BIO-MODIFICATION OF EUCALYPTUS CHEMITHERMO-MECHANICAL PULP WITH DIFFERENT WHITE-ROT FUNGI

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Modification of chemithermomechanical pulp (CTMP) by fungal treatment was investigated. Eucalyptus CTMP was treated with three different types of white-rot fungi, namely, *Phanerochaete chrysosporium* (*P.c*-1767), *Trametes hirsute* 19-6 (*T.h*-19-6), and *Trametes hirsute19-6w (T.h-19-6w),* under a stationary culture condition. Pulp total weight loss, lignin loss, and cellulose loss were determined to compare the different enzymes secreted by the three fungal strains. Pulp physical strengths, optical properties, and bleachability after the fungal treatment were investigated to compare the effect of fungal treatment on the pulp quality improvement. The results show that lignin reduction by both *T.h*-19-6 and *T.h*-19-6 (w) was about twice as much as that by *P.c*-1767. However, the selectivity of *T.h*-19-6 (w) towards lignin over cellulose was only 0.82, while that of *T.h*-19-6 was as high as 4.43. After *T.h*-19-6 treatment, pulp tensile, tear, and internal bonding strength increased by about 27%, 38%, and 40%, respectively.

Keywords: Eucalyptus CTMP pulp, White-rot fungi, Trametes hirsute 19-6, Bio-modification

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INTRODUCTION

Chemithermomechanical pulp (CTMP) has been used increasingly in traditionally wood-free paper grades due to its relatively low costs and some specific qualities such as high bulk, high opacity, and good printability (Zhou 2004; Cannel and Cockram 2000; Zhou and Zou 2003). However, the bonding ability of CTMP fibers is not as high as kraft pulp fibers, since most of the lignin is kept in the CTMP fibers. This sets a limit on the maximum addition rate of CTMP fibers in wood-free paper. It has been found that a large portion of the CTMP fiber surface is covered by lignin-rich middle lamella material (Cisneros et al. 1995; Börås et al. 1999; Li et al. 2006), and lignin on the fiber surface is particularly detrimental to inter-fiber bonding (Shao and Li 2006; Li and Reeve 2002).

Fiber modification refers to any processes that can alter the papermaking property of the fibers (Gruber 2000). Traditional fiber modification includes chemical modify-ation and mechanical modification. For CTMP fibers, the bleaching process is not only for increasing the brightness of the pulp fiber, but also for adjusting the bonding ability and the flexibility of the fibers (Zhou and Zou 2003). Mechanical modification, i.e. low-consistency (LC), refining is becoming a more popular means for papermakers to finetune the pulp property (Zhou and Zou 2003; Franzén 1986).

Use of lignin-degrading fungi prior to mechanical pulping, so-called bio-pulping, has been studied extensively by several research groups. Most studies were targeted on reducing the energy consumption of the refining process by fungal treatment of wood chips. It has also been found (Abuhasa et al. 1988; Bar-Lev et al. 1982; Pere et al. 1996; Leatham et al. 1990b) that by treating the CTMP fibers with fungi after the first-stage refiner, not only the energy consumption of the second-stage refiner is reduced, but also the physical strength of the resultant pulp is improved. Some studies (Hunt et al. 2004; Ferraz et al. 2002) proposed that the benefits of biopulping, including energy savings and increased handsheet strength, are due to the structure changes of the residual wood components and the increase of acid group content of wood. However, some studies (Akhtar et al. 1992; Leatham et al. 1990a) found that with fungal treatment, pulp strength was deteriorated. It was suggested (Setlife et al. 1990) that the contradictory results might be due to the difference in the types of fungi and in the treatment conditions used in different studies.

Fungal treatment of wood chips is environmentally friendly and saves energy consumption in the refining process, but the treatment time is quite long, and the process itself is complicated in comparison with the chemical pretreatment process (Scott et al. 1998; Scott et al. 1998). The present study attempts to explore the possibility of using lignin-degrading fungi to modify the CTMP fiber for improved strength properties. The rationale behind is that when pulp fibers, rather than wood chips, are treated, only a minor or a relatively short period of fungal treatment may remove most of the lignin-rich material from the fiber surface. It is expected that sole removal of the fiber surface lignin will improve the inter-fiber bonding strength significantly, and at the same time retain the other properties of the CTMP fibers. This paper reports the first step in this study, which is to compare the overall effect of different stains of white-rot fungi on eucalyptus CTMP fibers. The effect of fungal treatment on lignin removal, yield loss, pulp strength properties, optical properties, and bleachability are compared.

