EFFECTS OF FUNGAL TREATMENT ON STRUCTURAL AND CHEMICAL FEATURES OF HORNBEAM CHIPS

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Structural and chemical changes were investigated in Hornbeam (*Carpinus betulus*) chips that had been exposed to *Phanerochaete chrysosporium* BKM-1767 fungus. Samples subjected to fungal treatments for durations of 1, 2, and 4 weeks were investigated and compared with a control sample not subjected to fungal treatment. Results of scanning electron microscopy indicated that fungal hyphae were present on the surfaces of all chips exposed to the fungus. In the samples treated for a 2 or 4-week period, these hyphae additionally penetrated into vessels and lumens through ray cells, softening and destroying the cell walls. FT-IR spectra indicated that fungal treatment modified the chemical structure of the wood. Furthermore, there was a remarkable decrease in the amount of lignin in woods exposed to fungus. Lignin decreases after 1, 2, and 4 weeks of treatment were 2.83%, 11.4%, and 18.56%, respectively. Measurement of fiber dimensions indicated that cell wall thicknesses decreased after treatment, but that the lumen width increased compared with the control sample.

Keywords: Hornbeam; Phanerochaete chrysosporium BKM-1767; SEM; Lignin; FTIR

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INTRODUCTION

Many different organisms cause damage to wood, but fungi are among the most damaging. White rot fungi are a diverse group of organisms that are able to break down lignin. There are many morphologically different patterns of white rot that occur in wood due to variations in the way lignin and polysaccharides are removed. White rot fungi have complex extracellular lignolytic enzyme systems that include lignin peroxidase, manganese peroxidase, and laccase. These systems can selectively remove or alter lignin to permit removal of cellulose fibers (Breen and Singleton 1999). Some white rot fungi have the ability to selectively remove extensive amounts of lignin (Messner and Serbotnik 1994). Studies of wood and lignin decomposition by fungi are very complex and are often confounded by several factors. One of these is the nature of the lignin polymer. Unlike many other biopolymers, the lignin polymer does not contain repeating

units joined by bonds that are readily cleaved. When a complex macromolecule such as lignin is present in a wood matrix, it is possible to change the physical properties of the wood without changing the chemical properties of the component macromolecules to the same extent (Villalba et al. 2006). The development of the biopulping technologies (Akhtar et al. 1998; Ferraz et al. 1998; Messner and Serbotnik 1994; Chen and Schimidt 1995) has made the selection of fungal species for lignin degradation a frequent subject of experimental research (Akhtar et al. 1994; Rodriguez et al. 1997; Bettucci et al. 1992; Setliff et al. 1990). However, the number of fungal species that can be assayed is limited (Ferraz et al. 2000). Some fungi are effective in degrading hardwoods, whereas others are effective with both hardwoods and softwoods. *C. subvermispora*, *P. chrysosporium*, and *P. subserialis* are among the most effective species for both types of wood (Kirk et al. 1993). The ability of white rot fungi to decompose lignin, and especially their ability to selectively degrade lignin from wood, makes these fungi ideally suited for industrial applications where lignin or various phenolic compounds must be altered or removed (Blanchette et al. 1988). Fungal growth in wood chips causes concomitant changes in the chemical structure of the wood, which facilitate fiber separation, thus saving electrical energy and improving the strength properties of certain industrial products (Hakala et al. 2004). The fungus opens the wood cell wall structure, permits greater access to wood components, and can lead to energy savings in mechanical pulping (Villalba et al. 2006).

White rot fungus changes the structure of lignin, and these changes can improve refinement energy costs, strengthen paper, facilitate pulp bleaching, and ameliorate environmental issues. Fourier transform infrared spectroscopy (FT-IR) is one of the methods used to study structural changes in lignin. FT-IR spectroscopy has been used as a simple technique for analysis of wood samples and as a tool for monitoring structural wood changes during chemical or physical processing (Michell 1988; Owen and Thomas 1989; Michell 1994). Comparison of FT-IR spectra has shown that damage caused by white rot fungus to the lignin structure of hardwoods is more extensive than that to softwoods (Hakala et al. 2004). White rot fungi affect the lignin component of the wood more than they affect hemicellulose. Correlation of FT-IR spectra with the content of lignin, cellulose, and hemicellulose in treated chips indicated that damage caused by white rot fungus to the lignin structure is more extensive than that to cellulose and hemicellulose structures (Qin et al. 2004). In addition, a comparison among lignin spectra showed in treated wood chips showed that lignin structures are destroyed by white rot fungus (Yang et al. 2007).

