Biomechanical Pulping of Poplar with Crude Enzyme Secreted from *Trametes* sp. lg-9

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Crude enzyme secreted from *Trametes* sp. lg-9 was applied in bio-mechanical pulping of poplar. The Canadian Standard Freeness (CSF) of the pulp pretreated with crude enzyme by a charge of 8 IU g^{-1} pulp was 215 mL. However, the CSF of untreated pulp was 235 mL at the same refining revolutions of 15000. Also, the energy consumption during refining was significantly lowered. The brightness of enzyme-pretreated pulp was increased by 2%, and the light absorption coefficient and opacity were decreased slightly. The effect of H_2O_2 (P) and CH_3COOOH-H_2O_2 (PaP) bleaching were reinforced, and the brightness of pulp was further enhanced when the dosage of crude enzyme was great than 8 IU g^{-1} pulp. However, the content of fines was decreased, and the lowest value was 6.99% when the dosage of crude enzyme was 8 IU g^{-1} pulp. The results of this work will be valuable for future possible commercialization.

Keywords: Crude enzyme; Refining; Mechanical property; Brightness

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INTRODUCTION

Mechanical pulp accounts for about 25% of wood pulp production in the world. Mechanical pulping is an effective way to utilize wood fiber resources because of its high yield (Reddy and Yang 2005). However, mechanical pulping requires high energy consumption, and the produced paper possesses poor mechanical strength and brightness compared with chemical pulp. Therefore, the application of mechanical pulping is restricted (Hatton and Johal 1994; Mohlin 1997; Alami *et al*. 1997). In order to expand the application and enhance the properties of mechanical pulp, bio-mechanical pulping was developed. Fast-growing white-rot fungi have been widely used for biological pretreatment of wood chips due to their unique characteristics (Schoemaker *et al.* 1994; Katagiri *et al.* 1995; Nazarpour *et al.* 2013), and some satisfactory results have been achieved. Certain species of white-rot fungi such as *Lentinula edodes* can lower the lignin content of wood without substantially affecting the cellulose content of pulp (Ferraz *et al.* 2008). With the aid of white-rot fungi followed by non-chlorine bleaching, pulp brightness can be increased by up to 2% (Reid and Paice 1994; Ferraz *et al.* 2008). However, pretreatment with white-rot fungi has some disadvantages such as long treatment time, large reaction space, and low efficiency (Zhong *et al.* 2011; Wang *et al.* 2014). Extracellular enzymes secreted by white-rot fungi can be extracted easily and have been applied in pulping and papermaking (Vaithanomsat *et al.* 2010; Järvinen *et al.* 2012). It is important to find new sources of enzymes for bio-mechanical pulping.

Many reports describe the use of laccase treatment for improving properties of mechanical pulps, where procedure concerning laccase purification is cumbersome and time-consuming. In our study, *Trametes* sp lg-9 as a white-rot fungi species can rapidly produce a large amount of enzyme, which effectively enhances mechanical and bleaching properties of SCMP. Therefore, a simplified process on pulp treatment at low cost can be fabricated. Specifically, refining and bleaching of SCMP were improved due to crude enzyme treatment. Correspondingly, energy consumption in refining and effluent derived from chemical bleaching could be substantially reduced.The objective of the present work was to evaluate the effect of crude enzyme secreted from *Trametes* sp. lg-9 strains on properties of sulfonated chemical mechanical pulp (SCMP) and find the suitable strain for possible industrial application.

EXPERIMENTAL

Materials

Poplar SCMP preparation

Poplar chips (2.0 cm × 2.0 cm × 0.5 cm) were obtained from Hebei province, China. Sulfonation of chips was performed with 10% Na_2SO_3 and 10% NaOH at maximum temperature of 130 °C for 30 min, and the liquid-to-wood ratio was 4:1 (volume to weight). The temperature was raised to 130 °C within 60 min. Three-step refining was conducted in a high-consistency refiner, and refining consistency was maintained between 20% and 25%. The refining distances for three steps were 0.50 cm, 0.30 cm, and 0.15 cm, respectively. The pulp was diluted to 2.0% after refining and latency removal was performed at 90 °C for 30 min.

