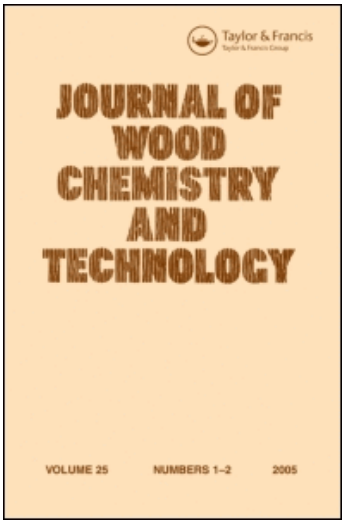

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Modification of Loblolly Pine Chips with *Ceriporiopsis subvermispora* Part 1: Effect of Fungal Treatment

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Modification of Loblolly Pine Chips with *Ceriporiopsis subvermispora* Part 1: Effect of Fungal Treatment

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Abstract: The effect of treating loblolly pine (*Pinus taeda*) chips with the fungus *Ceriporiopsis subvermispora* was investigated by assessing the wood changes at 2 and 4 weeks incubation relative to a control (no fungal treatment). Scanning electron microscopy indicated that during the first 2 weeks of colonization, *C. subvermispora* had grown over the surface of the chips and also within ray cells. A mass loss of 5% occurred after 2 weeks, 6% after four weeks. The extractives were reduced 23% in the first 2 weeks of fungal treatment and 32% after 4 weeks. Lignin loss was statistically insignificant at 2 weeks but reached 8% of the original lignin after 4 weeks. The carbohydrate content of the treated wood showed no significant differences from the control after 2 weeks and only minor losses after 4 weeks. The fraction of the wood soluble in 1% NaOH was 14.9% at 2 weeks compared to 11.5% for the control chips and increased to 18.8% after 4 weeks. This indicated only a mild decay due to the fungal treatment. At 2 weeks of fungal treatment, the lignin phenolic hydroxyl content was not significantly different from the control. However, after 4 weeks of treatment the phenolic hydroxyl groups increased by 14%. Small increases in the average pore size of the wood occurred with incubation time, including a broadening in the range of pore sizes.

Keywords: *Ceriporiopsis subvermispora*, white-rot fungus, loblolly pine, carbohydrates, lignin, extractives, pore size

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INTRODUCTION

Biopulping processes utilize naturally-occurring wood decay organisms to modify some of the wood structure and components. The fungus opens the wood cell wall structure and can lead to energy savings in mechanical pulping, enhanced paper sheet strength properties, and shorter cooking time in chemical pulping.[1] Selective removal or modification of the lignin component of the cell wall can also decrease the amount of chemicals needed in the pulping process, thereby reducing the potential environmental impact of the process while improving the economics.

Biotechnological processes are intrinsically friendly to the environment, and delignification is a biochemical process, basic to the earth's natural carbon cycle. Since white-rot fungi produce a powerful delignifying enzyme system, it is possible to take advantage of these microorganisms to improve utilization of wood resources in the papermaking process.[2] Pretreatment of wood chips with white-rot fungi prior to chemical pulping is a potential approach to decrease the cooking time to achieve a specific kappa number, or achieve a lower kappa number with a given cooking time. The latter effect could result in a lower bleaching chemical requirement. The fungal treatment also has the potential to improve paper sheet strength properties.

Thousands of strains and species of fungi have varying capacities to degrade lignin, cellulose, or hemicelluloses. The species of white-rot fungi that selectively degrade lignin are of particular interest for biopulping. The most desirable biological systems for biopulping should have the potential to minimize the process environmental impact, reduce energy consumption, and operating costs. The most promising and effective species found are *Ceriporiopsis subvermispora, Phanerochaete chrysosporium, Phlebia brevispora, Phlebia tremelosa, Phlebia subserialis*, and *Dichomitus squalens*. Some fungi are effective with hardwoods whereas others are effective with both hardwoods and softwoods. Among the most effective for both types of wood are *C. subvermispora, P. chrysosporium, and P. Subserialis*.[3]

EXPERIMENTAL

Fungal Treatment and Sampling

The fungal incubations were carried out in aerated, static-bed reactors consisting of 20-L autoclavable polypropylene containers (Figure 1). Before inoculation, the bioreactors containing chips were decontaminated using atmospheric steam for 30 min to avoid contamination with microorganisms that could prevent or compete with the growth of the *C. subvermispora*.