The present research studied the effects of fungal treatment on the structural and chemical properties of hornbeam chips. Structural and chemical changes to lignin and to other chip components were assessed as a function of fungal treatment, using scanning electron microscopy (SEM) and Fourier transform infra-red spectroscopy (FT-IR). Also, this paper focuses on the evaluation FT-IR as an analytical tool for monitoring wood degradation. Previous research has focused more on the effects of fungal treatment on refining energy and paper properties, and studies of the effects of fungal treatment on the structural and chemical features of chips are rare. Furthermore, the previous softwoods and hardwoods investigated were of low density, whereas hornbeam is a high-density hardwood that is useful in papermaking.

EXPERIMENTAL

Fungal Treatment

Hornbeam chips were obtained from the Mazandaran Paper Factory in Iran and were completely washed and dried in fresh air. After drying, they were put in plastic bags to prevent growth of infectious microorganisms. The fungus used in this research was *Phanerochaete chrysosporium* BKM-1767. In accordance with methods described in the literature (Kirk et al. 1993; Akhtar 1997), this fungus was first inoculated on a solid plate culture and stored at a temperature of 39 ºC for 5 days. Afterwards, it was inoculated in a liquid plate culture for an additional 5 days at a temperature of 39 ºC. In order to stop development after completing these preparation stages, the fungi were transferred to and stored in a refrigerator at a temperature of 4 ºC.

The bioreactor used in this study was an aerated, static-bed type reactor of cylindrical shape. It had a capacity of about 21 liters and was made of steel sheets. A pipe under the bioreactor allowed the passage of air into the reactor, with flow supplied by an aquarium pump.

According to literature methods (Kirk et al. 1993; Akhtar 1997), wood chips were autoclaved for 30 minutes to prevent infection by microorganisms. Under sterile conditions, about 1500 g chips (on a dry weight basis) were poured into the bioreactor. Inoculum liquid was mixed with unsterile Corn Steep Liquor (0.5 % of dry weight) and was poured over the chips. To ensure that the injection liquid affected all chips, they were mixed thoroughly. Using sterile water, they were kept in an environment with suitable humidity for fungus growth (about 55-60%). This bioreactor was put in an incubator with a temperature of 39 ºC and a relative humidity of 65%. After treatment periods of 1, 2, and 4 weeks, chips were placed in plastic bags and frozen to stop fungal activity.

Scanning Electron Microscopy

Wood samples were sputter-coated with 15 nm of gold-palladium alloy and were observed in a JXA- 840 Model JEOL scanning electron microscope, to explore the growth patterns of the fungi in the wood on a microscopic level. The control sample was similarly examined as a basis to assess the altered structure of the fungal treated wood.

Measurement of Weight Loss

Before the incubation, the wood chips were dried to constant weight at 40 ºC. After the incubation, the wood chips were washed by sterile distilled and were filtrated to remove the dissolved components and microorganisms. The washed chips were dried at 40 ºC to constant weight and weight loss was calculated based on the initial and final dry weights.

Chemical Analysis of Materials

Lignin was determined as described in TAPPI T-222 om-88. Cellulose was identified in accordance with TAPPI T-264 om-88. For FT-IR analysis, wood chips were milled. Milled samples were sieved to pass through a 100 mm screen. The 100 mm-sieved sample was dried at 30 ºC for 6 h. Seven mg of sample were homogenized with 225 mg KBR for 1 min and put in a macro-cup of the Spike Technologies attachment for

FT-IR spectroscopy. FT-IR spectra of wood chips before and after treatment were recorded with an MB-FT-IR 100 Bomem instrument over a frequency range of 400 to 4000 cm^{-1}.

