EFFECT OF PRIOR MECHANICAL REFINING ON BIOBLEACHING OF WHEAT STRAW PULP WITH LACCASE /XYLANASE TREATMENT

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Wheat straw pulp was mechanochemically processed in a PFI mill in order to improve the effect of laccase/xylanase system (LXS) treatment before bleaching. The delignification and bleachability of the prepared pulp were investigated. The delignification of the prepared pulp could be enhanced with the mechanochemical processing (refining) and LXS treatment. The delignification was increased by 29.8% with refining 7000 revolutions and 5 IU/g enzyme dosage. The LXS treatment after the mechanochemical process could save 28.6% effective usage of chlorine in the subsequent hypochlorite bleaching process, compared with the traditional bio-bleaching. The crystallinity of cellulose was increased by the co-treatment with mechanochemistry and LXS treatment. This result was further supported by the observations from SEM. This co-treatment with mechanochemistry and bio-treatment enhanced the delignification and bleachability of pulp.

Keywords: Mechanochemistry; Laccase/xylanase system; Refining; Biobleaching

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INTRODUCTION

China possesses a large variety of non-wood-fiber raw materials (Zhan *et al.* 2008). The utilization of non-wood fiber species is an effective way to solve the shortage of raw fiber materials in the paper industry. At present, chlorine and hypochlorite are still being used as pulp bleaching agents to produce bleached pulp in some of the non-wood fiber mills. The bleaching effluent contains large quantities of organic chloride, resulting in serious pollution, biological toxicity, mutagenesis, and carcinogens. It is known that bio-bleaching technology can reduce chemical agents in the subsequent bleaching process, decrease environment pollution, and improve pulp properties (Jacobs *et al.* 1998; Senior and Hamilton 1992; Clark *et al.* 1991; You *et al.* 2003). Delignification with laccase/mediator system (LMS) has been widely studied as a pre-bleaching stage. However, the requirement for an expensive mediator precludes it from practical application. The previous research of our team had demonstrated that laccase/xylanase system (LXS) from white-rot fungus (*Lentinus lepideus*) had the same ability to delignify as the laccase/mediator system (LMS) (You *et al.* 2008, 2009a,b). But the combined approach involving both mechanical refining and LXS treatment has not been investigated.

Conventionally, the PFI mill is used for refining bleached pulp in the laboratory. The fiber flexibility and handsheet performance will be improved after PFI refining. In the process, PFI milling fibrillates the fibers and creates more fines. The principle of PFI milling is similar to the powder mechanochemistry process. The definition of mechanochemistry is based on theoretical considerations of Ostwald (Nobel Prize 1909) concerning the relationship between chemical and mechanical energy. The investigations continued, as summarized in a series of review papers (Peters 1962; Baláž 2008). Mechanical force can be used to steer chemical reactions along pathways that are unattainable by conventional approaches (Hickenboth *et al.* 2007). The related devices include high-energy mills, such as mixing mills, vibration mills, colloid mills, centrifugal grinding, *etc*. (Baláž 2008; Yuan and Sun 2009). This method has attractive application in high-tech fields (Hickenboth *et al.* 2007; Beyer and Clausen-Schaumann 2005; Chen *et al.* 2001; Yuan and Sun 2009).

The combination of mechanical refining with enzyme treatment has been motivated to improve delignification and bleachability in subsequent bleaching stages. The feasibility of subjecting different raw materials to enzyme treatment were examined in our previous studies, which considered wheat straw pulp treated with recombinant xylanase (You *et al.* 2010) and the effect of refining on delignification with LXS treatment of masson pine (*Pinus massoniana*) (Lian *et al.* 2011). The results indicated that a substantial delignification without severe yield loss can be achieved by moderate refining prior to enzyme treatment. In this paper, the effects of mechanochemical processing (refining) on the surface structure and properties of the wheat straw pulp were investigated in detail. The effects of the process combined with laccase/xylanase system (LXS) on the bleachability of the pulp is also considered.

EXPERIMENTAL

Materials and Methods

Wheat straw

Wheat straw collected from Nanjing, China was air-dried and stored in plastic bags for moisture balance.

Original pulp

The wheat straw was cooked with a soda-AQ method using a 14 liter rotating batch digester (model: ZQL-I) under constant cooking conditions, such as active alkali charge 11% as Na_2O based on wheat straw and maximum cooking temperature of 160˚C.