EXPERIMENTAL

Pulp Samples

Eucalyptus urophylla chemi-thermomechanical pulp was provided by the Chinese Academy of Forestry, Nanjing, China. The brightness of the pulp was 49.6% ISO, and the CSF freeness was 700 ml. The pulp was air-dried to a moisture content of 15% and stored at 4 ^oC until use.

Fungi

The white-rot fungus *Phanerochaete chrysosporium* (*P.c*-1767) used was the same as that described by Pease and Tien (1992). *Trametes hirsute* 19-6 (*T.h*-19-6) and *Trametes hirsute*19-6w (*T.h*-19-6w) were isolated from bamboo in the wild field by the State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou, China. The *P.c*-1767 produces lignin peroxidase (Lip), manganese peroxidase (Mnp), cellulase, and hemicellulase. *T.h*-19-6 and *T.h*-19-6 (w) produce manganese peroxidase (Mnp), laccase, hemicellulase, and cellulase. *T.h*-19-6 produces only a minor portion of cellulase. All the fungal strains were cultured on PDA bevel culture medium, and a spore suspension of 9-day-old culture was used as the inoculum. Seeding of the pulp was carried out as 0.5 ml/g of pulp (o. d.).

Fungi Culture Conditions

The incubation was performed in 1000 ml flasks with 30 g of CTMP pulps (o. d.), 15 ml of spore suspension, and some of the culture medium, without using a buffer. Sterilized water was added to give a final pulp consistency of 25% (w/v). The solution, flask, and pulp samples were sterilized in an autoclave (30min, 121 ^{o}C). The control samples contained sterile water instead of the spore suspension. The flask was incubated under a stationary culture condition as shown as in Table 1.

Table 1. The Culture Conditions for White-Rot Fungi

	Temperature (^{o}C)	Time (days)	Initial pH
P.c-1767	35	7	4.8
T.h-19-6	28	7	4.5
T.h-19-6w	28	7	4.5

The flasks were flushed with oxygen for 10 min every day. After incubation, all pulp samples were filtered with a 400-mesh nylon screen and washed with water to remove the superficial mycelium and then dried in air. The residual lignin content was determined as Klason lignin (TAPPI Standard T222), and cellulose content was determined according to TAPPI Standard T249. Three replicate experiments were performed for each test, and the average values are reported.

Handsheet Properties

Handsheet properties, including tensile, tear, and internal bonding strength, were determined according to TAPPI standard methods T 205, T220, T248 respectively. Optical properties (brightness, opacity, scattering coefficient) were determined with a Technidyne TechniBrite^{TM} Micro TB-1C Photospectrometer according to the ISO standard.

Electron Microscopy

Scanning Electron Microscope (SEM) images of pulp fibres were obtained using a JEOL JSM-6400 scanning electron microscope, operated in secondary electron mode at an accelerating voltage of 10kv. CTMP fibres were dehydrated with an ethanol dehydration series and then dried with critical point drying method. The fibres were coated with gold for 120s using a S150 sputter coater prior to scanning.

Transmission Electron Microscope (TEM) images of pulp fibres were obtained using a JEOL 2011 Transmission Electron Microscope, operated at 120 kV. Images were taken with a Gatan digital camera. Prior to analysis, the fibre samples were embedded in Spurr-resin after dehydration with an ethanol dehydration series. Ultra-thin sections (70 nm) were cut with a diamond knife onto distilled water. Sections were collected onto uncoated, copper grids. Some grids with sections were post-stained with uranyl acetate and lead citrate to enhance contrast.

Bleaching

A two-stage bleach sequence ($Na_2S_2O_4$ + H_2O_2) was used. The dosages of $Na_2S_2O_4$ and H_2O_2 were 1.5% and 3.0% (w/w), respectively. Detailed bleaching conditions are listed in Table 2. Before H_2O_2 bleaching, the pulp was treated with an EDTA solution (0.3%) at 3% consistency to remove metal ions. $MgSO_4$ (0.5g/L) was used to stabilize the peroxide and to prevent degradation of carbohydrates.