Measurement of Fiber Biometry Characteristics

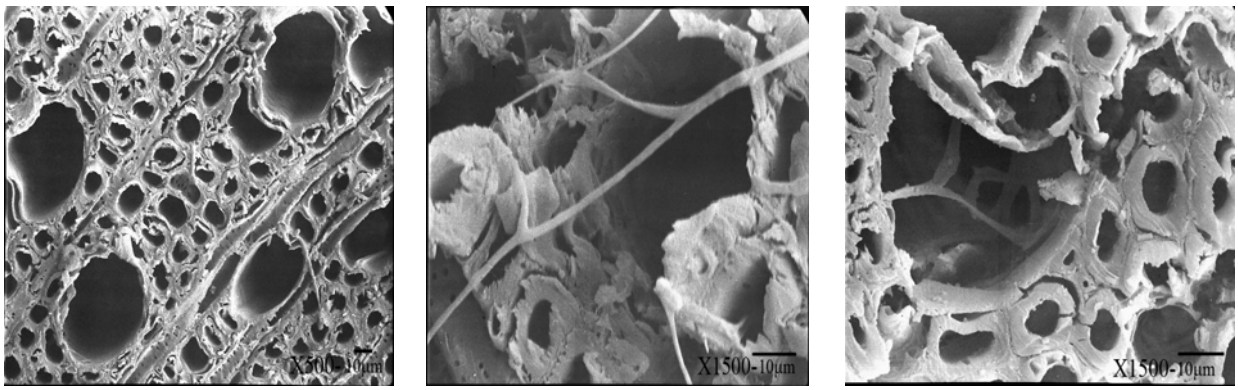
From each treatment, several chips were chosen randomly and were cut about 10 mm pieces. The Franklin method (1954) was used to separate fibers. Initially, the pieces of chips were treated in a mixed solution (50/50,v/v) of acetic acid (solution 63%) and hydrogen peroxide (solution 33%) for a period of 24 hours at 60 ºC. Then, the samples were washed by distilled water, and the fibers were separated by gentle shaking. The fibers were stained with 1% aqueous safranin-o solution and placed on 9 glass microscope slides for each treatment. The fiber length, fiber diameter, and lumen width were measured with a microscope equipped with a Leica Image Analysis System (Quantimeta 100+). The fiber wall thickness was calculated as a difference of fiber diameter and lumen width divided in half. Fibers were measured for samples subjected to treatments of 1, 2, and 4 weeks in duration. For each treatment 10 fibers were measured on each slide, resulting in 90 fibers measured. From these data, the average fiber dimensions were calculated and then the following derived indexes were determined:

Slenderness ratio = Length of fiber / Diameter of fiber
Flexibility ratio = (Lumen width of fiber / Diameter of fiber) 100
Runkel ratio = 2 (Wall thickness / Lumen width) 100

RESULTS AND DISCUSSION

Structural Changes

Figure 1(a) shows transverse sections of chips after one week of treatment. There was no apparent change in vessels and cell walls.



a **b** **c**

Fig. 1. a. Transverse sections of wood chips after 1 week of, **b.** Transverse sections of wood chips incubated with P. chrysosporium for 2 weeks. Fungal hyphae are apparent, **c.** Cell wall structure of wood chips after 4 weeks of treatment with P. chrysosporium.

Figure 1(b) shows transverse sections of wood chips after two weeks of treatment. Fungus hyphae were clearly present inside the vessels and lumens. They also had penetrated to the adjacent cells through pits.

Figure 1(c) shows transverse sections of wood chips after four weeks of treatment. Fungal hyphae were present inside the vessels and lumens, and cellular walls had been destroyed.

In all three cases, fungal hyphae were visible all over the chips, together with plentiful calcium oxalate crystals. White rot fungi adapt readily to their environment and consume the available sugars and other foods, which are plentiful in ray cells. In 1998, Messner et al. reported that the amount of sugar in the chips decreased due to fungal treatment. For treatments lasting 2 and 4 weeks, the fungus made important changes in the cell structure of the wood. This structure was weakened and the cell walls thinned. Using a microtome, layers of treated samples were easily separated. However, boiling in water for 3 to 4 hours was required for untreated samples to soften them for separation. These results are similar to results that were published by Villalba et al. (2006), except that these authors used a sample of softwood (Loblolly Pine) and a different type of fungus (*Cepiporiopsis subvermispora*) in their research.

Measurements of fiber dimensions indicated that the lengths, widths, and thicknesses of cell walls in treated wood decreased, but that the widths of cell lumens increased. Significant changes (at the 5% level or more) were observed in cell wall thicknesses after treatment for 1, 2, or 4 weeks compared with the control treatment. Table 1 presents the results of the fiber dimension measurements.

Table 1. Mean Values of Indices Derived from Fiber Dimensions - Comparison between the Fibers of Control (untreated) Wood Chips and Treated Wood Chips.

4 Weeks	2 Weeks	1 Week	Control	Fiber properties
1205.8	1210.7	1226.7	1253.23	Length (mm)
22.01	47.22	23.23	23.97	Diameter (μm)
5. 73	6.19	6.36	6.64	Cell wall thickness (μm)
11.25	11.01	10.81	10.7	Lumen width (μm)
54.79	53.86	52.81	52.28	Slenderness ratio (%)
51.11	48.99	46.53	44.62	Flexibility ratio (%)
101.87	112.44	117.69	124.11	Runkel ratio (%)

Remarkably, the cell lumens increased in size in treated chips. This phenomenon is possible because the fungus attacked the cell walls through the lumens, so the thicknesses of cell walls decreased. Except for the Rankle coefficient, two other coefficients increased. The decrease in cell wall thickness and the increase in cell lumen width made the Rankle coefficient decrease. No previous reports of fiber dimensions in fungal-treated wood chips are known to the authors as available for comparison.