Methods

Culture medium and collection of crude enzymes

Trametes sp. lg-9 is a kind of basidiomycetes, which was obtained *via* selective medium separation by the College of Life Science in Shandong University. Sequence homology analysis of *Trametes* sp. lg-9 was conducted in the National Center for Biotechnology Information (NCBI) and identified as *Trametes* sp. in conjunction with morphology analysis of mycelium and spores. In 2008, *Trametes* sp. lg-9 was stored in the Management Center for Preservation of Common Microbial Strains in China (No. CGMCC 2422). *Trametes* sp. lg-9 was a high-yielding strain of laccase. It was cultured in potato medium (200 g potato/L and 20 g glucose/L) in 250 mL flasks for 11 days. The culture of *Trametes* sp. lg-9 was centrifuged at a speed of 5000 rpm for 30 min at 0 °C, and supernatant filtered through 0.45 μm sterile filter and stored at 4 °C for future use.

Determination of laccase activities

Crude enzymes (1 mL) from *Trametes* sp. lg-9 and 0.5 mL of 0.5 mM 2, 2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS, having a molar absorptivity ε_{420} = 38,000 $M^{-1}\times cm^{-1}$) were mixed with reaction buffer (1.5 mL of 0.1 M acetic acid buffer, pH 5.0) at 45 °C for 3 min. The laccase activity was indicated by the oxidation of ABTS and determined by measuring optical density of the reaction mixture at 420 nm (OD_{420}) using a thermostated spectrophotometer. The buffer containing ABTS without any enzyme was used as the blank control. One international unit (IU) of laccase activity was defined as the amount of enzyme required to oxidise 1 mmol of ABTS per minute.

Determination of CSF and lignin content of pulps

The Canada Standard Freeness (CSF) was determined according to the Technical Association of the Pulp and Paper Industry (TAPPI) T227 om- 04 test method (2004). The lignin content of pulps consisted of acid-insoluble lignin (TAPPI standard method T 222 om-02) and acid-soluble lignin determined by UV absorption at 205 nm (2002).

Pretreatment of pulp with crude enzymes

The required dosage of crude enzymes was mixed with pulp in polyethene bags after adjusting the pH to desired value. The pulp was kept at a constant temperature and mixed every 15 min.

H_2O_2 bleaching of pulp

The bleaching buffer consisted of 2.5% H_2O_2, 2.0% NaOH, 4.0% Na_2SiO_3, 0.05% $MgSO_4$, and 0.3% EDTA in single P (peroxide hydrogen) bleaching. Pulp was mixed with the bleaching buffer in a plastic bag at pulp consistency of 8.0% and placed in a 70 °C water bath for 120 min. In respect of PaP (peracetic acid-peroxide hydrogen) bleaching, the conditons of Pa stage are 0.3% peracetic acid, 70 °C, pulp consistency of 12%, 90 min. The pulps were thoroughly washed and transferred to the P stage, and the conditons of P stage were 2.0% H_2O_2, 1.8 % NaOH, 3.5% Na_2SiO_3, 0.05% $MgSO_4$, and 0.3% EDTA with pulp consistency of 8.0% at 70 °C for 120 min.

Pulp refining

Refining of pulp was performed with a PFI refiner, and the linear pressure was 3.4 N/mm, and refining energy consumption was recorded using an attached electricity meter. Percentage of energy consumption reduction (PECR) was calculated as decrease of refining energy consumption based on the sample without crude enzyme treatment.