Table 2. Two-Stage Bleaching Conditions

	Initial pH	Pulp consistency (%)	Temperature (^oC)	Time (min)
$Na_2S_2O_4$	8.0	1.5	60	60
H_2O_2	11.0	3	70	90

RESULTS AND DISCUSSION

Pulp Chemical Composition Change after Fungal Treatment

White-rot fungi can degrade all the major components of wood, i.e., cellulose, hemicellulose, and lignin, since they can secrete lignin-degrading enzymes, cellulase, and hemicellulase at the same time (Eriksson 1990). However, different fungal strains may secret these enzymes in different proportions. Table 3 lists the changes of the chemical composition of the CTMP after fungal treatment. It can be seen that all three strains had significant effects on the CTMP fibers. The total weight loss by the *T.h*-19-6 (w) was the highest, about 7%, and the total weight losses by *P.c*-1767 and *T.h*-19-6 were only about 3%. The cellulose loss by *T.h*-19-6 (w) was even greater than the lignin loss, with a selectivity factor of 0.82. In contrast, lignin loss by *T.h*-19-6 was much larger than cellulose loss, more than four times. This indicates that the *T.h*-19-6 secreted a higher level of lignin-degrading enzymes than cellulase and hemicellulase. *P.c*-1767 removed much less lignin, but the total weight loss was almost the same as that by *T.h*.-19-6, which means that *P.c*-1767 also hydrolyzed a significant amount of hemicellulose. For the purpose of CTMP fiber modification, it is desirable that only lignin is removed. Removal of cellulose and hemicellulose not only reduces pulp yield, but may also decrease the physical strengths and interfibre bonding ability of the fibers, since cellulose constitutes the framework of the fiber structure, and hydrophilic hemicellulose promotes fibre swelling, and inter-fiber hydrogen bonding. Therefore, of the three fungal strains, the *T.h*-19-6 was the most suitable one for the fiber modification.

Table 3. Changes of Pulp Chemical Composition after Fungal Treatment

Fungi strains	Total weight loss (%)	Lignin loss (%)	Cellulose loss (%)	Lignin selectivity (lignin loss/cellulose loss)
Control	0.35	0.12	0.49	0.24
P.c-1767	3.28	3.95	1.02	3.78
T.h-19-6	3.46	7.70	1.74	4.43
T.h-19-6w	7.24	6.23	7.62	0.82

Pulp Physical Strength Properties

In the present study, the freeness of CTMP used was CSF 700 ml, which is toward the high end of the freeness range of commercial CTMP pulps. If handsheets are made by the CTMP fibers only, the physical strength will be very low, and it will not be easy to compare the effect of fungal treatment. Therefore, the control pulp and fungal-treated pulps were further refined in a PFI mill to a target freeness level of CSF 300 mL. As shown in Table 4, treatment with white-rot fungi *P.c*-1767 and *T.h*-19-6 improved the strength properties of handsheets produced from the CTMP pulps. In particular, tensile index and internal bonding were increased significantly after white-rot fungus 19-6 treatment.

Table 4. Pulp Physical Strength Properties after Fungal Treatment

Fungi Strains	Control	*P.c*-1767	*T.h*-19-6	*T.h*-19-6w
Freeness (ml)	325	320	320	325
Tensile index (N.m/g)	24.20	26.68	30.76	22.54
Tear index (mN.m^2/g)	3.28	3.98	4.52	3.12
Internal bonding (J/m^2)	59.18	77.23	82.79	60.92

As discussed previously, both *P.c*-1767 and *T.h*-19-6 removed relatively small amounts of lignin, being about 4% and 8%, respectively. It is expected that if a small portion of lignin on the fiber surface can be removal, then the increase in inter-fiber bonding will be significant. *T.h*-19-6 (w), although it removed about 6% of lignin, it also removed about the same amount of cellulose. Therefore, the effect on the physical strength may be balanced by these two counteracting factors.