Chemical Changes

Wood chips subjected to treatments of 2 and 4 weeks' duration lost more weight than untreated samples and those treated for only 1 week. This weight loss is attributed to loss of lignin in the treated chips. The fungus first consumes sugar in ray cells, decreasing

the sugar content of these cells and causing the chips to lose weight. Blanchette et al. (1988) reported that wood chips lost 38% of their weight after 12 weeks of treatment with the fungus *Phanerochaete chrysosporium* BKM-F1767. In their report, 46% of the initial weight was lost after exposure to fungus HHB-11741, 31% was lost due to fungus HHB-6251, 17% was lost due to fungus FPL-V-1706, and 38% was lost due to fungus ME-PC-8. The weight loss process for these chips was similar to the process published by Hakala et al. (2004). In their reports, the chips lost 0.8%, 6.3%, and 19% of their initial weight after 2, 6, and 10 weeks of exposure to *Physisporinus rivulsos* T241 I, respectively. Hernandz et al. (2005) reported 2 to 3% loss in weight after 2 weeks of treatment with *Streptomyces cyaneus*.

Table 2. Chemical Analyses of Control (untreated) and Treated Wood Chips

4 Weeks	2 Weeks	1 Week	Control	Analysis
18.56	11.4	2.83	0	Weight Loss (%)
19	20.67	22.67	23.33	Lignin (%)
56.5	55	53	50.67	Cellulose (%)

The greatest decrease in lignin content occurred after 4 weeks of treatment. These samples suffered an 18.56% decrease relative to the control sample. For treatments lasting "1" and "2" weeks, this decrease was about 2.83% and 11.4%, respectively. Blandchete et al. (1988) stated that 12 weeks of treatment with *Phanerochaete chrysosporium* resulted in a 73% decrease in lignin. In their report, the HHB-11741 fungus caused a 51% decrease in lignin, HHB-6251 fungus caused a 28% decrease, FPL-V-1706 fungus caused a 27% decrease, and finally ME-PC-8 fungus caused a 23% decrease. Such decreases are similar to those reported by Hakala et al. (2004). In that report, the lignin decreases due to *Physisporinus rivulosus* T 241 I exposure for 2, 6, and 10 weeks were 1.9, 16, and 39%, respectively. Using the fungus *Phanerochaete chrysosporium* F-1767, an 8.1% decrease in the amount of lignin was reported after 10 weeks.

FTIR Results

The IR spectra of wood chips were obtained over a wavelength range of 400 to 4000 cm^{-1}. The characteristic spectra of wood structures are most apparent within the range 1000-1800 cm^{-1}, and the major spectral peaks in this range have been identified (Pandey 1999; Yang et al. 2007).

FT-IR spectra of chips treated with *Phanerochaete chrysosporium* for 1, 2, and 4 weeks were compared with an untreated sample. The comparison was performed over the wavelength range corresponding to the fingerprint of lignin (1000-1800 cm^{-1}). Figures 2 through 5, show spectra of untreated chips and chips treated for one, two, and four weeks.