Fiber quality analysis

Fiber quality analysis was carried out by a LDA02 Fiber Quality Analysis System (OpTest Company, Hawkesbury, Canada) and operated according to the manufacturer's instructions. The fiber length of the pulp was expressed as an arithmetic mean length (L_n), a length weighted mean length (L_w), and a weight weighted mean length (L_{ww}), as defined by Eqs. 1, 2, and 3, respectively.

$$\mathrm{L_n} = \frac{\sum n_i L_i}{\sum n_i} \tag{1}$$

$$\mathrm{L_w} = \frac{\sum n_i L_i^2}{\sum n_i L_i} \tag{2}$$

$$\mathrm{L_{ww}} = \frac{\sum n_i L_i^3}{\sum n_i L_i^2} \tag{3}$$

where i is the 1, 2 …N categories of fiber length, n_i is the fiber count in the "ith" length category, and L_i is the contour length in the "i^{th}" category.

Fines data were reported as arithmetic fines (F_n) and length weighted fines (F_w). F_n is the number of fibers that are shorter than 0.2 mm (Eq. 4), and F_w is the weight fraction estimate of the fines, assuming that coarseness is constant for the fibers (Eq. 5).

$$\mathrm{F_n}\,(\%) = 100 \times \frac{\sum n_i}{N} \tag{4}$$

$$\mathrm{F_w}\,(\%) = 100 \times \frac{\sum n_i L_i}{L_T} \tag{5}$$

where n is the count of fibers less than 0.2 mm, N is the total number of fibers, L_i is the fines length of the 'i' class, and L_T is the total fiber length.

Handsheet making and testing of paper

Handsheets were prepared in a RK-3A Rapid-Köthen Blattbildner-Sheet Former (PTI-Flank, Laakirchen, Austria) at a grammage of 60 g/m^2. The sheets were conditioned at 23 ± 1 °C and 50 ± 1% relative humidity overnight. The mechanical and optical properties of handsheets were determined according to ISO methods. Specificlly, brightness was determined according to ISO 12625-7 (2014); light scattering coefficient and absorption coefficient were determined according to ISO 9416 (2017). Breaking length was determined according to ISO 1924-2 (2008). Tearing Index was determined according to ISO 1974 (2012), and folding endurance was determined according to ISO 5626 (1993).

RESULTS AND DISCUSSION

Effects of Crude Enzyme Pretreatment on Refining Properties of SCMP

The Canadian Standard Freeness value of pulp describes the effect of pulp refining, and it is mainly influenced by fibrillation and the amount of fines. The effects of pretreatment with crude enzymes secreted by *Trametes* sp. lg-9 on CSF of SCMP are shown in Fig. 1. The pretreatment with crude enzymes had a dramatic effect on the CSF of SCMP. At 15000 PFI revolutions, the CSF of SCMP treated with crude enzyme at a charge of 8 IU g^{-1} pulp was 215 mL, which was much lower than 235 mL observed in untreated pulp. Moreover, increasing the dosage of crude enzymes favored the refining of pulp, which was confirmed by the continuous decrease of pulp CSF. However, when the dosage of enzyme was higher than 8 IU g^{-1} pulp, the CSF of pulp changed slowly. Refining properties have a close relationship with energy consumption; therefore, the crude enzyme pretreatment could save energy to a certain degree. As shown in Fig. 1, percentage of energy consumption reduction (PECR) increased as a function of dosage of crude enzyme. Firstly, the increase was small, and then PECR significantly increased when the dosage of crude enzyme was greater than 4 IU g^{-1} pulp. For example, PECR with crude enzyme dosage of 6 IU g^{-1} pulp and 8 IU g^{-1} pulp was 2.7% and 4.3%, respectively. PECR significantly increased to 9.8% with a crude enzyme dosage of 24 IU g^{-1} pulp. And the amplitude of PECR gradually became smaller as a function of dosage of crude enzyme. Compartively, commericial laccase from *Trametes villosa* (Novozymes) was used to pretreat pulp. For laccase treatment, laccase (8 IU g^{-1} pulp) was added into pulp slurry with 1.0% HBT, and the other conditions were the same as that crude enzyme pretreatment. The CSF and PECR of commercial laccase preated pulp were 210 mL and 4.5%, respectively. The refining efficiency with commercial laccase was slightly superior to that treated with crude enzyme at the identical enzyme dosage. However, purification of commercial enzyme is complex, and use of mediator increased the cost of pretreatment.

