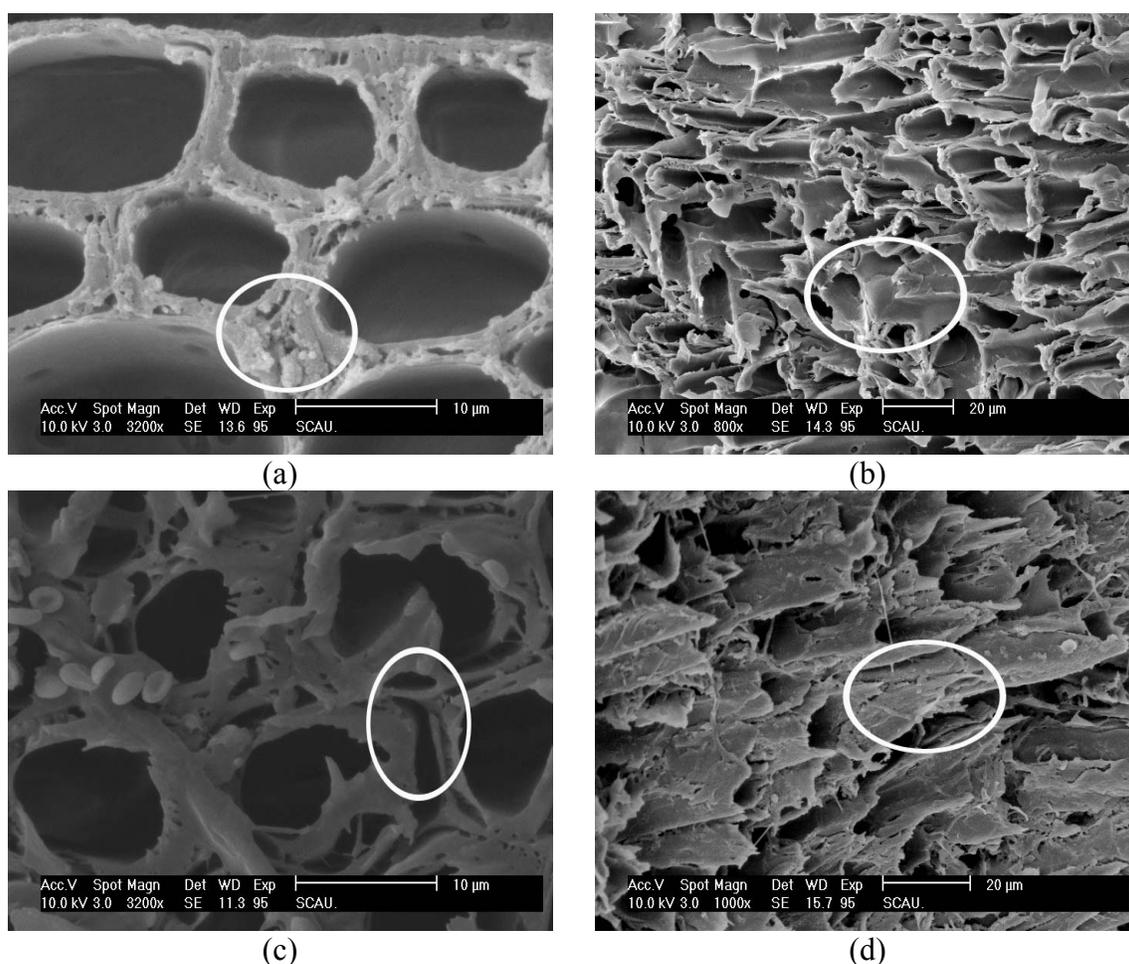
**Figure 2.** Solubilization of xylan in the enzymatic pretreatment stage as a function of temperature. 50AXU/g o.d.chip, 1.5hrs, pH 8, eucalyptus wood chips



**Figure 3.** Solubilization of xylan in the enzymatic pretreatment stage under the different pH, 50AXU/g o.d.chip, 1.5 hrs, 60 °C, eucalyptus wood chips

### Cross-Section of Wood Chips by SEM

Figure 4 shows the SEM images of eucalyptus wood chips without (a, b) and with (c, d) xylanase pretreatment. It can be seen that the xylanase hydrolysis reaction brought about modification of the wood chips. Loose structures and cracks were observed on the chip surfaces after xylanase hydrolysis compared with those on the control sample. This structural change was attributed to the hydrolysis of hemicellulose into oligosaccharides by means of xylanase catalysis. Therefore, xylanase hydrolysis may increase the accessibility of chemical liquid to chips and contribute to a preferable fiber separation during the subsequent refining process. It is also expected that the structural change of the wood chips by means of the enzyme treatment will reduce the energy consumption during refining.



