Degradation Mechanism and Chemical Component Changes in *Betula platyphylla* Wood by Wood-Rot Fungi

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In this study, four species of wood-rot fungi-Piptoporus betulinus, Fomes fomentarius, Irpex lacteus, and Coriolus versicolor-were compared regarding their ability to degrade the wood of white birch and used to assess the degradation mechanisms. Chemical analyses were conducted following the Chinese national standard methods and included Fourier transform infrared spectroscopy (FTIR). The wood samples were inoculated with the four wood-rot fungi for a predetermined duration in the wood-decaying test. In the wood weight loss test, both F. fomentarius and P. betulinus showed the greatest reduction, but through different mechanisms: F. fomentarius mainly decomposed lignin, whereas P. betulinus mainly acted on cellulose. F. fomentarius, I. lacteus, and C. versicolor exhibited a shift at 3417 cm⁻¹ related to O-H stretching in hydroxyl groups, along with decreased absorption at 3410, 3406, and 3405 cm⁻¹, most likely due to the degradation of the related functional groups of lignin side chains. The wood decayed by *P*. betulinus displayed a change in the relative position of celluloseassociated bands at 1161 and 898 cm⁻¹. F. fomentarius can be considered a potential agent for the biopulping of white birch because of its high ability to degrade lignin, high holocellulose content, low content of 1% NaOH, and ethanol-benzene extractives.

Keywords: Wood-rot fungi; Betula platyphylla; Decay; FTIR analysis

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INTRODUCTION

Betula platyphylla Suk. (white birch) is a widespread tree species that is popular in the pulp and paper industry. The species is mainly found in temperate or subarctic areas of Asia, such as Japan, China, Korea, and Siberia (Li *et al.* 1995).

Wood biodegradation is a relatively slow natural process performed by certain specific microorganisms, mainly fungi (Eriksson *et al.* 1990). Basidiomycetes are the most important degraders of lignocellulosic biomass. The two most common modes of wood degradation by basidiomycetes are brown-rot and white-rot decay (Blanchette 1991). White-rot fungi attack both lignin and polysaccharides, either simultaneously or successively (Cohen *et al.* 2002). Brown-rot decay is characterized by extensive degradation of cellulose and hemicellulose with limited lignin degradation (Deacon 2009; Stamets 2005). The mechanism of wood-rot decay and the change in chemical composition of the wood has been investigated by various methods to understand the behaviors and mechanism of wood-rot decay. Recently, Li *et al.* (2011) examined the changes in the main chemical composition of wood with different decay levels caused by *Wolfiporia cocos*.

To better understand changes in the functional groups of decayed wood, Fourier transform infrared spectroscopy (FTIR) has also been used to analyze wood structural changes during chemical or physical processing (Li 2002). FTIR plays an important role in wood sciences because of the simple sample preparation, the small sample size required for analysis, and the detailed structural information provided. FTIR spectra and the band assignment of wood samples have been extensively studied (Li *et al.* 2011; Rana *et al.* 2010; Shi *et al.* 2012). Indeed, Popescu *et al.* (2010) applied FTIR to investigate changes in the structure of lime wood (*Tilia cordata* Mill.) decayed by *Trichoderma viride* Pers. They found qualitative and quantitative changes in the carbohydrate components of wood decayed by soft-rot, which could be observed in two regions of the FTIR spectra (the OH stretching vibrations in the 3800 to 2700 cm⁻¹ region and the "fingerprint" region from 1800 to 400 cm⁻¹).

This paper presents the detailed chemical components of *Betula platyphylla* decayed by four wood-rot fungi and the changes in wood functional groups detected by FTIR analysis. The mean weight loss, holocellulose and total lignin losses, and change in wood chemical composition caused by wood-rot fungi were investigated to provide useful information on the physiological status and decaying mechanism of wood-rot fungi during different exposure periods and to compare the decay ability of different wood-rot fungi to cause decay.

EXPERIMENTAL

Wood Sample Preparation

Three hundred 5 mm diameter wood cores were obtained with an incremental core borer at breast height from white birch trunks growing on Maoer Mountain in Heilongjiang Province in northern China and kept frozen at -20 °C. The test samples were cut from these wood cores in 5 mm lengths, sterilized at 121 °C for 1 h, and then maintained at ambient pressure for 30 min in 40% to 60% relative humidity for the wood-decaying test.