The fungal inoculum, unsterilized corn steep liquor, and the water required to bring the chips to 55% moisture were mixed with the chips and the chips were placed in separated layers in the bioreactors. The bioreactors

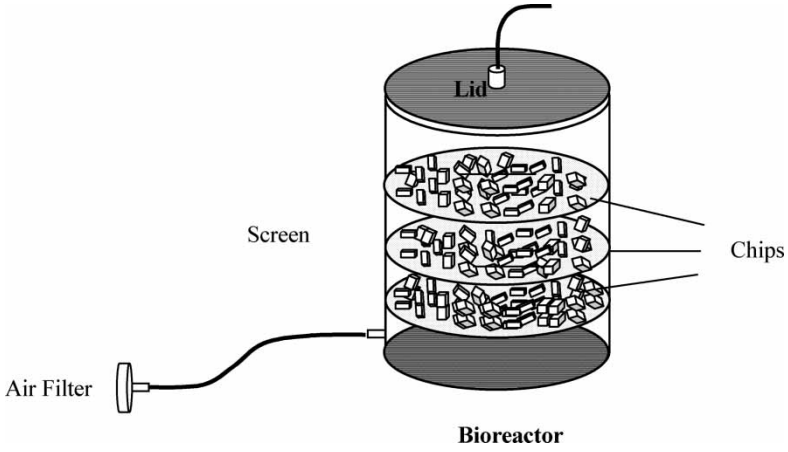

Figure 1. Aerated static-bed bioreactor showing the layering of the chips to facilitate removal of samples at biweekly intervals.

were then placed in an incubation chamber at 27°C receiving 28 L/h filtered, moisture saturated (room temperature) air for 2 and 4 weeks. After each treatment period, chip fractions were removed from the bioreactors, sealed in plastic bags, and frozen to stop fungal activity.

Control (no fungal treatment) and treated wood samples for analysis were obtained in accordance with TAPPI T257 cm-85, "Sampling and Preparing Wood for Analysis".

Scanning Electron Microscopy

Wood samples were sputter-coated with 15 nm of gold-palladium and then observed in a JEOL 5800 LV scanning electron microscope to explore the growth patterns of the fungi in the wood at a microscopic level. The control sample was similarly examined as a basis to assess the altered structure of the fungus-treated wood.

Lignin Analysis

Klason lignin was determined in accordance with TAPPI T-222 om-88, "Acid-Insoluble Lignin in Wood and Pulp". The acid-soluble lignin was determined as described in TAPPI UM 250, "Acid-Soluble Lignin in Wood and Pulp". The total lignin content of a sample was calculated as the sum of the acid-soluble and acid-insoluble lignin.

The periodate method was used to analyze the lignin phenolic hydroxyl groups. The method, developed by Adler et al.,[4] is based on oxidation of

phenolic guaiacyl or syringyl compounds to ortho-quinone structures with aqueous sodium periodate. In the oxidation, nearly 1 mole of methanol is released per mole of phenolic hydroxyl group. The methanol is determined by quantitative gas chromatography.[5]

Extractives Analysis

The wood extractives were measured according to TAPPI T204 om-88 "Solvent extractives of wood and pulp" using acetone as a solvent. Some ethanol-benzene extractions were also performed.

Sodium Hydroxide Solubility

Solubility of the wood chips in 1% NaOH was determined as described in TAPPI T212 om-88.