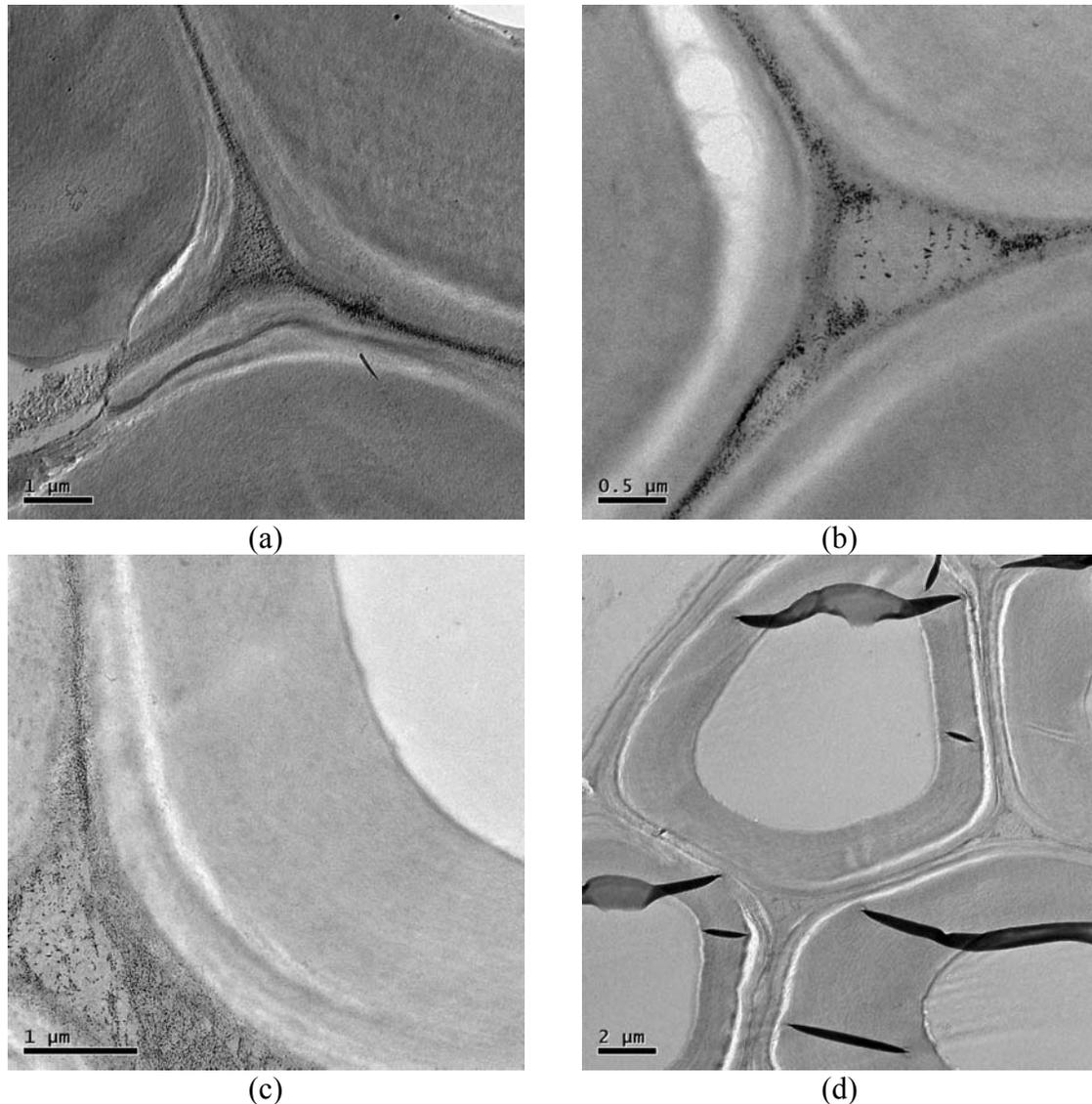
**Figure 4.** SEM images of wood chips: (a) and (b) without xylanase treatment. (c) and (d) with the xylanase treatment

### Cross-Sections of Fibers by TEM

Transmission electron microscopy (TEM) was used to observe the effects of xylanase pretreatment on fiber separation. It can be seen that after xylanase pretreatment, fiber bundles obtained after the first-refining stage of Bio-CTMP exhibited more

separation in the P+S1 layer than in the middle lamella region (Fig. 6). Since the S layer has more cellulose than the middle lamella, it is expected that the separation in the S layer will produce fibers with more cellulose on their surfaces, thus higher inter-fiber bonding ability.