Isolation and Cultivation of the Fungal Species

The four wood-decay fungi, including three white-rot fungi (*Fomes fomentarius*, *Coriolus versicolor*, and *Irpex lacteus*) and a brown-decay fungus (*Piptoporus betulinus*), were obtained from Maoer Mountain in Heilongjiang Province of northern China (Fig. 1). The purified mycelia were isolated by the tissue isolation method and identified on the basis of morphological characteristics (Dai and Tolgor 2007) and then maintained on a medium consisting of 78% white birch sawdust, 20% wheat bran, 1% CaSO₄•H₂O, and 1% sugar in 60% relative humidity at 4 °C.

Before the wood-decaying test, each fungus was incubated on potato dextrose agar (PDA) medium until the mycelium reached the edge of the petri dish. Then, each wood core sample with three replications was placed on the mycelium of the petri dishes under stationary conditions at 28 °C and 75% to 85% relative humidity until the wood sample was completely penetrated by hyphae. Because of different fungal growth rates, the treatment times required for the wood samples to be completely penetrated by the four wood-rot fungi differed as follows: 50 d for *F. fomentarius*, 38 d for *C. versicolor*, and 98 d for *I. lacteus* and *P. betulinus*.

All the fungal tests were conducted under sterile conditions in an acclimatized chamber. Before and after incubation, the wood samples in the weight loss test were oven-dried at 100 ± 5 °C to constant weight. All the experiments were performed in triplicate.



Fig. 1. The fruit bodies of the four wood-decay fungi in the forest

Weight Loss of the Wood Samples

After they were completely penetrated by hyphae in the weight loss test, all of the samples were incubated continually for 30 d.

The weight loss (W_L) of each wood sample was calculated using the mean percentage of the weight losses, as shown in Eq. 1.

$$W_{\rm L} = \frac{W_1 - W_2}{W_1} \times 100\% \tag{1}$$

 W_1 is the oven dry weight of the sample before the decay treatment and W_2 is the ovendry weight of the sample after the decay treatment.

The holocellulose loss (X) of each wood sample was calculated using the mean percentage of the holocellulose losses, as shown in Eq. 2.

$$X = \frac{Y - (1 - W) \times F}{Y} \times 100\%$$
⁽²⁾

W is the weight loss of the decayed wood sample, F is the holocellulose content of the decayed sample, and Y is the holocellulose content of the fresh wood sample. The lignin losses of each wood sample were also calculated using the same method. All the experiments were performed in triplicate.

Analysis of the Main Chemical Composition of the Wood Samples

After all of the samples were completely penetrated by hyphae, they were incubated continually for 30 d in the chemical composition test.

The chemical composition of the wood samples, including holocellulose, Klason lignin, acid-soluble lignin, ethanol-benzene extractives, and 1% NaOH extractives, was

determined using Chinese national standard (GB) methods, namely GB/T2677.10-1995, GB/T2677.8-1994, GB/T10337-1989, GB/T2677.6-1994, and GB/T2677.5-1993, respectively, which are described below (Shi and He 2003). The relative total lignin content was considered to be the sum of the Klason lignin and the acid soluble lignin.

For the 1% NaOH extractives, the samples (2.0 g) and 100 mL of 1% NaOH were added to a 300 mL Erlenmeyer flask equipped with a reflux condenser and successively extracted for 1 h. The obtained residue was dried at 105 °C to a constant mass.

For the ethanol–benzene extractives, the samples (3.0 g) were successively extracted with 170 mL ethanol-benzene mixture (1:2,v/v) for 6 h using a Soxhlet apparatus. The content of solids in the solutions was measured in duplicate samples after evaporating the solutions to dryness in a water bath and completing the desiccation at 105 °C to a constant mass.

For the Klason lignin determination, 1.0 g of the samples, extracted by ethanolbenzene according to GB/T2677.6, was mixed with 72% H_2SO_4 (15 mL) for 2 h at 18 to 20 °C. The mixture was then diluted with 560 mL of distilled water, heated under reflux for 4 h and filtered. The residue of Klason lignin obtained in this manner was washed with hot water and dried at 103 °C to a constant mass.

The acid-soluble lignin was determined by UV spectrophotometry analysis of the filtrate obtained from the acid-insoluble lignin experiment. The UV absorbance reading was taken at 205 nm.

For the holocellulose determination, the samples (2.0 g), extracted by ethanol– benzene according to GB/T2677.6, were placed in a flask containing distilled water (65 mL). Sodium chlorite (0.6 g) and glacial acetic acid (0.5 mL) were added to the flask and the mixture was refluxed at 75 °C for 1 h. After 1 h, sodium chlorite (0.6 g) and glacial acetic acid (0.5 mL) were again added. The procedure was repeated four times until the material turned white. The obtained residue was washed several times with distilled water and three times with acetone before being dried at 105 °C for 24 h. All the samples were analyzed in duplicate.