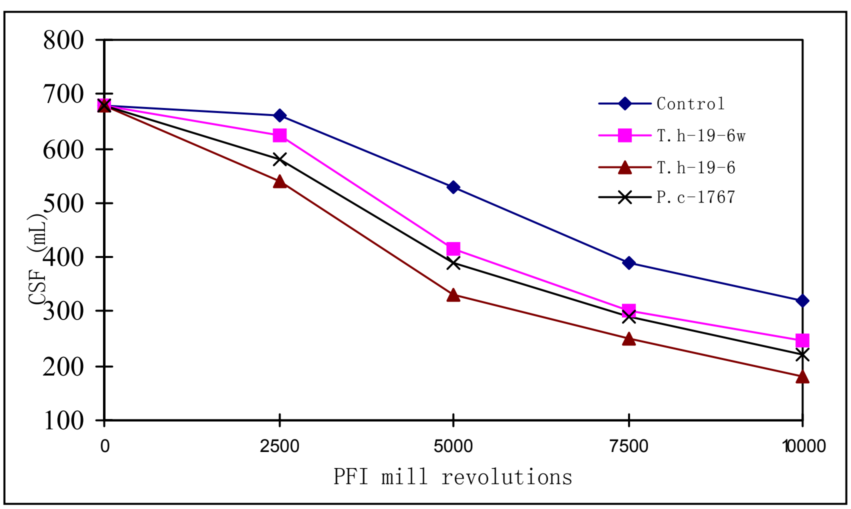


Fig. 1. Freeness change PFI mill revolutions of the control and fungal-treated eucalyptus CTMP pulps.

Figure 1 shows the freeness change as a function of PFI revolutions for the control pulp and fungal-treated pulps. It can be seen that in comparison with the control pulp, all three fungal treated pulps were easier to be refined to lower freeness levels, and *T.h*-19-6 treated pulp was the easiest. This implies that, for a targeted freeness level, *T.h*-19-6 treatment will reduce the energy consumption in the LC refining process. In commercial practice, LC refining becomes necessary for fine-tuning the pulp properties for most paper grades. Therefore, the fungal treatment will benefit the LC refining.