Carbohydrate Analyses

Carbohydrate analyses utilized a modification of a previously reported ^{1}H NMR method.[6,7] The procedure involved hydrolyzing the wood samples in acidic solution, filtering the insoluble lignin from the mixture, and analyzing the sugar monomers in the filtrate by ^{1}H NMR. Spectra for the wood hydrolyzates were recorded on a Bruker AVANCE 600 MHz NMR system. The total concentration of each sugar was determined from the sum of the integrals of its α- and β-anomeric proton doublets (the α doublet occurs above 5.00 ppm and the β doublet occurs below 4.95 ppm). To calculate the amount of individual sugars in the samples, each NMR integral total was multiplied by a survival-response factor determined from each individual sugar's response to the analytical procedure. The individual sugar content of the samples as %glycan was calculated as follows:

$$\%\text{Glycan} = 100\,(A_g/A_i)\,(W_i/W_s)\,(F_c/F_r)$$

where A_g is the integral of the sugar, A_i is integral of the internal standard (D-rhamnose), W_i is the weight of the internal standard, W_g is the oven-dry weight of the sample, F_c is the factor to convert glycose to glycan (0.88 for pentose, 0.90 for hexose, and 0.89 for monodeoxy-hexose), and F_r is the experimental hydrolysis survival-response factor.

Pore Size and Surface Area

The pore volume, surface area, and pore size distribution of the extractives-free wood samples were determined from adsorption data obtained using

the density functional theory (DFT). Adsorption isotherms experiments were conducted with liquid nitrogen in a BET Instrument model ASAP 2000 from Micromeritics, Inc. The extractives-free wood samples were outgassed at 393K under a vacuum of 5×10^{-3} mm Hg for 15 h. BET measurements were carried out using nitrogen at its boiling point, 77K, on ground wood samples (1 g).

RESULTS AND DISCUSSION

Structural and Physical Changes

Figure 2 shows a transverse section of loblolly pine after 2 weeks of incubation where a hypha, which originated in the ray parenchyma cells, has penetrated the adjacent fiber cell via a bore hole through the cell wall. It is likely that the fungal hyphae penetrate the cell walls at their weakest point, i.e. the bordered pits. A micrograph taken after 4 weeks of colonization (Figure 3) shows degraded middle lamellae and disrupted cells that are separated from one another. The fungus has caused significant modifications to the cell structure, such as a progressive thinning of the cell wall and a softening or relaxing of the tube-like cell structure. In addition, extensive fungal hyphae growth is seen throughout the wood structure, both inside and outside the cell lumen.

Table 1 summarizes the weight losses and chemical and physical analyses of the control and treated wood samples.

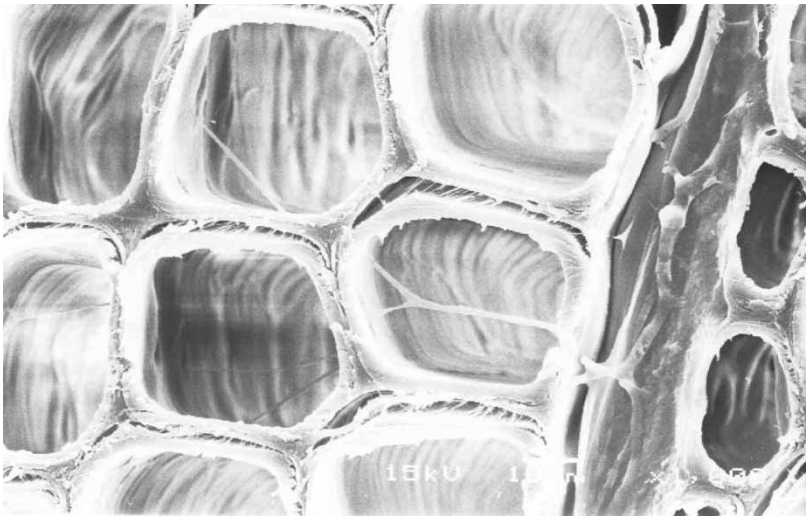

Figure 2. Transverse section of loblolly pine incubated with *C. subvermispora* for 2 weeks showing branched hyphae colonizing tracheids.