Anthraquinone (AQ) was used at a level of 0.03% on OD wheat straw, for which the ratio of cooking liquor to straw was 6:1 (v/w). Wheat straw was first impregnated with the cooking liquor at ambient temperature for 30 min. Then the temperature was raised to cooking temperature in 2 h and maintained for 10 min.

The kappa number of original pulp was 14.5, the brightness was 35.8 % ISO, and the cellulose viscosity was 853.0 mL/g.

Laccase/xylanase

Laccase was directly produced by controlling the culture conditions of white-rot fungus (*Lentinus lepideus*). This source provides a laccase/xylanase system (LXS). The enzyme activities of laccase and xylanase in LXS were 172.80 IU/mL and 1.20 IU/mL, respectively, and the cellulase activity was low (0.06 IU/mL). The activities of lignin peroxidases or Mn-peroxidases were not detected in the system. The LXS had the same ability to delignify as a laccase/ mediator system (LMS) (You *et al.* 2008).

Refining

The pulp (20 g, O.D.) was refined in a PFI mill (model PL11, Xianyang Electromechanical Company, Shanxi). Refining conditions were: pulp consistency of 10%, refining pressure of 3.34 N/mm, and a gap between the working surfaces of 0.2 mm. Beating degree of refined pulp was tested by a Schopper-Riegler tester (Machine Factory of the Shaanxi University of Science and Technology) (ISO 5267-1:1999).

Enzyme treatment

Based on previous research (You *et al.* 2008), 20 grams of oven-dried pulps were prepared at a pulp consistency of 5%, mixed with LXS in acetate acid-sodium acetate buffer solution (0.1 M, pH 4.2), and were placed into sealed bags. Then, they were incubated at 45 ^{o}C for 1.5 h with agitation using a rotator. The ratios of LXS to pulp were 3, 6, and 9 IU/g pulp (o.d). After enzyme treatment, the pulp was filtered on a Buchner funnel, washed with distilled water, and then dispersed to determine pulp properties. The control pulp was treated under the same conditions without adding any enzyme, as shown in Table 1.

Pulp bleaching procedure

Pulps were disintegrated with 10000 revolutions at room temperature in a disintegrator before bleaching. The pulp suspensions were filtered on a Buchner funnel and then placed in double-layer polyester bags, mixed with the bleaching chemicals to a final consistency of 5% at temperature of 38 ^{o}C, and then held for 2 h in a thermostatic water-bath. After bleaching, pulps were filtered and washed with distilled water. Sample codes are presented in Table 1.

Table 1. Pulp Treatment

NO	Sample code	Conditions
1	Original	Pulp without mechanical pulping and no treatment
2	Refining	Pulp with prior mechanical pulping and no treatment
3	Control	Pulp with prior mechanical pulping and treatment(no enzymatic)
4	LXS	Pulp without mechanical pulping + enzymatic treatment
5	Refining + LXS	Pulp with prior mechanical pulping + enzymatic treatment
6	H	Pulp with prior mechanical pulping + chemical bleaching
7	LXS+H	Pulp without mechanical pulping + enzymatic + chemical bleaching
8	Refining + LXS+H	Pulp with prior mechanical pulping + enzymatic + chemical bleaching

Kappa number determination

The kappa number of pulp was measured after alkali extraction. The alkali extraction was carried out under the following conditions: pulp consistency 10%, alkali dosage 1%, temperature 70 ^{o}C, and time 1.5 h. The alkali extracted pulp was washed, and the kappa number was determined according to T236 cm-85 (Kappa number of pulp).

Determination of pulp properties

The yield, brightness, and the strength properties of handsheets were measured by related TAPPI test methods. Viscosity of pulp was analyzed according to ISO 5351/1.

Determination of cellulose crystallinity

A small amount of control and enzyme-treated pulp were made into tablets, and then crystallinity was determined by use of a model DX-2000 X-ray diffractometer (Dandong Fangyuan Instrument Limited Company, Liaoning). Detecting conditions were: tube pressure 30KV, tube currency 20 mA, Cu-target. The following formula was used to calculate relative crystallinity,

$$\text{Relative crystallinity} = (I_{002}-I_{\mathrm{AM}})/I_{002} \tag{1}$$

where I_{002} is the maximum intensity of the diffraction angle of 002 crystal lattice, and I_{AM} is the scattering intensity of amorphous background diffraction when $2\theta = 18°$.

SEM observation

Images of the untreated and treated pulps were taken with a Fei Quanta 200 scanning electron microscopy (SEM), provided by Philips.