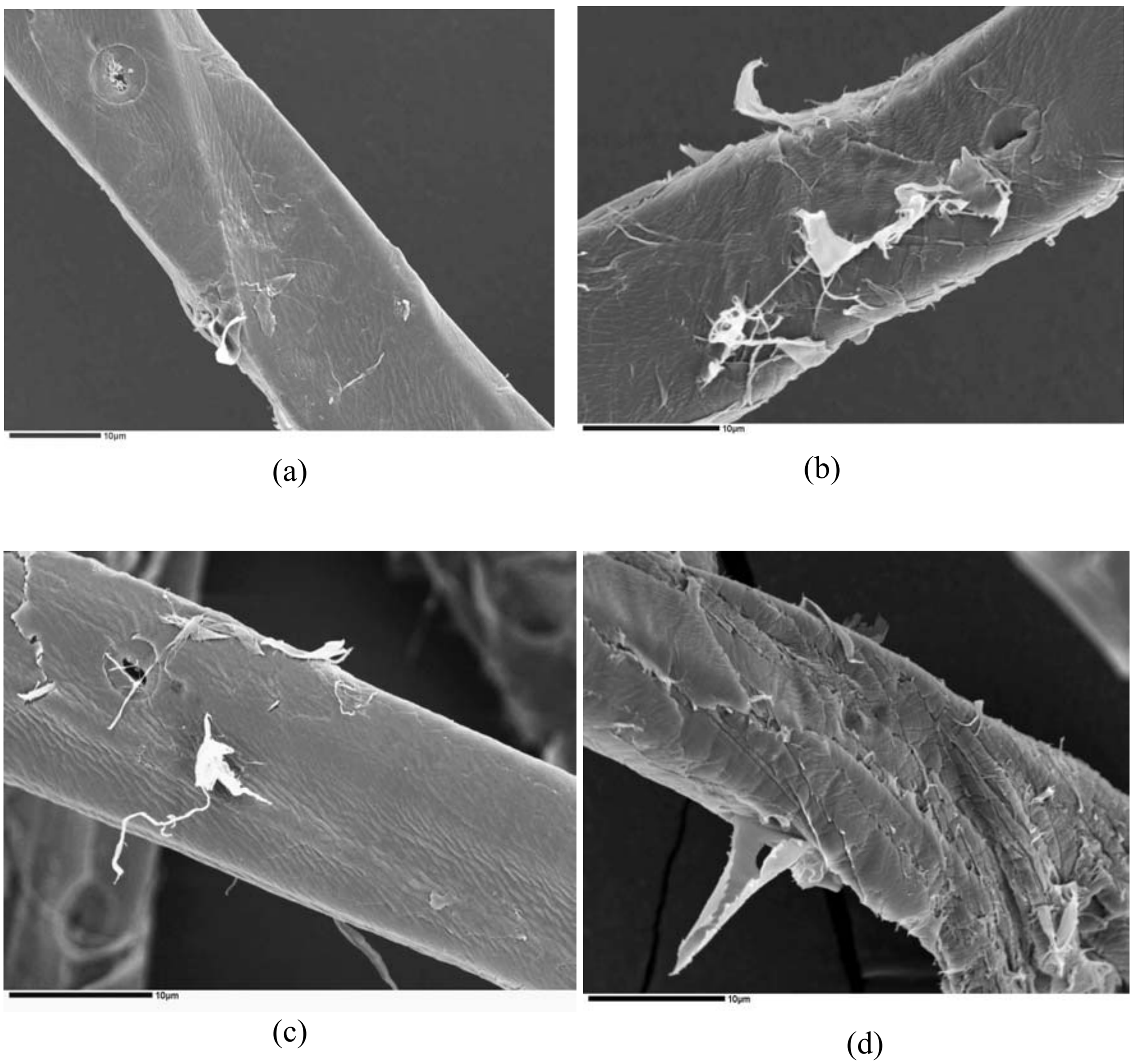
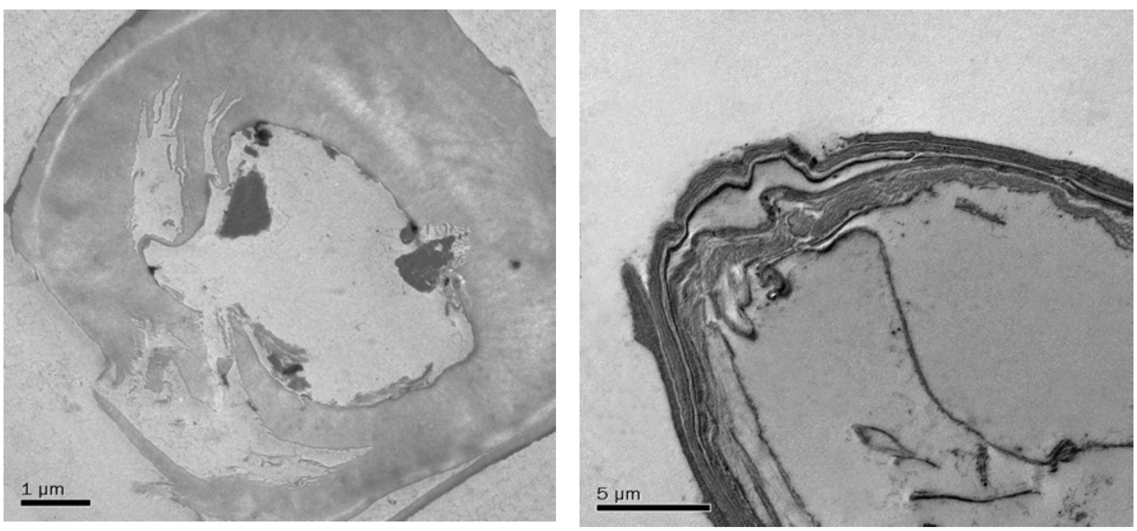


Fig. 2. SEM images of eucalyptus CTMP fibers before and after *T.h*-19-6 fungal treatment. (a) image of a control CTMP fiber; (b) a control CTMP fiber after PFI treatment; (c) a fungal-treated CTMP fiber; and (d) a fungal-treated CTMP fiber after PFI treatment.

In fact, the beneficial effect of the fungal treatment was better realized by the subsequent LC refining process. As shown in Figure 2, without PFI treatment, the CTMP fibers of control pulp and fungal-treated pulp looked almost the same. After PFI treatment, a slight increase in external fibrillation could be observed on the control fibers.

In contrast, a substantial change could be observed on the fungal-treated fibers. The entire structure of the fiber had been loosened, and cracks between microfibrils could be seen. The fiber also appeared twisted and more flexible due to the overall structural change. The difference in the structural change after PFI refining is not surprising. It is understood that the fungal treatment dissolves some of the fiber wall components, but the total weight loss was relatively small, being about only 3.46% in case of *T.h*-19-6 treatment. Such a small amount of material loss may not be seen from the structural change, as shown in Fig. 2. However, even a small degree of lignin and/or cellulose degradation in the fiber wall will loosen the bonding between microfibrils. When a subsequent mechanical treatment such as PFI refining is used, the weak points in the fibre structure due to fungal treatment will be broken, leading to a significant structure change. This can be better seen from the cross-sectional images of fiber wall as shown in Fig. 3. Before PFI treatment only some damage such as cracks or voids due to fungal treatment could be seen of the fiber walls. After PFI treatment the entire fiber wall was delaminated, resulting in extensive internal fibrillation of the fiber wall. This also, on the other hand, explains the increased pulp physical strength after the fungal treatment



(a) (b)

Fig. 3. TEM images of eucalyptus CTMP fibers after T.h-19-6 fungal treatment.
(a) without PFI refiner treatment; and (b) after PFI treatment.