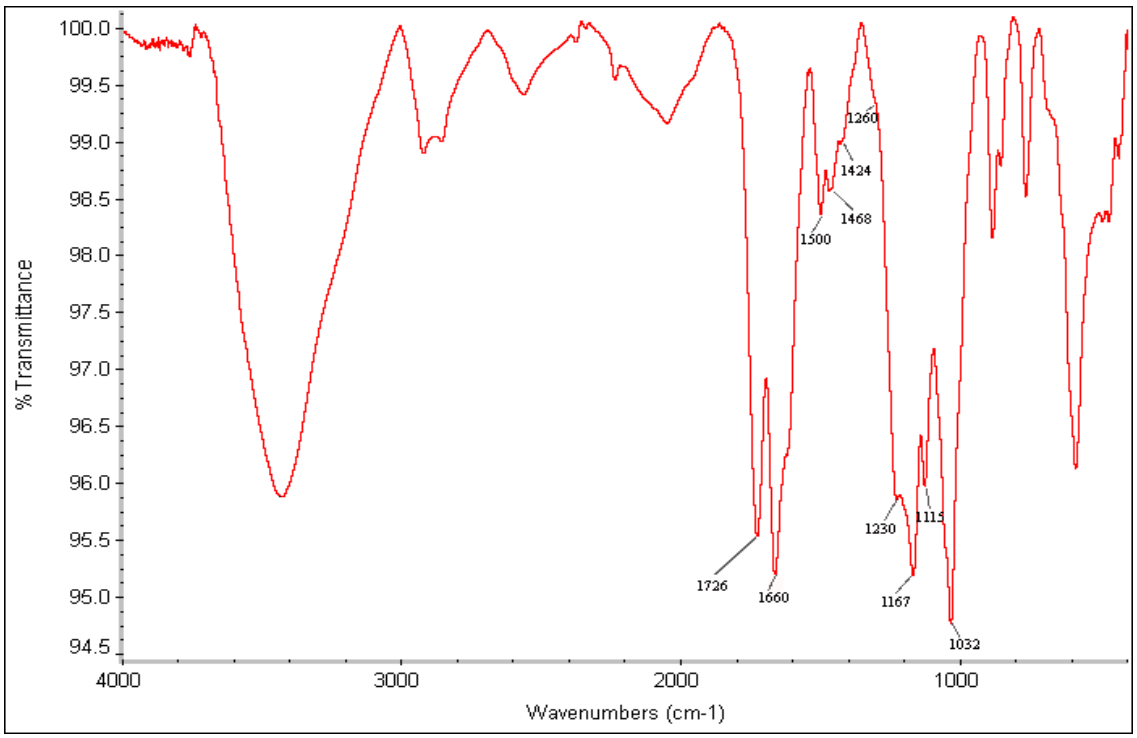


Fig. 2. FT-IR spectrum of untreated chips

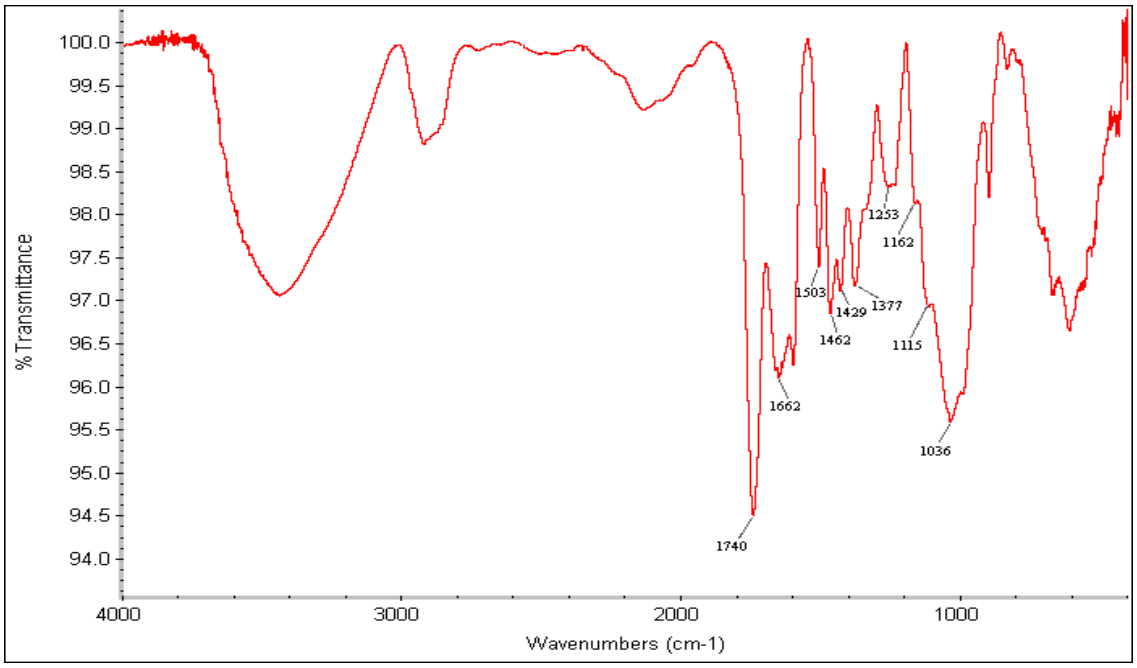


Fig. 3. FT-IR spectrum of chips treated for 1 week with *Phanerochaete chrysosporium*