RESULTS AND DISCUSSION

Effect of Refining on Delignification Ability of LXS Treatment

The previous research demonstrated that the laccase/xylanase system (LXS) from white-rot fungus (*Lentinus lepideus*) had the same ability to delignify as a laccase/ mediator system (LMS) (You *et al.* 2008). In order to improve delignification, as well as to improve the bleachability in subsequent bleaching stages, the pulps were refined in a PFI mill, and then they were treated with LXS. Table 1 shows the effect of refining revolutions on delignification with LXS Treatment.

Table 2. Effect of Refining Revolutions on Delignification with LXS Treatment

PFI revolutions	Beating degree (°SR)	Kappa number	Delignification (%)	Brightness (% ISO)	Viscosity (mL/g)	Yield (%)
0	23	10.8	25.5	38.3	959	95.2
7000	30	9.7	33.1	37.9	978	94.4
14000	37.2	9.5	34.5	37.4	982	93.6
28000	43.7	9.4	35.2	37.1	1017	92.6

As illustrated in Table 2, the kappa number and yield of the treated pulp with LXS system decreased with increasing refining revolutions. The kappa number of pulp was reduced from 10.8 to 9.7, and the extent of delignification was increased from 25.5% to 33.1% as the refining revolutions were increased from zero to 7000. This effect is attributed to the repeated changes in shape of the cell wall in the course of refining. The primary wall (P) and the S_1 layer were exploded, and the fiber was cut to some extent. Because P and S_1 layers contain a higher proportion of lignin, this lignin was exposed outside after refining, which helps to promote the reaction between enzyme and lignin, resulting in a higher degree of delignification and yield loss. In addition, the higher refining revolutions could increase the specific surface area, promoting the reaction of laccase and lignin, and the degree of delignification by LXS was higher. As the refining revolutions was increased to 28000 revolutions, the kappa number decreased to 9.4. This means that extending the refining revolutions did not have an appreciable effect on delignification, whereas the reduction in pulp yield was about 92.6%, and there was a 2% loss versus 7000 revolutions of refining. Obviously a balance must be accepted for a reduction in kappa number, delignification, and acceptable yield losses with the refining treatments.

Contrary to kappa number and pulp yield, refining appeared to have little effect on viscosity and brightness of the pulp, for laccase can oxidize residual lignin and hence the absorption coefficient of pulp increased and brightness decreased. These effects appeared to offset the increase due to the lowered lignin content. Furthermore, excess refining could consume more energy and contribute to washing problems of the pulp in subsequent bleaching. Thus, a refining level of 7000 revolutions was selected for subsequent work.

Effect of Enzyme Dosage and Refining on Delignification

Figure 1 shows the effect of different LXS enzyme dosage on kappa number of pulp. In both cases, no matter whether there was refining treatment, the kappa number decreased rapidly at low enzyme dosage.

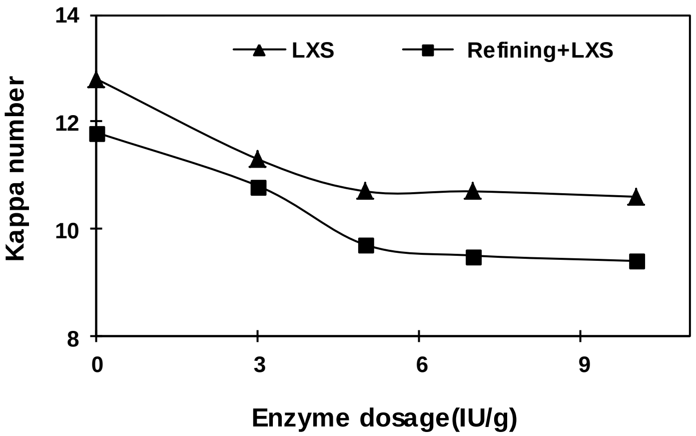


Fig. 1. Effect of LXS dosage on kappa number

Kappa number exhibited no further obvious differences in response to increased enzyme dosages higher than 5 IU/g. Compared with the original pulp, the kappa number decrease for LXS pulp was 9.4%, while it was 33.1% in the case of Refining +LXS pulp.

Figure 2 also clearly demonstrates that delignification was greatly improved with refining at the same enzyme dosage. For refined pulp, the delignification already reached a higher value even at the LXS dosage of 5 IU/g; however, there was little improvement in lignin removal by further increasing enzyme dosage.