FTIR of the Wood Samples

All of the samples for FTIR analysis were incubated continually for 120 d after the wood samples were completely penetrated by hyphae. The relative intensities of some of the carbohydrate and lignin bands were found to rapidly change after a 15-week period of decay (Li *et al.* 2011). Thus, in the FTIR analysis, the 120-d sample decay treatment can provide more information than shorter treatments on wood structure changes.

The infrared (IR) spectrum of the decayed samples was recorded within the 600 to 4000 cm^{-1} region on a Nicolet Impact 410 FTIR spectrometer using the KBr pellets technique and sound wood as the control. The decayed wood was removed from the medium, cleaned of hyphae using a double-sided razor, dried at 103 °C to a constant mass, then ground in an agate mortar and mixed with potassium bromide to a concentration of approximately 0.5–1%. All the spectra were measured at a spectral resolution of 4 cm⁻¹ with 32 scans.

For each sample, three different subsamples were measured, and the resultant mean spectra were used for further analyses. OPUS version 6.5 software was employed for vector normalization and offset correction and for the computation of the mean FTIR spectra and their standard deviations.

Statistical Analysis

Data processing was performed using SPSS variance analysis (ANOVA), followed by Duncan's multiple range test. The significance of differences among the samples in 1% NaOH extractives, ethanol-benzene extractives, and holocellulose was tested by using Fisher's least significant difference (LSD) test at the 5% probability level after a one-way analysis of variance. The changes in the FTIR absorption of the lignin/cellulose functional group are shown in Figs. 4 and 5 as the difference between the sample and the control.

RESULTS AND DISCUSSION

Weight Loss of the Decayed Wood

The weight loss values of the wood samples decayed by the four different woodrot fungi are given in Table 1 and ranged from 34.97% to 62.55%. The variance analysis showed that there was a significant (p < 0.01) difference (F value ranging from 1.56 to 2.47) in the mean weight loss mentioned above. F. fomentarius degraded the wood samples the most quickly, with the least variation among the samples and a weight loss reaching 62.55%. Although showing the lowest weight loss at 34.97%, I. lacteus also had the highest variation among the samples. There was a great difference among the fungi in their ability to degrade lignin. The maximum lignin loss rate was 66.84%, shown by F. fomentarius, and the minimum was 38.94%, shown by C. versicolor. Different fungi have different growth rates and different wood degradation abilities (Liu et al. 2008). The growth rate of the wood-rot fungi was not related to their wood degradation ability; for example, C. versicolor is a widely reported wood-rot fungus (Evans 1985; Hamzeh et al. 2012; IqbalZafar et al. 1989) that has the most rapid growth but showed a medium amount of wood loss in the present study and relatively low lignin and cellulose losses. The discrepancy between its growth rate and its ability to degrade wood most likely occurred because white birch is not a natural host of C. versicolor. Pandey and Pitman (2003) reported that the weight losses of samples exposed to C. versicolor were high and that the simultaneous decay resulted in little change in the relative intensities of the lignin and carbohydrate bands, with only a slight preference for lignin. The holocellulose loss was the highest in the wood samples decayed by P. betulinus, reaching 73.63%, the second highest for F. fomentarius, and the lowest for I. lacteus.

Characteristics	I. lacteus	C. versicolor	F. fomentarius	P. betulinus
No. of valid samples	296	295	288	292
Mean weight loss (%)	34.97	44.72	62.55	56.77
Standard deviation	24.71	18.52	10.52	14.90
Standard error	0.08	0.06	0.04	0.05
Holocellulose loss (%)	36.43	59.58	61.79	73.63
Total lignin loss (%)	46.72	38.94	66.84	50.89
Note that the treatment time was 30 d.				

White-rot fungi break down the lignin in wood, leaving the lighter-colored cellulose behind; some of them break down both lignin and cellulose (Blanchette and Biggs 1992; Eriksson *et al.* 1990). According to the results of the current study, *C. versicolor* has the ability to break down both lignin and cellulose, whereas the other two white-rot fungi (*I. lacteus* and *F. fomentarius*) selectively degrade lignin rather than holocellulose.

Changes in the Main Chemical Composition of the Decayed Wood

The chemical composition of the wood decayed by the four species of wood-rot fungi is shown in Fig. 2. The highest cellulose content (up to 50.89%) was measured in the wood samples decayed by *F. fomentarius*, followed by the second highest content in those decayed by *I. lacteus*, which had content 2.13% lower than that of the control. *P. betulinus*, a brown-rot fungus, mainly removed cellulose, only 30.43% of which was left after the degradation treatment.