Pulp Optical Properties

One of the major concerns for bio-mechanical pulping is the pulp brightness. While most studies on Kraft pulp have reported that fungal treatment increases pulp brightness (Reid and Paice1994; Fujita and Kondoi 1991), the results from fungal treatment of mechanical pulp show a decrease in pulp brightness (Abuhasan et al. 1988; Sykes 2003; Pellinen et al. 1989). It was suggested that the brightness loss is caused by melanin synthesized during the secondary stage of growth and lignin modification by enzymes (Fukui et al. 1991). It was also suggested that the discoloration of mechanical pulp seems to be connected to the presence of sulfonate groups, since Kraft pulp did not turn dark (Pellinen et al. 1989). It has also been shown by some recent studies (Scott et al. 2002; Guerra et al. 2005; Guerra et al. 2006) that although the brightness of fungal treated Eucalyptus mechanical pulp was reduced, high brightness levels of bio-mechanical pulp could be attained after bleaching.

Brightness and opacity were determined to evaluate the effect of fungal treatment on optical properties of CTMP pulp (Table 5). The data shows that fungal treatment has a slight effect on opacity but causes a sizable decrease in the pulp brightness. Compared with other studies in the literature, the brightness reduction by fungal treatment can be as high as by 140% (Abuhasan et al. 1988). The difference may be attributed to the different fungal strains, wood species, and the CTMP processing conditions.

Table 5. Pulp Optical Properties after Fungal Treatment

Fungi strains	Opacity %	Brightness (prior to bleaching) %ISO	Brightness (after bleaching) %ISO
Control	99.3	49.6	72.6
P.c-1767	99.5	36.9	58.6
T.h-19-6	99.5	37.8	70.8
T.h-19-6w	99.6	35.4	54.8

The bleachability of fungal treated pulps was evaluated by using a two-stage bleaching sequence. Sodium hydrosulfite was used in the first stage, and alkaline hydrogen peroxide was used in the second stage. As shown in Table 5, the brightness of the pulp treated by *T.h*-19-6 increased by 33 brightness units to 71% ISO, which was almost the same as the final brightness of the control pulp (73% ISO). However, brightness of the pulps treated by *P.c*-1767 and *T.h*-19-6 (w) was only 58.6% ISO and 54.8% ISO, respectively. The results indicate that different fungi have different effects on the pulp bleachability. Treatment of CTMP by *T.h*-19-6, although it reduced the initial pulp brightness, the pulp could be further bleached to a high brightness level by a conventional bleaching process. The result is encouraging since in many cases, the brightness of mechanical pulps is reduced by fungal treatment and it is difficult to bleach the pulps to a higher bright brightness level.

SUMMARY

1. The properties of CTMP fibers can be improved by a fungal treatment process. Removal of a small amount of lignin from the CTMP fibers can improve the pulp strengths significantly; however, removal or degradation of cellulose will deteriorate pulp strengths.

2. For the three types of white-rot fungi, namely, *Phanerochaete chrysosporium* (*P.c*-1767), *Trametes hirsute* 19-6 (*T.h*-19-6) and *Trametes hirsute19-6W (T.h-19-6w)*, *T.h*-19-6 has the highest selectivity towards the degradation of lignin over cellulose. The amount of lignin removed by *T.h*-19-6 is more than four times the amount of cellulose. After *T.h*-19-6 treatment, pulp tensile, tear and internal bonding increased by 27%, 38% and 40% respectively. With *T.h*-19-6 (w)

treatment, cellulose loss is greater than lignin loss. Therefore, pulp physical strengths decreased after the fungal treatment.

3. Electron micrographs of fibers revealed that fungal treatment loosened the fiber wall structure due to the removal and/or degradation of the fiber wall components. A subsequent PFI refining treatment further promoted the fungal treatment effects, resulting in both external and internal fibrillation, which is the reason behind the increased bonding strength.

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