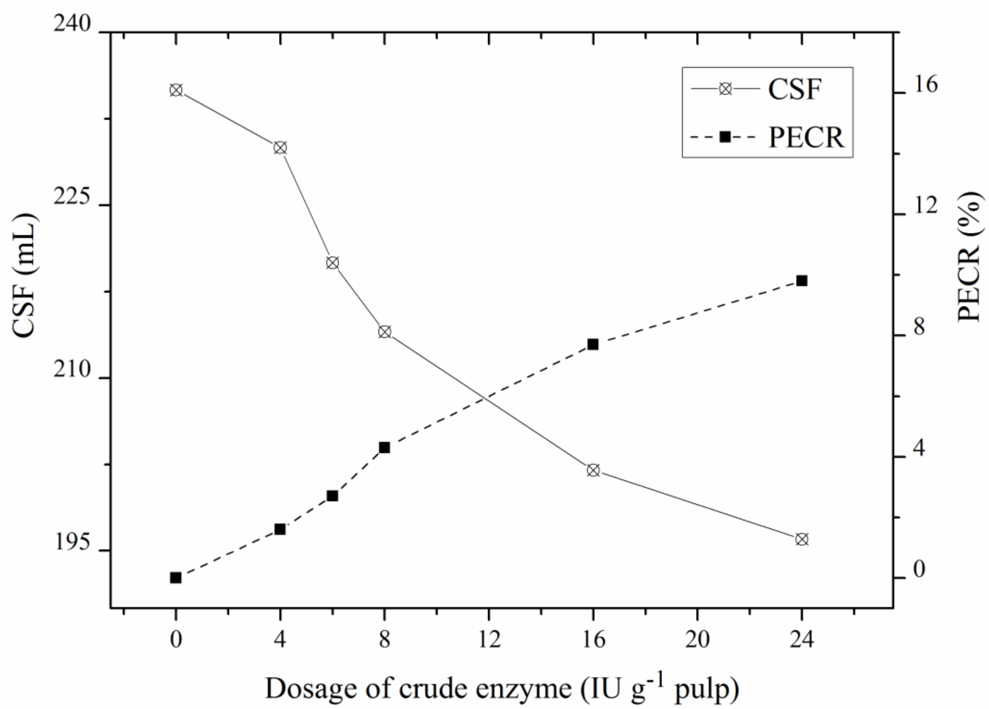

Fig. 1. Effects of crude enzyme pretreatment on CSF and PECR with refining revolutions of 15000

Fiber morphology analysis was performed to further investigate the influence of crude enzyme pretreatment on pulp refining property, and the results are shown in Table 1. The fiber length of the pretreated pulp increased slightly compared with that of untreated pulp fiber, and the fiber width of pretreated pulp decreased as well.

Table 1. Fiber Morphology of Refined SCMP Pretreated with Crude Enzyme

Enzyme Dosage (IU g^{-1} pulp)	CSF (mL)	Fiber Mean Length (mm)			Width (m)	Fines Content (%)	
		Arithmetical	Length weighted	Weight weighted		Arithmetical	Length weighted
0	235	0.447	0.729	0.946	25.4	33.37	9.13
4	230	0.461	0.743	0.961	25.2	32.13	8.45
6	220	0.489	0.775	0.982	25.1	30.21	7.56
8	214	0.503	0.792	1.004	24.9	28.62	6.99
16	202	0.492	0.786	1.003	24.2	30.46	7.73
24	196	0.473	0.764	0.996	24.0	32.19	8.26

Pretreatment conditions: 40 °C, 90 min, 8% pulp consistency, pH 5 to 6

The fiber mean length firstly increased with crude enzyme dosage in the range of 0-8 IU g^{-1} pulp. The stiff fiber could be softened at a relatively low crude enzyme dosage due to lignin modification. Consequently, cutting of fiber was reduced with a high level of fibrillation. However, fibers are inclined to be cut due to the harsh degradation of fiber at high enzyme dosage (> 8 IU g^{-1} pulp), which can account for the reduced fiber mean length at high crude enzyme dosage. The content of fines was reduced, and the fines content was the lowest when the dosage of crude enzymes was 8 IU g^{-1} pulp. Notably, there was a gradual increase in fines content when the crude enzymes charge was more than 8 IU g^{-1}

pulp. This result implied that moderate pretreatment with crude enzyme could promote fibrillation and avoid excessive cutting of fiber during the refining process.