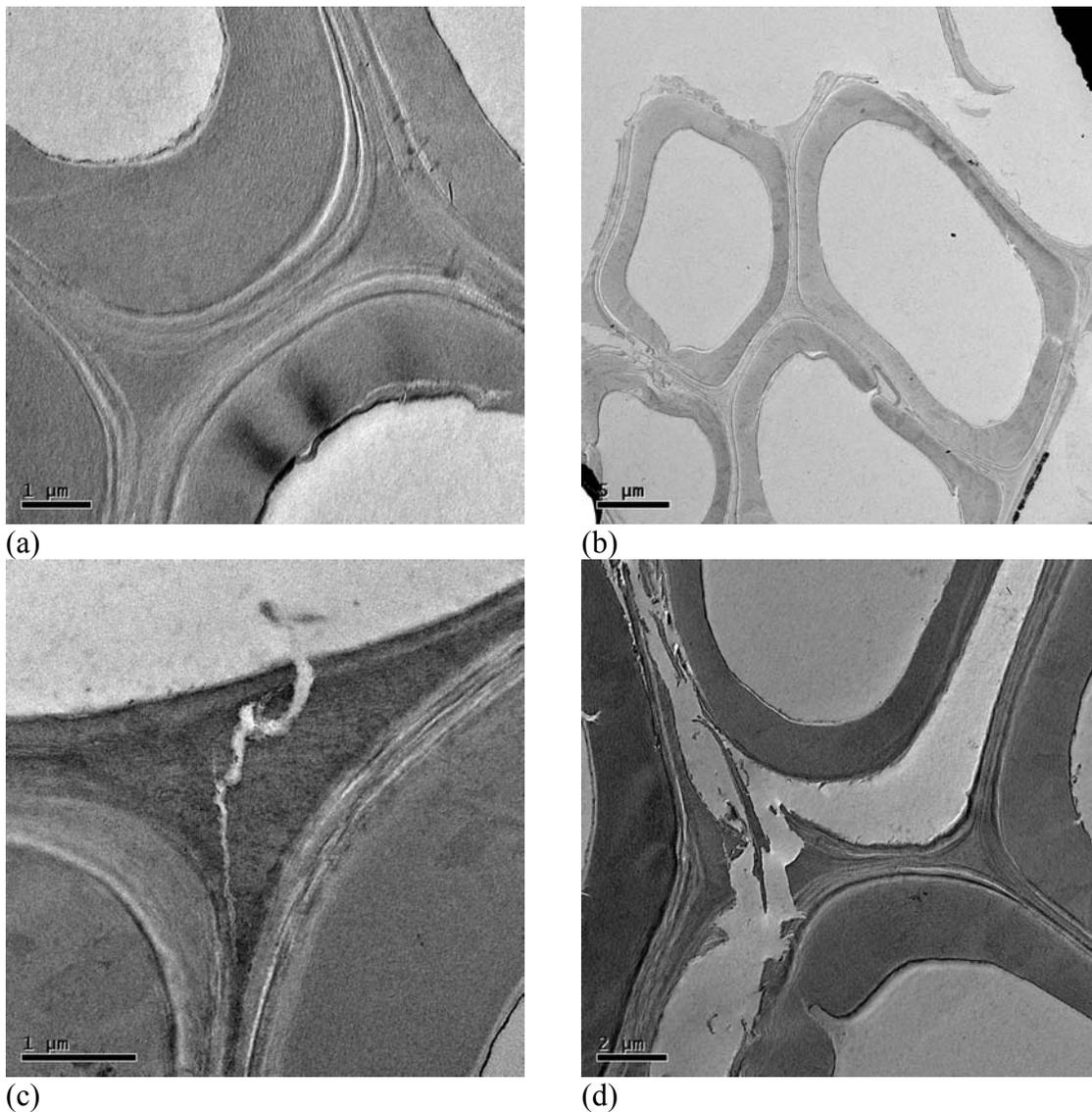
Several studies have been reported about fracture zones in softwood as affected by different mechanical processes, including refiner mechanical pulp (RMP), thermomechanical pulp (TMP), and CTMP (Kurdin 1979; Franzén et al.1986; Corson et al. 1989; Cisneros et al. 1995; Salmén et al. 1999; Hamad et al.1997; Kure 1997).