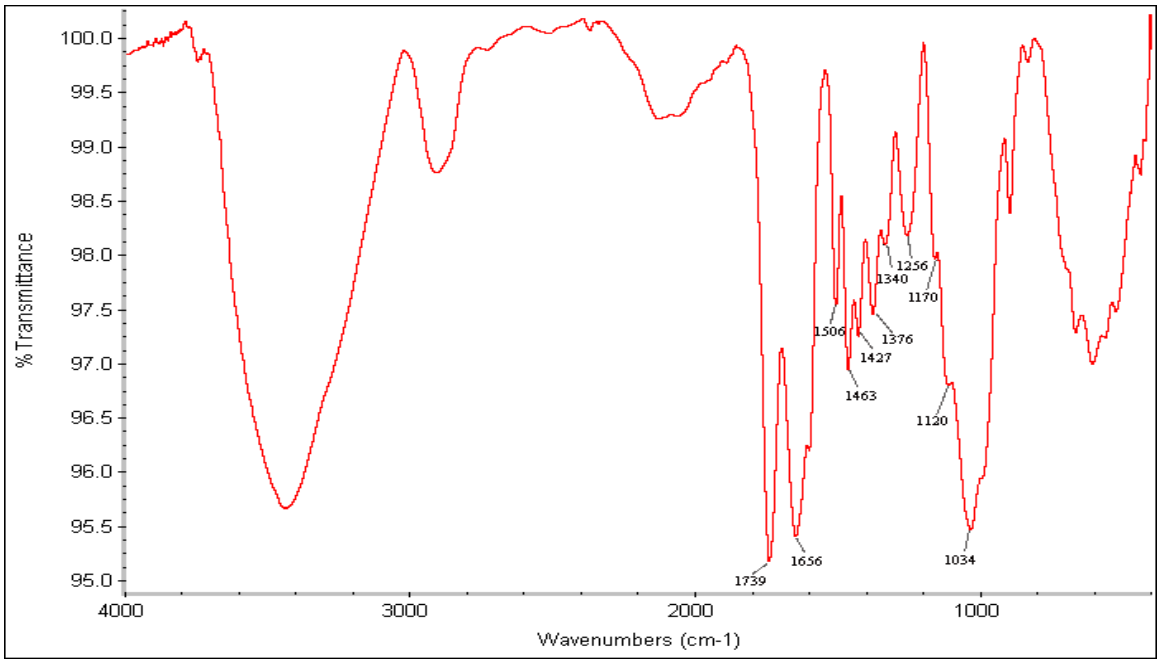


Fig. 4. FT-IR spectra of chips treated for 2 weeks with Phanerochaete chrysosporium.

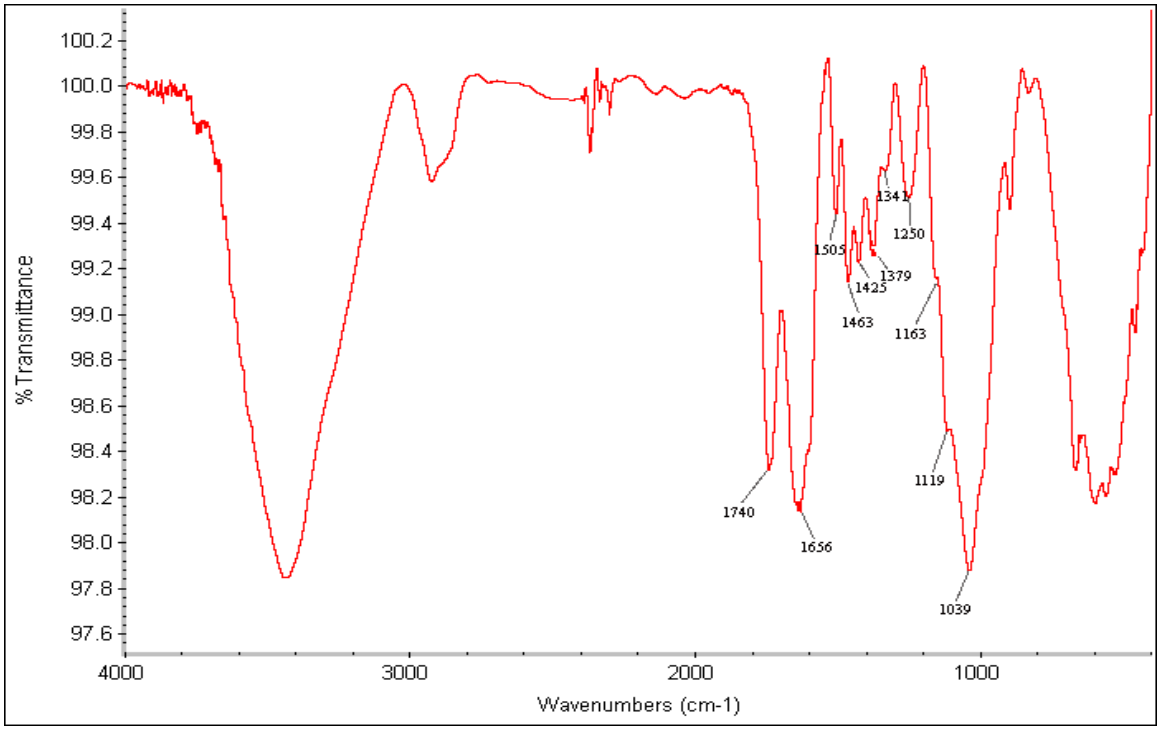


Fig. 5. FT-IR spectra of chips treated for 4 weeks with Phanerochaete chrysosporium.