Effect of crude enzyme pretreatment on mechanical and optical properties of SCMP

The effects of crude enzyme pretreatment on mechanical and optical properties of SCMP are shown in Table 2. The handsheets of pretreated pulp presented better mechanical properties than those obtained with untreated pulp. They also had lower light absorption coefficients and opacities, and higher values of brightness. Crude enzyme pretreatment had, in general, a positive effect on fiber bonding, which was shown by the increasing values of breaking length, tearing index, and folding endurance. These results could be explained by the varied fiber morphology of different pulps. As mentioned previously, the pulp pretreated with crude enzyme had longer fiber and lower fines content, and both traits could contribute to fiber bonding. Brightness increased with the increase of enzyme charge. The brightness of paper depended largely on the number of chromophonic groups present in fiber. Lignin is the main source of this group, and it is extremely recalcitrant to degradation. Efficient degradation of lignin is the most important characteristic of laccase enzyme, and *Trametes* sp. lg-9 produces a high yield of laccase. Therefore the improvement of brightness was probably because crude enzyme pretreatment destroyed lignin-carbohydrate complexes and dissolved lignin.

Table 2. Effects of Crude Enzyme Pretreatment on Properties of SCMP

Crude enzyme dosage[a] (IU g^{-1} pulp)	0	4	6	8	16	24
CSF (mL)	235	230	220	214	202	196
Lignin, (%)	21.2	21.0	20.8	20.5	20.4	20.0
Brightness (%)	57.8	58.3	59.2	59.8	60.1	60.2
Opacity (%)	89.1	89.0	88.8	88.7	87.7	85.4
Light Scattering Coefficient ($m^2 \cdot kg^{-1}$)	36.3	36.3	36.2	36.2	36.0	35.7
Absorption Coefficient ($m^2 \cdot kg^{-1}$)	1.61	1.59	1.56	1.55	1.52	1.50
Breaking Length (km)	4.13	4.19	4.23	4.29	4.87	4.61
Tearing Index ($mN \cdot m^2 \cdot g^{-1}$)	4.15	4.18	4.20	4.24	4.33	4.23
Folding Endurance (times)	5	6	6	7	7	7

Pretreatment conditions: 40 °C, 90 min, 8% pulp consistency, pH 5 to 6

The opacity of the paper made from the crude enzyme treated pulp was slightly lower compared with the untreated pulp. A change in opacity of paper often relates to a change in the brightness of paper. Higher brightness presents lower opacity, but opacity can also relate to light scattering ability of a paper. Paper containing less fines can have lower light scattering coefficient and also lower opacity. These results were similar to previous reports (Pang *et al*. 2005).

Effects of Crude Enzyme Pretreatment on Bleaching Properties of SCMP

Bleaching of pulp is necessary for whitening of paper. It is based on the removal of residual lignin from cellulose fibers, but bleaching should have no adverse effect on cellulose fiber quality. H_2O_2 bleaching is an effective method to improve the brightness of SCMP. The effect of crude enzyme pretreatment on bleaching properties of SCMP is shown in Table 3. The present experiments with different charges of crude enzyme treatment revealed that the brightness of pulp increased with increasing crude enzyme charge. This change of brightness displayed steadily and slowly when the enzyme charge increased up to 16 IU g^{-1} pulp. At the same time, the corresponding physical properties were slightly reinforced, exhibited by the increases of breaking length, tearing index, and folding endurance of paper sheets. The purpose of pulp bleaching was to enhance the brightness of pulp, and this function was reinforced by the pretreatment with crude enzyme. However, the pretreatment had negative effects on opacity, light scattering coefficient, and absorption coefficient.