**Figure 5.** TEM images of cross section of a eucalyptus Bio-CTMP fiber bundle after first-stage refining

The position at which the fracture takes place between fibers depends on the structure of the various cell wall layers composing the fiber (Corson 1989). Meanwhile,

the refining temperature also affects the fracture region. In the case of softwood species, specifically with thin fiber walls, the weakest point of the wood structure is between the S1 and S2 layers (Kurdin 1979). Under the mechanical shear forces, fibers generally will separate along this line. During the RMP refining process the fracture occurs in an uncontrollable manner due to stiff lignin (Salmén et al. 1999). For the TMP process, which is carried out under higher temperature and pressure conditions, the fracture zone is moved further outward to the secondary S1 and the primary wall of the fiber (Salmén et al. 1999). When a chemical pretreatment is carried out, softened lignin will make fiber separation easier, and the weakest point in the wood structure will shift from the S1



**Figure 6.** TEM images of cross section of a eucalyptus CTMP fiber bundle after first-stage refining

and S2 to the middle lamella (Kurdin 1979). As a result, most of the fibers separated from the middle lamella are covered with a thin layer of lignin, pectin, and a small quantity of hemicellulose, which come from the middle lamella. Recent studies have shown that remnants of the lignin-rich middle lamella are exhibited on the fiber surface, not only for softwood, but also for hardwood (Börås et al. 1999; Koljonen et al. 2003; Li et al. 2006). This indicates that in the traditional CTMP, fiber surfaces are covered by a greater quantity of lignin and extractives. Such hydrophobic material is expected to hinder inter-fiber bonding (Shao et al. 2006).

In this study, in which wood chips were treated by xylanase, the degradation of some xylan in the secondary wall would be expected to create some weak points in the fiber wall region. Therefore, in the subsequent refining processes, fiber separation will occur more easily in the secondary wall region. TEM images further confirmed this modification and its effects. In comparison, for the conventional CTMP process, almost all separation occurred in the middle lamella and very few fractures opened up between the primary wall and the S1 or the S1 and the S2 layers, as illustrated in Fig. 7.

### Fiber Surface Topography by AFM

Figure 7 shows the AFM image of the fiber surface. It can be seen that the topographical structure of the Bio-CTMP and CTMP fibers was quite different. On the surface of CTMP fibers (Fig. 8 a, b, c) irregular particles or granules are seen. In contrast, on the surface of Bio-CTMP fibers (Fig. 8 d, e, f), the fibrillar structure can be clearly seen with much less granules. Furthermore, individual fibril diameters in the range of 10-30 nm as shown in Fig. 8 (b and c), which is also in agreement with Fardim et al. (2005). Granules and grains were considered to consist of lignin, extractives, or their complex composites in several studies (Börås et al. 1999; Koljonen et al. 2003; Li et al. 2006). Therefore, less lignin and extractives on the Bio-CTMP fiber surface supports the idea that the location of most fiber separations is not in middle lamella. In addition, randomly oriented microfibrillar network structures typical for the primary cell wall were observed in Fig. 8 (e), which is the feature of primary wall, and more parallel microfibrils were observed in Fig. 8 (d and f), which features the secondary wall. Therefore, the results connected with the analysis of SEM and TEM indicated that more separation occurred in the primary and secondary walls after xylanase pretreatment.

### Fiber Classification and Fiber Quality Analysis

Table 1 lists the fiber classification results. In comparison with CTMP, Bio-CTMP had higher R14, R30, and R50 fractions. In particular, the long fiber fraction (R14) was increased by 55 %, from 0.49 % to 0.76 %. In the mean time, the fines fraction (P200) was slightly decreased. The results indicated that due to xylanase treatment, the fiber wall structure was loosened, in particular in the secondary wall, thus, in the subsequent refining process the mechanical forces could more easily split fibers, causing less damage to fiber length. This conclusion also was supported by results of the FQA test. As shown in Table 2, the length of the fines of Bio-CTMP was larger than that of the CTMP, and at the same time, Bio-CTMP had less fines. This further confirms that due to xylanase treatment of the wood chips, fiber separation in the subsequent refining took

place along the weak area of the fiber wall. Therefore, the ruptures at transverse section of fibers decreased, and long fibers were preserved in Bio-CTMP.