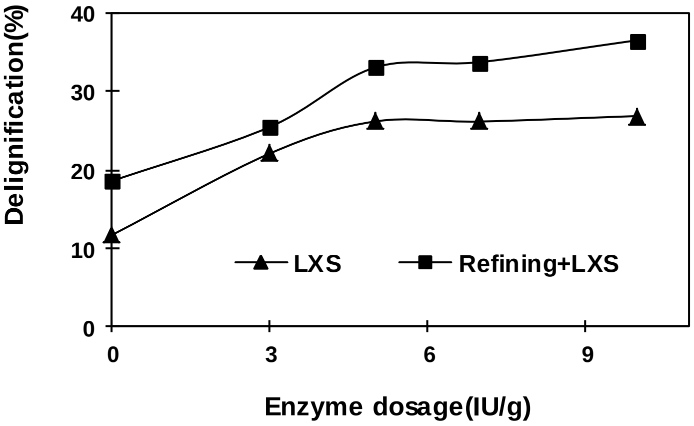


Fig. 2. Effect of enzyme dosage on delignification

The effects of enzyme dosage and refining on pulps yield are shown in Fig. 3. A substantial reduction of the yield of pulp, from 96.6% to 94.8%, was obtained with the LXS without refining. Correspondingly, a similar yield was obtained for the pulp refining before LXS. Also, there was a small yield loss, about 2.5%, from 95.9% to 93.4%. This was probably due to the fact that fines, created during refining, were lost. Thus, refined pulp had lower yield than the pulp without refining at all enzyme dosage levels.

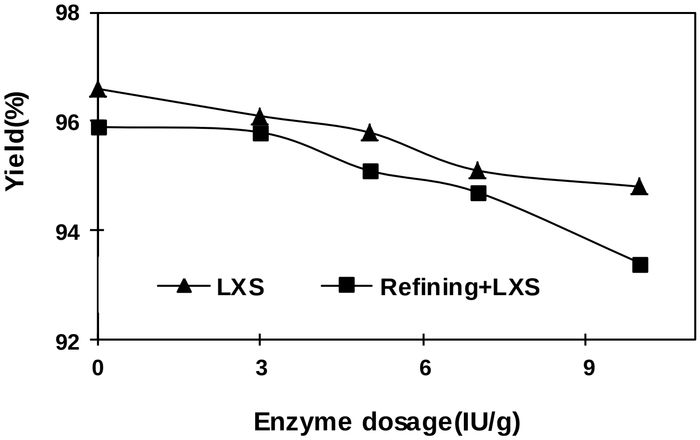


Fig. 3. Effect of enzyme dosage on yield

Table 3. Effect of Enzyme Dosage on Brightness and Viscosity

Enzyme dosage (IU/g)	Brightness (% ISO)		Viscosity (mL/g)	
	LXS	Refining + LXS	LXS	Refining + LXS
0	35.9	36.4	986	1008
3	36.1	36.3	990	1003
5	36.2	36.2	994	999
7	35.5	36.2	996	993
10	35.3	36.1	969	977

A comparison of the brightness and the viscosity of the pulps is presented in Table 3. There were no significant differences in brightness and viscosity between the two kinds of pulp (LXS and Refining + LXS), with a total of ten samples.

Effect of Treatment on Bleachability of Pulp

The purpose of bleaching is to remove the residual lignin in pulp with an acceptable yield. The utilization of chlorine bleaching agent in the small and medium pulp mills results in serious water pollution. The introduction of enzymatic pretreatment in such a bleaching process could reduce the consumption of effective chlorine. In this study, the wheat straw pulp was first refined for 7000 revolutions to obtain an optimal beating degree of 30°SR. The pulp was sequentially treated at the optimized condition of LXS and then bleached with hypochlorite. The brightness and the physical properties of handsheets were carried out to evaluate the effects of refining on pulp bleachability.

Table 4. Comparison of Different Pretreatment on Pulp Brightness and Physical Properties

Bleaching sequence	Effective usage of chlorine (%)	Brightness (% ISO)	Yield (%)	Breaking length (km)	Bursting index (kPa·m^2/g)	Tearing index (mN·m^2/g)
H[1]	7	72.6	90.6	3.7	2.1	6.3
	5	72.4	92.0	3.9	2.2	6.4
	3.5	68.1	93.8	4.0	2.3	6.5
LXS[2] + H	7	76.3	89.6	4.5	2.9	6.4
	5	75.4	91.2	4.6	3.0	6.5
	3.5	73.2	92.6	4.7	3.1	6.6
Refining[3] + LXS + H	7	78.2	89.2	4.6	3.0	6.5
	5	77.2	90.3	4.6	3.0	6.5
	3.5	74.2	91.2	4.8	3.1	6.6
	3	73.2	92.6	4.8	3.2	6.7
	2.5	72.3	92.8	4.9	3.3	6.9