In general, chips treated with *P. chrysosporium* exhibited lower peak band intensities over the range of 1000-1100 cm^{-1} (C-O bands), compared with untreated samples. The decrease was most marked in the sample treated for four weeks and cannot be related to a decrease in sugar content. Ferraz et al. (2000) reported that spectral band intensities over 1000-1100 cm^{-1} (C-O bands) increased in chips treated with brown rot fungus. This was attributed to an increase in glucans in chips treated with brown rot fungus.

No significant change was observed in the spectral intensities of the 1730 cm^{-1} (unconjugated C=O) band for samples treated for one and two weeks. The spectral intensity decreased in the case of a four week treatment. This decrease could be related to a decrease in the formation of non-coupled C=O groups in the side chain of the lignin macromolecule in chips treated with fungus.

In samples treated for one and two weeks, band intensities at 1660 cm^{-1} (conjugated C=O band) were similar to that of the control. The intensity decreased after a four-week treatment. This decrease could be related to the function of the *P. chrysosporium* fungus. Chemical analysis and decreasing chip weights indicated that fungal activity was concentrated on lignin in the chips. This destruction results in a lower-intensity peak associated with the conjugated C=O band in lignin. Yang et al. (2007) reported that peaks related to lignin in fungal-treated chips were weaker than those in untreated samples.

A detailed review of the spectra between 1000 and 1200 cm^{-1} highlights the differences between treated and untreated samples. Because of fungal activity, peak intensities of bands at 1115 cm^{-1}, 1167 cm^{-1}, and 1230 cm^{-1} decreased as treatment time increased. The band at 1230 cm^{-1} is related to the C-O bond in the aromatic ring of lignin and to the bending vibration of the hydroxyl phenol. After fungal treatment, the formation of free radicals and dimers on lignin does not occur. Therefore, the connection of phenyl propane and the formation of macromolecular chains of lignin are disturbed. In similar research, Hakala et al. (2004) reported that the destruction of lignin structures by white rot fungus is more complete in hardwoods than in softwoods. Qin et al. (2004) reported that more lignin was destroyed in treated chips to a greater extent, compared to cellulose and hemi-cellulose. FTIR spectra reported by Yang et al. (2007) showed that lignin structures in treated chips were more degraded compared to those in untreated chips.

CONCLUSIONS

1. Electron microscopy indicated that fungal activity was not limited to chip surfaces. Fungus penetrated into chip vessels and lumens. The fungus then developed inside fibers and permeated into adjacent cells through pits.
2. Results indicated that fungal activity makes the cell walls soft and thin in hardwood. Together with destruction of cell walls, these changes can have positive effects on the papermaking process.

3. Morphological studies indicated that in treated chips, cell wall thicknesses decreased but lumen widths increased. This indicates that cell wall thinning was a direct result of fungal activity.
4. Chemical analysis showed that lignin decreased by 2.83%, 11.4%, and 18.56% after treatment for one, two, and four weeks, respectively.
5. FT-IR spectra showed that lignin was destroyed by fungal activity and that this destruction was accompanied by weight loss.
6. Notably, this study illustrates the effects of white rot fungus on hornbeam, which is a high density hardwood. Previous studies have focused on softwoods or low density hardwoods.

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REFERENCES CITED

Akhtar, M. (1994). "Biomechanical pulping of aspen wood chips with three strains of *Ceriporiopsis subvermispora*," *Holzforschung* 48, 199-202.

Akhtar, M., Blanchette, R. A., Myers, G., and Kirk, T .K. (1998). E*nvironmentally Friendly Technologies for the Pulp and Paper Industry*, Wiley, New York.

Akhtar, M., Lentz, M. J., Blanchette, R. A., and Kirk, T. K. (1997). "Corn steep liquor lowers the amount of inoculum for biopulping," *TAPPI J.* 80(6), 161-165.

Bettucci, L., Speranza, M., and Piaggio, M. (1992). "Selecting white-rot fungi for biopulping," *Proceedings of the Braz. Symp. Chem. Lignins and other Wood Components* 3, 394-401.