Table 3. Effects of Crude Enzyme Pretreatment on H_2O_2 Bleaching Properties

Crude enzyme dosage (IU g^{-1} pulp)	0	4	6	8	16	24
CSF (mL)	230	225	218	212	200	194
Brightness (%)	73.7	74.0	74.2	74.7	75.7	75.9
Opacity (%)	83.9	83.2.	82.5	82.1	81.4	80.5
Light Scattering Coefficient ($m^2 \cdot kg^{-1}$)	35.9	35.7	35.5	35.4	34.6	33.8
Absorption Coefficient ($m^2 \cdot kg^{-1}$)	0.89	0.87	0.84	0.81	0.77	0.72
Breaking Length (km)	4.46	4.54	4.62	4.68	4.97	4.84
Tearing Index ($mN \cdot m^2 \cdot g^{-1}$)	4.28	4.30	4.30	4.31	4.46	4.35
Folding Endurance (times)	6	6	7	7	9	7

Pretreatment conditions: 40 °C, 90 min, 8% pulp consistency, pH 5 to 6

Delignification and bleaching of mechanical pulps can be effectively achieved using peracetic acid (Pa) treatment. Thus, PaP bleaching sequence can be applied to SCMP bleaching, and effects of crude enzyme pretreatment on PaP bleaching properties were investigated (Table 4). Crude enzyme pretreatment also can effectively activate the residual lignin in SCMP, and brightness enhanced remarably after PaP bleaching. However, the increase was negligible when crude enyzme dosage is great than 16 IU g^{-1} pulp. Due to transformation of chromphores in pulp, the absorption coefficient of bleached pulp substantially decreased. In agreement with peroxide bleaching, the physical properties were slightly reinforced. Therefore, crude enzyme pretreatment has excellent applicability for SCMP bleaching in view of enhancement on optical and physical properties.

Table 4. Effects of Crude Enzyme Pretreatment on PaP Bleaching Properties

Crude enzyme dosage (IU g^{-1} pulp)	0	4	6	8	16	24
CSF (mL)	230	220	216	212	198	192
Brightness (%)	74.1	74.5	74.9	75.4	75.9	76.1
Opacity (%)	83.4	83.0	82.5	81.7	81.2	80.2
Light Scattering Coefficient ($m^2 \cdot kg^{-1}$)	34.7	34.3	33.8	33.2	32.8	32.6
Absorption Coefficient ($m^2 \cdot kg^{-1}$)	0.75	0.74	0.73	0.72	0.70	0.69
Breaking Length (km)	4.52	4.56	4.65	4.74	4.99	4.89
Tearing Index ($mN \cdot m^2 \cdot g^{-1}$)	4.35	3.38	4.40	4.42	4.58	4.48
Folding Endurance (times)	7	7	7	8	9	8

Pretreatment conditions: 40 °C, 90 min, 8% pulp consistency, pH 5-6

CONCLUSIONS

1. *Trametes* sp. lg-9 has potential as an important source of enzymes to improve the properties of mechanical pulp. Pretreatment with crude enzyme secreted from *Trametes* sp. lg-9 improved refining pulp properties and reduced energy consumption.
2. The mechanical properties of pulp were improved as well. Moderate pretreatment with crude enzyme secreted from *Trametes* sp. lg-9 reduced the fines content.

ACKNOWLEDGMENTS

The authors are grateful for the financial support from Natural Science Foundation of Shandong Province (ZR2017MC007), National Key Research and Development Project (2017YFB0307900), the National Science Foundation of China (No. 31670595 and 31670590.), State Key Laboratory of Pulp and Paper Engineering (Project number 201720), the Taishan Scholars Project Special Funds.

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Article submitted: October 26, 2017; Peer review completed: December 29, 2017; Revised version received and accepted: March 7, 2018; Published: March 20, 2018.
DOI: 10.15376/biores.13.2.3420-3428