Notes: 1. H (hypochlorite) bleaching conditions: initial pH 10.5, pulp consistency 5%, temperature 38 °C, time 2 h; 2. LXS treatment conditions: pH 4.2, temperature 45℃, pulp consistency 5%, time 1.5 h, enzyme dosage of 5 IU/g; 3. Refining conditions: pulp consistency 10%, 7000 revolutions

Table 4 presented results for the bleachability of pulp subjected to the enzyme treatment and refining. Compared with the control pulp, the brightness of pulp increased by 3.7% and 5.6% ISO, respectively, with (LXS + H) bleaching and the (Refining + LXS + H) at an effective usage of chlorine 7%. Also, 50% and 64.3% effective usage of chlorine was saved at the same brightness of control pulp (72.7% ISO). Furthermore, compared with traditional bio-bleaching, an effective usage of 28.6% chlorine could be saved at a similar yield, and the strength of pulp was also enhanced. This means that it may significantly enhance delignification and swelling of fiber by co-treatment with refining and enzymatic treatment.

All of the results demonstrated that the environment pollution of the bleaching waste water could be reduced by refining before pretreatment with enzyme.

Effect of Pretreatment on Crystallinity of Cellulose

The X-ray diffraction method was used to determine the crystallinity of cellulose, and the calculated crystallinity of samples are given in Table 5.

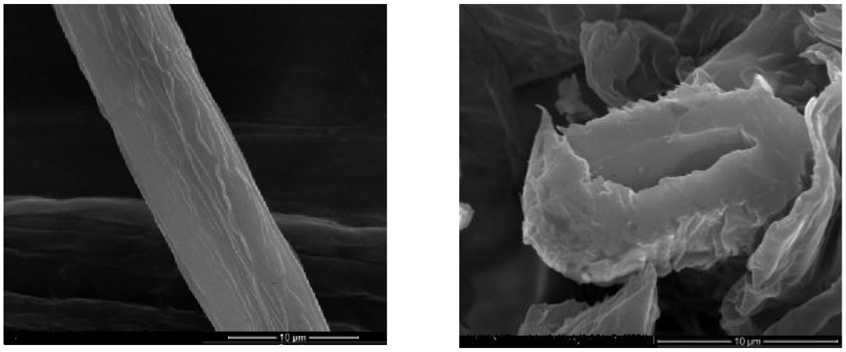
Table 5. Crystallinity of the Treated Pulp

Pulp	Crystallinity %
Original	60.0
LXS	62.3
Refining	57.4
Refining+ LXS	61.9

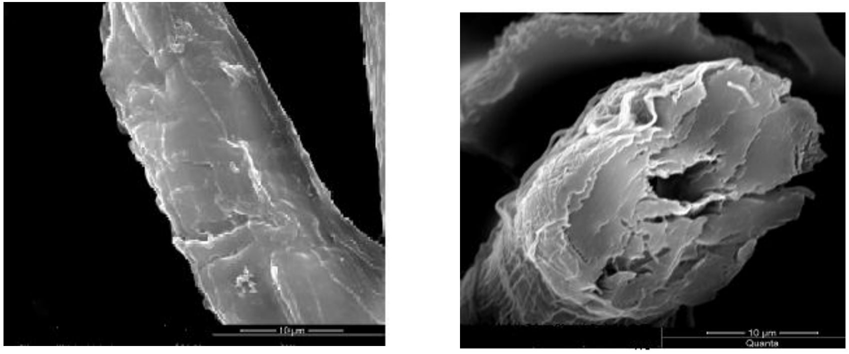
The original pulp had a crystallinity index of 60.0%, and after refining, it had a lower crystallinity index of 57.4%, due presumably to the creation of fines during the refining. This was consistent with the work of Yang *et al.* (2000). The pulp treated with only LXS (no refining) increased the crystallinity index to 62.3%. With refining + LXS, the crystallinity was 61.9%. The crystallinity was raised to the same level as the LXS pulp. This could be due to two reasons. Firstly, refining could disperse the fibers and also liberate some fines. This could cause amorphous areas to be more exposed, hence decreasing the relative proportion of crystalline cellulose at the surface. Secondly, the increase in crystallinity was likely due to a removal of fines, xylan, and lignin of the refined pulp during the LXS treatment; this idea was supported by the absorbance value and the reducing sugar content in the filtrate (You *et al.* 2008). This mechanism also explains why the pulp strength could be enhanced after LXS and refining treatment.

