The Influence of Fungal Treatment on Structural, Biometry and Chemical Properties of Hornbeam Chips

Jafar Ebrahimpour Kasmani, Majid Kiaei and Ahmad Samariha

Islamic Azad University, Savadkoh Branch, Young Researchers Club, Iran
Department of Wood and Paper Science and Technology, Islamic Azad University, Chalous Branch, Iran
Islamic Azad University, Science and Research Branch, Young Researchers Club, Tehran, Iran

Abstract: The structural, biometry 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. There was a significant difference between experimental samples (treated and untreated samples) in cell wall thickness, chemical properties and loss weight. So that, the cell wall thickness and lignin values were decreased and cellulose was increasing after treatment. Results of scanning electron microscopy showed that fungal hyphae were present on the surfaces of all chips exposed to the fungus. In the treated samples for a 2 or 4-week period, these hyphae additionally penetrated into vessels and lumens through ray cells, softening and destroying the cell walls.

Key words: Biometry properties • Chemical properties • Hornbeam • Phanerochaete chrysosporium BKM-1767

INTRODUCTION

Many different organisms cause damage to wood, but fungi are among the most damaging. White rot fungi are diverse groups 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 rots 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 [1]. Some white rots fungi can remove selectively extensive amounts of lignin [2]. Studies of wood and lignin decomposition by fungi are very complex and are often confounded by several factors. One of these is the natures 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 [3]. The development of the biopulping technologies [4-7] has made the selection of fungal species for lignin degradation a frequent subject of experimental research [8-11]. However, the number of fungal species that can be assayed is limited [12]. 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 [13]. The ability of white rot fungi to decompose lignin and especially their ability to degrade selectively lignin from wood, makes these fungi ideally suited for industrial applications where lignin or various phenolic compounds must be altered or removed [14]. Fungal growth in wood chips causes concomitant changes in the chemical structure of the wood, which simplify fiber separation, thus saving electrical energy and improving the strength properties of certain industrial products [15]. The fungus opens the wood cell wall structure, permits greater access to wood components and can lead to energy savings in mechanical pulping [3]. This presents research studied the effects of fungal treatment on the structural, biometry and chemical properties of hornbeam chips. Previous research has focused more on the effects of fungal treatment on refining energy and paper

Corresponding Author: Jafar Ebrahimpour Kasmani, Islamic Azad University, Savadkoh Branch, Young Researchers Club, Iran. Postal Code: 47491/74351, Tel: +98-9112178835, E-mail: jafar_kasmani@yahoo.com.
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 in Iran.

MATERIALS AND METHODS

**Fungal Treatment:** Hornbeam chips were obtained from the Mazandaran Paper Factory in Iran and were completely washed and dried the fresh air. After drying, they were put in plastic bags to prevent the growth of infectious microorganisms. The fungus used in this research was *Phanerochaete chrysosporium* BKM-1767. In accordance with methods described in the literature [13, 16], this fungus was first inoculated on a solid plate culture and stored at a temperature of 39°C for 5 days. Afterwards, it was inoculated in a liquid plate culture for an additional 5 day at a temperature of 39°C. To stop development after completing these preparation stages, the fungi were transferred to and stored in a refrigerator at a temperature of 4°C. The bioreactor used in this study was an aerated, static-bed type reactor of cylindrical shape. It had a capacity to about 21 liters and was made of steel sheets. A pipe under the bioreactor allowed the passage of air into the reactor, with the flow supplied by an aquarium pump. According to literature methods [13, 17], 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 affect 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°C and 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 that sputter-coated with 15 Nm of gold-palladium alloy and were observed in a JXA- 840 Models 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.