Blanchette, R. A., Burnes, T. A., Leatham,G. F., and Effland, M. J. (1988). "Selection of white- rot fungi for biopulping," *Biomass J*. 15, 93-101.

Breen, A., and Singleton, F. L. (1999). "Fungi in lignocellulose breakdown and biopulping," *Current Opinion in Biotechnology* 10, 252-258.

Chen, Y.R., and Schimidt, E. L. (1995). "Improving aspen kraft pulp by a novel low-technology fungal pretreatment," *Wood Fiber Science J*. 27, 198-204.

Ferraz, A., Baeza, J., Rodriguez, J., and Freer, J. (2000). "Estimating the chemical composition of biodegraded pine and eucalyptus wood by DRIFT spectroscopy and multivariate analysis," *Bioresource Technology J*. 74, 201-21.

Ferraz, A., Christov, L., and Akhtar, M. (1998). "Fungal pretreatment for organosolv pulping and dissolving pulping production," *Environmentally Friendly Technologies for the Pulp and Paper Industry*, Wiley, New York, 421-447.

Franklin, G. (1954). "A rapid method of softening wood for microtome sectioning," *Tropical woods J*. 88, 35-36.

Hakala, T. K., Maijala, P., Konn, J., and Hatakka, A. (2004). "Evaluation of novel wood-rotting polypores and corticioid fungi for the decay and biopulping of Norway Spruce wood," *Enzyme and Microbial Technology J*. 34, 255-263.

Hernandez, M., Hernández-Coronado, M. J., and Perez, M. I. (2005). "Biomechanical pulping of spruce wood chips with *Streptomyces cyaneus* CECT 3335 and handsheet characterization," *Holzaz J*. 59, 173-177.

Kirk, T. K., Koning, J. W., Burgess, R. R., Akhtar, M., Blanchette, R. A, Cameron, D. C., Cullen, D., Kersten, P. J., Lightfoot, E. N., Mayers, G. C., Sachs, I., Sykes, M. B., and Wall, M. B. (1993). "Biopulping A glimpse of the future?," Madison: Wisconsin. Rep. FPl- RR- 523.

Messner, K., and Srebotnik, E. (1994). "Biopulping: An overview of developments in an environmentally safe papermaking technology," *FEMS Microbiol. Rev. J*. 13, 351-364.

Messner, K., Koller, K., Wall, M. B., Akhtar, M., and Scott, G. M. (1998). "Fungal treatment of wood chips for chemical pulping," *Environmentally Friendly Technologies for the Pulp and Paper Industry*, Wiley, New York.

Michell, A. J. (1988). "Infra-red spectroscopy transformed – New applications in wood and pulping chemistry," *Appita*. 41, 375-380.

Michell, A. J. (1994). "Vibrational spectroscopy – A rapid means of estimating plantation pulp wood quality," *Appita* 47, 29-37.

Owen, L. L., and Thomas, D. W. (1989). "Infrared studies of hard and soft woods," *Appl. Spectroscopy* 43, 451-455.

Pandey, K. K. (1999). "A study of chemical structure of soft and hardwood and wood polymers by FTIR spectroscopy," *Applied Polymer Science J*. 71, 1969-1975.

Qin, T. F., Huang, L. H., and Zhou, Q. (2004). "The chemical groups and bonds characterization of juvenile wood and mature wood lignins of Chinese Fir," *Scientia Silvae Sinicae J*. 40, 137-141.

Rodriguez, J., Ferraz, A., Nogueira, R.P., Ferrer, I., Esposito, E., and Duran, N. (1997). "Lignin biodegradation by the ascomycete *Chrysonilia sitophila*," *Appl. Biochem. Biotechnol*. 62, 233-242.

Setliff, E.C., Marton, R., Granzow, S.G., Eriksson, K.L. (1990). "Biomechanical pulping with white-rot fungi," *Tappi J*. 73(8), 141-147.

Villalba, L. L., Scott, G. M., and Schroeder, L. R. (2006). "Modification of loblolly pine chips with *Ceriporiopsis subvermispora*. Part 1: Effect of fungal treatment," *Wood Chemistry and Technology J*. 26(4), 339-348.

Wolfaardt, F., Taljaard, J. L., Jacobs, A., Male, J. R., and Rabie, C. J. (2004). "Assessment of wood-inhabiting basidiomycetes for biokraft pulping of softwood chips," *Bioresource Technology J*. 95, 25-30.

Yang, J. S., Ni, J. R., Yuan, H. L., and Wang, E. (2007). "Biodegradation of three different wood chips by *Pseudomonas* sp. PKE 117," *Intepnational Biodeterioration & Biodegradation J*. 60, 90-95.

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