SEM of Fiber Morphology with Treated Pulp

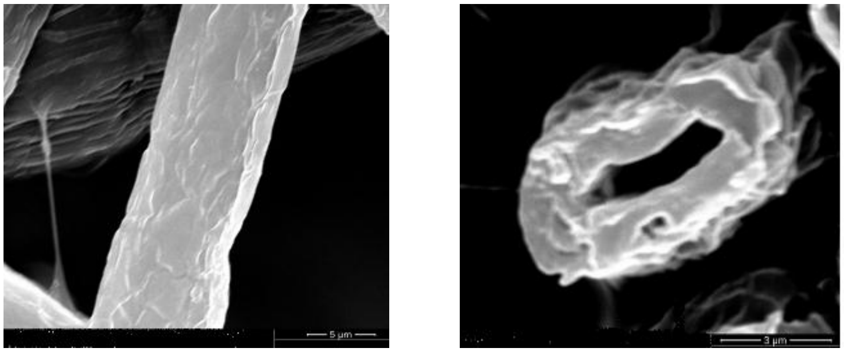
SEM images were used to help observe the changes of the microstructure of wheat straw pulps affected with co-action of refining and LXS treatment. Figure 4 shows the SEM images of the surface and cross-section of pulp with co-treatment of refining and LXS treatment. As can be clearly seen, without refining, the fiber surface and cross-section of original pulp were smooth and solid-like (4a), whereas the enzyme-treated pulp had a rough fiber surface and, in the cross section of the cell wall, tiny holes and cracks, presumably resulting from dissolution of lignin and xylan by LXS (4b).



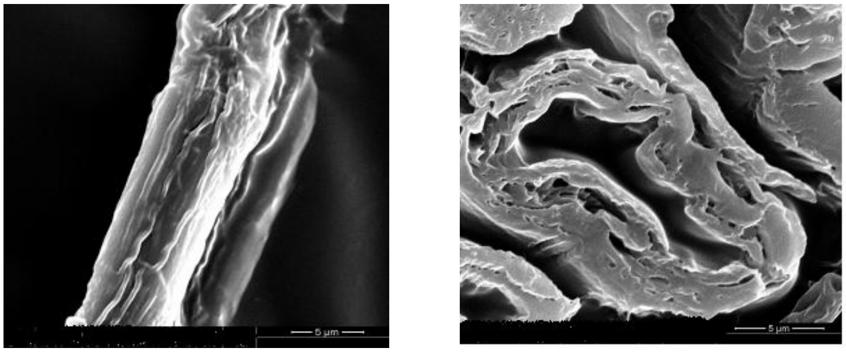
(4a) Original ----surface and cross section of fiber



(4b) LXS ---surface and cross section of fiber



(4c) Refining ---surface and cross section of fiber



(4d) Refining + LXS ---surface and cross section of fiber

Fig. 4. SEM images of the treated and untreated pulp

Refining of pulp clearly caused delamination and swelling of fiber, as could be seen by comparing images (4c) with images (4a). The refining increased accessibility to LXS treatment and resulted in further delamination and swelling of fiber as shown in Fig. (4d). These observations supported the findings that more lignin and xylan were dissolved by LXS treatment after refining. The increased delamination and swelling as a result of LXS treatment after refining may also enhance permeability of bleaching chemicals and dissolved lignin in subsequent bleaching operation, resulting probably in a reduced bleaching chemical charges and effluent pollution load. It was shown that refining contributed to an improvement in enzyme treatment. The work also demonstrated the mechanochemical effect, by use of SEM imaging.

CONCLUSIONS

1. Wheat straw pulp was refined in a PFI mill before pretreatment with a laccase/xylanase system (LXS). Compared with LXS pulp, the properly refined pulp before LXS treatment could increase delignification by 29.8%.
2. Compared with traditional bio-bleaching, the co-treatment of refining and LXS can significantly improve its bleachability in subsequent bleaching stages. This approach could save 28.6% of the effective usage of chlorine, and the strength of pulp was also enhanced.
3. The increase of crystallinity of cellulose showed that refining contributed to an improvement in enzyme treatment. The observations from SEM supported the results that more lignin and xylan were dissolved by co-treatment of refining and LXS.

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