The lignin content of the samples decayed by *I. lacteus* and *F. fomentarius*, at 21.58% and 23.32%, respectively, was far less than that of the control, but this difference was not significant. For *C. versicolor* and *P. betulinus*, the lignin content increased slightly but insignificantly, whereas the holocellulose content decreased. These results are similar to those obtained by Mohareb *et al.* (2012). Notably, the white-rot fungus *C. versicolor* showed a strong ability to degrade cellulose that is comparable with the ability of the brown-rot fungi such as *P. betulinus*. This phenomenon warrants further study.

The 1% NaOH extractive of the wood decayed by each of the fungi was much higher than that of the control. The highest value, 52.29%, was measured in the wood samples decayed by *P. betulinus*. This result is similar to those obtained for Japanese red

pine decayed by *Ceriporia lacerata*, *Stereum hirsutum*, and *Polyporus brumalis*, all white-rot fungi (Lee *et al.* 2007). The contents of the ethanol-benzene extractives of the decayed samples were also higher than that of the control, except for the samples treated with *I. lacteus*.

The wood samples decayed by *F. fomentarius* were characterized by a relatively low lignin content of 23.32%, 1% NaOH extractives of 36.3%, and ethanol-benzene extractives of 6.8%, whereas the holocellulose content remained high at 50.89%. Lignin and the aforementioned extractives have a negative influence on chemical recovery during the alkaline pulping process, which is supposed to be a positive feature when considering the pulp yield and chemical consumption for their removal (Mansouri *et al.* 2012). As the results indicate, *F. fomentarius* was considered the most suitable fungus for the biological pretreatment process in pulping production.

FTIR Spectra of the Decayed Wood

The FTIR spectra of the wood samples decayed by the fungi for 120 d showed large changes in the peak intensity and peak position, compared with the sound samples without decay(Fig. 3). Table 2 shows the assignment of the peaks in the fingerprint region.



Fig. 3. FTIR spectra of white birch wood decayed by four different wood-rot fungi for 120 d after vector normalization and offset correction. Each spectrum is the mean of three replicate samples.

The FTIR spectra of the wood samples decayed by three white-rot fungi—*F*. *fomentarius, I. lacteus,* and *C. versicolor*—exhibited clear peak position shifts downward, which were caused by degradation of the lignin side chains, to 3410, 3406, and 3405 cm⁻¹ relative to the control at 3417 cm⁻¹. By contrast, there was a smaller peak shift upward to 3420 cm⁻¹ for the wood decayed by *P. betulinus*, the brown-rot fungus (Figs. 3

and 4). This result indicated that the hydroxyl groups of lignin side chains are almost unaffected by brown-rot fungi, which is consistent with the decay properties of whiteand brown-rot fungi. The variation in the band peak positions at 1636 and 1245 cm⁻¹ was much greater for the wood decayed by *I. lacteus* (shifted to 1594 cm⁻¹ and 1236 cm⁻¹, respectively), demonstrating that the conjugated aryl ketone and phenol ether bonds of lignin have been significantly reduced by the fungi.

Wave Number(cm ⁻¹)	Absorption Peak Location and Assignment
3417	O-H stretching vibration in aromatic and aliphatic OH groups of lignin
1636	C=O stretching in conjugated aryl ketones of carbonyl groups
1330	syringyl ring breathing with CO stretching of lignin (Fengel and Wegener 2003; Hergert 1971)
1245	C-O-C stretching in phenol ether bonds of lignin (Gierlinger <i>et al.</i> 2008)
1161	anti-symmetrical bridge C-O-C stretching in pyranose rings of cellulose and hemicellulose (Liang and Marchessault 1959)
898	C-O-C stretching at β-1,4-glycosidic linkages of cellulose and hemicellulose (Kacurakova <i>et al.</i> 2000)
669	out-of-plane OH bending of cellulose and hemicellulose (Liang and Marchessault 1959)

The cellulose degradation of the wood decayed by the three white-rot fungi slightly changed the peak positions (1151 cm⁻¹ for *F. fomentarius*, 1159 cm⁻¹ for *I. lacteus*, and 1151 cm⁻¹ for *C. versicolor*) compared with the control peak at 1161 cm⁻¹. However, there was an obvious decrease in the peak position of the wood treated by the brown-rot fungus *P. betulinus* (1122 cm⁻¹) (Fig. 5).



Peak position of the control

Fig. 4. Cellulose functional group changes in the FTIR absorption spectra. Each peak position change of the cellulose functional group represents the sample minus the control value.