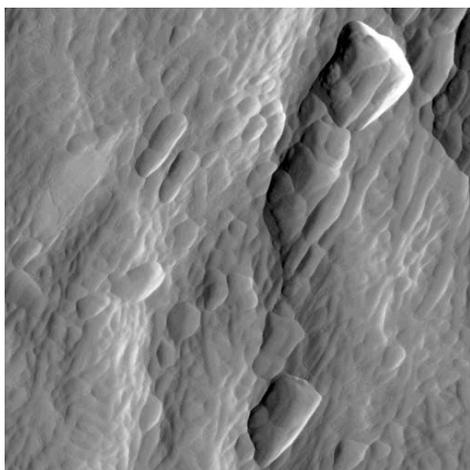
## CONCLUSION

Pretreatment of wood chips with xylanase prior to CTMP refining can hydrolyze a certain amount of xylan in the wood chip, in particular in the secondary wall of the fibers. Due to the degradation and dissolution of some xylan in the fiber wall, the fiber wall structure was loosened, which may benefit the chemical penetration into the fiber wall in the chemical pretreatment. The enzymatic treatment also loosens the structure of the fiber wall, thus opening some venues or generating some weak points for the subsequent refining to split or separate the fibers.

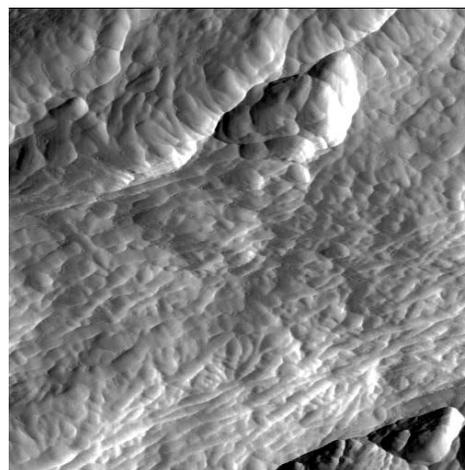
TEM images showed that the rupture lines mostly occurred in the fiber secondary wall region after xylanase treatment. In comparison, the rupture occurred mostly in the middle lamella region for control fibers.

Fiber surface observation with AFM showed that xylanase treatment, more fibril structure remained on the fiber surface, which becomes dominated by the primary and secondary wall structures of the fibers. In the case of control fiber samples, more granules or particles could be seen on the fiber surface, featuring the remnants of lignin-rich middle lamella materials on the fiber surface.