**Biometry and Chemical Properties:** Cellulose and lignin were identified in accordance with TAPPI T-264 om-88 and TAPPI T-222 om-88. From each treatment, several chips were chosen randomly and were cut about 10 mm pieces for measurement fiber properties. The Franklin method (1954) [18] 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 24 hours at 60°C. Then, distilled water washed the samples and gentle separated the fibers 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 (Quantimetra 100+). The fiber wall’s thickness was calculated as a difference of fiber diameter and lumen width divided to two. 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:

- **Felling 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

**Weight Loss:** Before the incubation, the wood chips were dried to constant weight at 40°C. 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°C to constant weight and weight loss was calculated based on the initial and final dry weights. To determine the relationship between the experimental variable (fungal treatments) and biometry and chemical properties, all the data measured were subjected to an analysis of variance and Duncan’s mean separation test.

RESULTS

Figure 1(a) shows transverse sections of chips after one week of treatment. There was no apparent change in vessels and cell walls. 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. The fiber dimensions as well as calculated runkel, flexibility and felting coefficients are summarized in Table 1 and the percentage of cellulose, lignin, ash, extractive soluble in alcohol-acetone and weight loss is summarized in Table 2. Each value in Tables 1 is the average of 90 fibers measurements for each of fungal treatments and each value in Table 2 is the average of three measurements for each of fungal treatments. Standard deviation of the measurements is also given in both tables to show the variations of the given property. Additionally, the weight loss was repeated one time. Analysis of variance shows that there are significant differences between fungal treatments and chemical properties and cell walls thickness at the 95 percent confidence level. However, these differences between fungal treatments and other biometry properties
weren't significant. By increasing time of fungal treatments, the cellulose and lignin values increased and decreased, respectively. Among treated wood chips (1, 2 and 4 weeks), the ash value in treatment of 1 week is more than other treatments (2 and 4 weeks), while the extractive and weight loss values in treatment of 4 weeks are higher than other treatments (1 and 2 weeks).

**DISCUSSION**

This study examined the effects of fungal treatment on biometry, structural and chemical properties of Hornbeam chips. In all three treatments (1, 2 and 4 weeks), fungal hyphae were visible all over the chips, together with plentiful calcium oxalate crystals. White rots fungi adapt readily to their environment and consume the available sugars and other foods, which are plentiful in ray cells. Messner et al., (1998) 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 Vilaiba et al., (2006), except that these authors used a sample of softwood (Loblolly Pine) and a different type of fungus (*Cephaloera subvermispora*) in their research. Measurements of fiber dimensions showed that the lengths, widths and cell walls thickness of cell walls in treated wood decreased, but the widths of cell lumens increased. Significant changes were observed in cell wall thickness after treatment for 1, 2, or 4 weeks compared with the control treatment. 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 thickness of cell walls decreased. Except for the Rinkle coefficient, two other coefficients increased. The decrease in cell wall thickness and the increase in cell lumen width made the Rinkle coefficient decrease. No previous reports of fiber dimensions in fungal-treated wood chips are known to the authors as available for comparison.

Wood chips subjected to treatments of 2 and 4 week duration lost more weight than untreated samples and those treated for only 1 week. These samples suffered an 18.56% decrease relative to the control sample. 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. Furthermore, the greatest decrease in lignin content occurred after 4 weeks of treatment. For treatments lasting one and two weeks, this decrease was about 2.83% and 11.4%, respectively. 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% were lost due to fungus MB-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 *physioporinus rivulosus* T241 I, respectively. Hernandez et al., (2005) reported 2 to 3% loss in weight after 2 weeks of treatment with *streptomycetes cyanes*. Blanchette 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 MB-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 *physioporinus 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 for lignin was reported after 10 weeks.

**CONCLUSION**

Electron microscopy showed 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. Results showed 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. Results of biometry properties showed these in treated chips, cell wall thickness decreased butlumen widths increased. This shows that cell wall thinning was a direct result of fungal activity. Chemical analysis showed that lignin decreased by 2.83%, 11.4% and 18.56% after treatment for one, two and four weeks, respectively. 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. In future, the effect of bio-pulping on pulp and paper properties of hornbeam will be investigated.

REFERENCES