Furthermore, a small decrease in the peak position at 898 cm^{-1} was observed for the wood samples decayed by *P. betulinus*, but there were almost no changes in the

position of the same peak for the wood decayed by all three white-rot fungi, which also confirms that the brown-rot fungus prefers to degrade cellulose. The control peak position at 669 cm⁻¹, which represents OH bending and stretching out-of-plane, significantly shifted for the wood samples decayed by all four wood-rot fungi to between 602 and 607 cm⁻¹, showing that cellulose and hemicellulose were decayed by both the white-rot and brown-rot fungi (Fig. 5).



Fig. 5. Lignin functional group changes in the FTIR absorption spectra. Each peak position change of the lignin functional group represents the sample minus the control value.

The results indicate that although there were no considerable changes in the aromatic skeleton of lignin during the wood degradation, the side chains of lignin-related aromatic compounds were still partly degraded by the three white-rot fungi, *i.e.*, as observed in the absorption peak at 3417 cm⁻¹. However, *F. fomentarius* and *C. versicolor* had greater ability than *I. lacteus* to degrade the lignin of white birch wood. These two fungi also partly decayed the cellulose- and hemicellulose-related groups of the wood, *e.g.*, the distinct decrease of the absorption peak at 669 cm⁻¹. *P. betulinus*, the brown-rot fungus, was nearly unable to degrade the lignin functional groups of the wood, although some changes could be seen at 1245 cm⁻¹ and 1330 cm⁻¹ on the FTIR spectra. This fungus showed an obvious preference for cellulose- and hemicellulose-related groups because of the dramatic decline in the absorption peaks at 898 cm⁻¹ and 1161 cm⁻¹ compared with the control. The same phenomenon has been found for the brown-rot decay process of *Coniophora puteana* (Pandey and Pitman 2003).

The pattern of lignin degradation of the wood decayed by the four wood-rot fungi varied regarding the FTIR peak absorption intensity and position change and indicated that *F. fomentarius*, *I. lacteus*, and *P. betulinus* caused more lignin degradation than *C. versicolor*, similar to the results of the wood chemical composition analysis.

White-decay fungi are able to break down the lignin functional groups of wood, leaving the lighter-colored wood behind, and to simultaneously act on cellulose and hemicellulose. By contrast, brown-rot fungi prefer to break down hemicellulose- and cellulose-related groups, and this degradation gives the wood a brown discoloration. Nevertheless, the brown-rot fungi are not believed to have the ability to degrade lignin (Deacon 2009). However, the current study shows that this conclusion is not absolutely

correct because the FTIR peak positions of the wood samples treated with *P. betulinus* and *F. fomentarius* were detected at 1327 cm⁻¹ and 1325 cm⁻¹, both slightly decreased compared with the control at 1330 cm⁻¹. These findings further enhance the understanding of hemicellulosic biodegradation and may have important implications for microbial-assisted pulping and for biofuel and biomaterial production.

CONCLUSIONS

- 1. *C. versicolor* decayed both lignin and cellulose, whereas *I. lacteus* and *F. fomentarius* selectively degraded lignin rather than holocellulose.
- 2. *F. fomentarius* degraded the wood samples the most quickly, with the weight loss reaching 62.55%, and produced the least variation among the samples. The 34.97% weight loss caused by *I. lacteus* was the lowest, but this loss was associated with the greatest variation among the samples.
- 3. The maximum lignin loss rate was 66.84%, caused by *F. fomentarius*, and the minimum value was 38.94%, caused by *C. versicolor*. The loss of holocellulose was the greatest in the wood samples decayed by *P. betulinus*, reaching 73.63%.
- 4. The samples decayed by *I. lacteus* and *F. fomentarius* contained far less lignin than the control, only 21.58% and 23.32% of the control, respectively. These fungi can be considered potential agents for the biopulping of white birch wood because the wood content was higher in holocellulose, lower in lignin, and lower in the 1% NaOH and ethanol-benzene extractives after biopretreatment.
- 5. The peak position of 669 cm⁻¹ for the control wood samples was significantly shifted by the decay caused by the four wood-rot fungi to between 602 and 607 cm⁻¹, showing that both white- and brown-rot fungi decayed cellulose and hemicellulose.
- 6. Although there were no considerable changes in the aromatic skeleton of lignin during the lignin degradation, the side chains of lignin-related aromatic compounds were still partly degraded by the three white-rot fungi, as indicated by the decay-induced shifts in the absorption peak positioned at 3417 cm^{-1} in the control.
- 7. *P. betulinus* preferred cellulose- and hemicellulose-related groups, judging from the dramatic decline in the absorption peaks occurring at 898 cm⁻¹ and 1161 cm⁻¹ compared with the control, but also degraded the lignin of the wood.

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