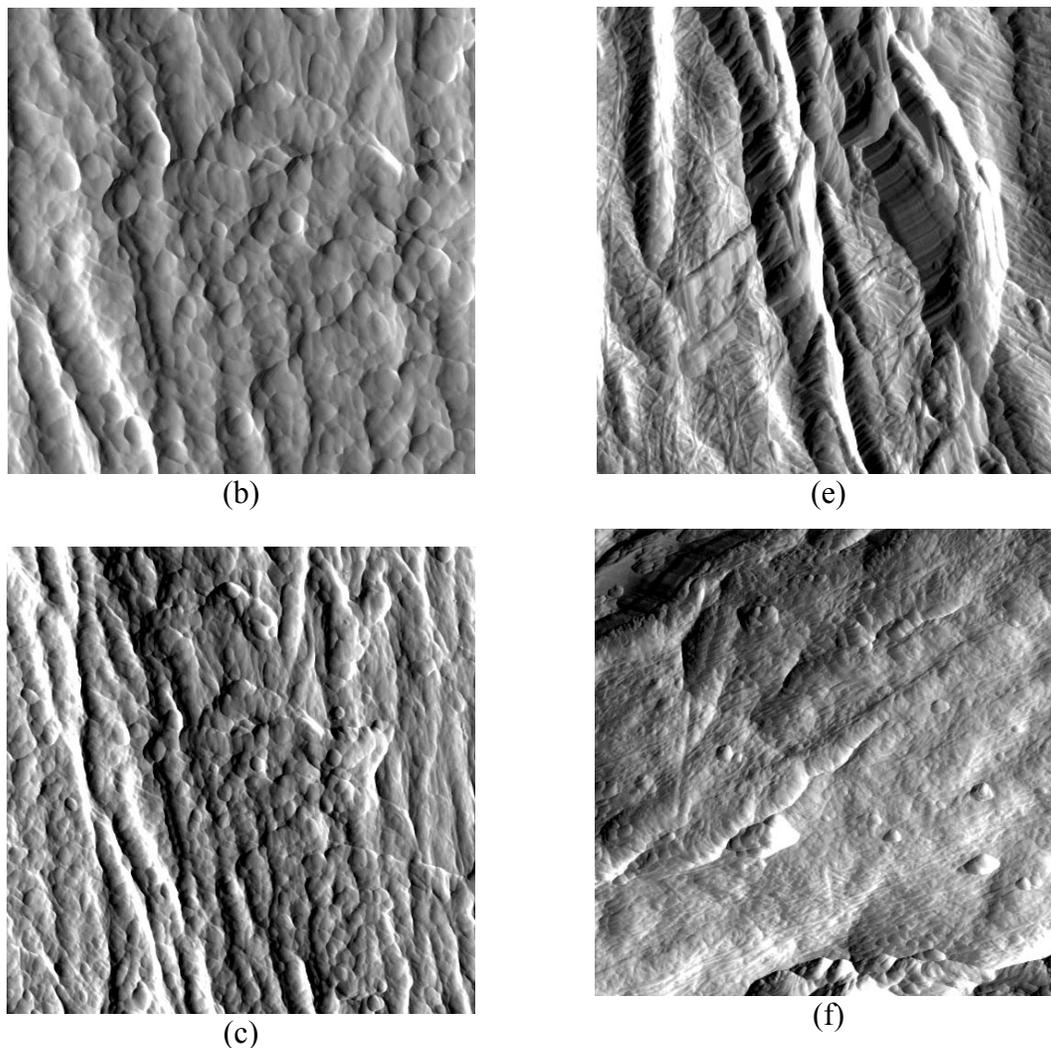
Fiber length and fiber classification results showed that with xylanase treatment, fiber length increased and the fines percentage was reduced. This supports the observation with TEM and AFM, which is that xylanase treatment loosens the fiber structure so that less random cleavage occurred in the subsequent refining. More separation in fiber secondary wall will generate fibers with less lignin on the surface, which is expected to benefit inter-fiber bonding.



(a)



(d)



**Figure 7.** AFM amplitude images of samples from CTMP (a, b, and c) and Bio-CTMP fibers (d, e, and f). Image size are as follows: (a)(d)  $2\mu\text{m}\times 2\mu\text{m}$ , (b)(e)  $3\mu\text{m}\times 3\mu\text{m}$ , (c)(f)  $5\mu\text{m}\times 5\mu\text{m}$

**Table 1.** Bauer-McNett Classification of CTMP and Bio-CTMP Pulp Fibers

Tyler series	R14	R30	R50	R100	R200	P200
CTMP/%	0.49	6.68	25.15	37.91	6.77	23.00
Bio-CTMP/%	0.76	6.71	26.41	37.69	6.07	22.37

**Table 2.** Fiber Length and Fines Percentage

	Fines* (%)		Mean length (mm)	
	Arithmetic	Length weighted	Arithmetic	Length weighted
CTMP	27.21	7.25	0.459	0.653
Bio-CTMP	23.64	5.85	0.494	0.690

\*Fines: length less than 0.2 mm

## ACKNOWLEDGMENTS

Financial support from the Natural Sciences and Engineering Research Council of Canada (NSERC), Canadian Foundation for Innovation (CFI), Atlantic Innovation Fund (AIF), Canada, and New Brunswick Innovation Fund (NBIF) to this study, is gratefully acknowledged. We also acknowledge the Novo-Nordisk Corporation for providing xylanase as well as financial Natural Science Foundation of China (50776035, U0733001), Foundation of Scientific Research for Universities (20070561038) and Initiative Group Research Project (IRT0552) from the Ministry of Education of China, National High Technology Project (863 project) (2007AA05Z408) and National Key R&D Program (2007BAD34B01) from the Ministry of Science and Technology of China.

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Article submitted: April 17, 2008; Peer review completed: June 3, 2008; Revised version received and accepted: July 6, 2008; Published: July 8, 2008.