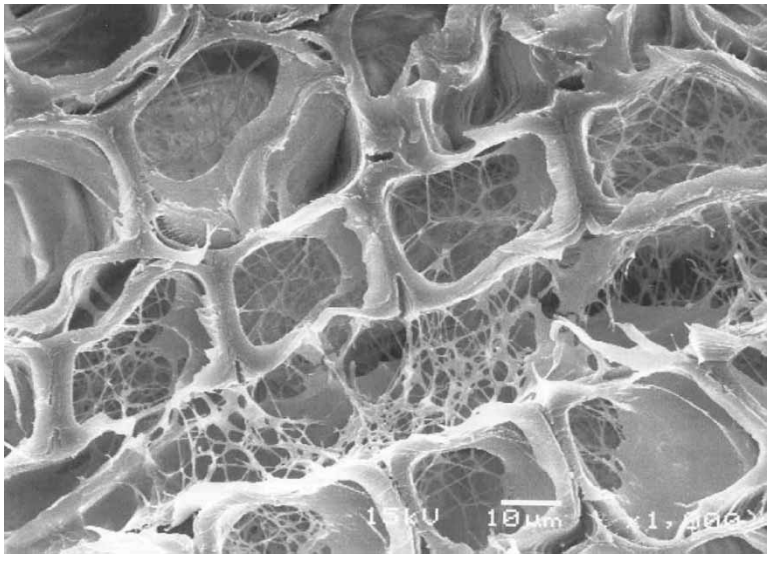

Figure 3. Cell wall structure of loblolly pine after 4 weeks of colonization with *C. subvermispora*. Mycelial growth has filled most of the voids.

The weight losses, i.e. 5.1 and 5.9% after 2 and 4 weeks fungal treatment, respectively, are similar to those reported previously for fungal treatment of loblolly pine with *C. subvermispora*.[8,9] Although the values seem low, a significant biopulping effect can be obtained at only a few percent weight loss. The weight loss may predominantly be attributed to the degradation of the nutrient-rich ray cells. At early stages of degradation, in the first 2 weeks, the vast majority of the wood fibers remain morphologically unchanged from those in sound wood. This suggests that the major components, e.g. lignin and cellulose, are still present in the wood cell walls after the fungal treatment.

Table 1. Chemical and physical analyses of control (untreated) and treated wood samples

Analysis	Control	2 Weeks	4 Weeks
Weight Loss, %	0	5.1[a]	5.9[a]
Klason Lignin, %	28.7	28.2	26.4[a]
Acid Soluble Lignin, %	0.17	0.51[a]	0.75[a]
Acetone Extractives, %	2.61	2.02[a]	1.78[a]
1% NaOH Solubility, %	11.5	14.9[a]	18.8[a]
Total Carbohydrate, %	66.7	64.0[a]	62.3[a]
Average Pore Diameter, Å	23	31[a]	37[a]

[a]Statistically significant compared to the control.

Figure 4 shows the changes in the average pore diameter and surface area in loblolly pine wood as a result of inoculation with *C. subvermispora*. The average pore diameter for loblolly pine wood was 22 Å, a value only slightly greater than that obtained for loblolly pine using the solute exclusion technique (20 Å).[10] There were significant differences in the average pore diameter of the control (22 Å) and treated pine chips, i.e. 31 and 37 Å, respectively at 2 and 4 weeks treatment, and the pore size distribution became broader with increasing exposure to the fungus. However, the pore size changes were smaller than those reported by Blanchette et al.,[10] where the average pore size increased from 12 Å to 60 Å after 4 weeks of incubation time. It must be pointed out, however, that these changes were associated with 40% weight loss. The limited increase in pore size in the present work is in agreement with the moderate weight loss experienced in the treated samples (5–6.5%). By comparing lignin loss with pore size changes, it seems that a significant degree of the delignification of the decayed wood is associated with little enzyme penetration, as evidenced by the small change in the average pore size. This effect was observed in *C. subvermispora*, as opposed to *P. chrysosporium* which showed much less delignification but more extensive enzyme penetration.[11]

Chemical Changes

The greatest reduction of the extractives (23%) content occurred in the first 2 weeks of treatment with the fungus, followed by a 32% loss at 4 weeks.

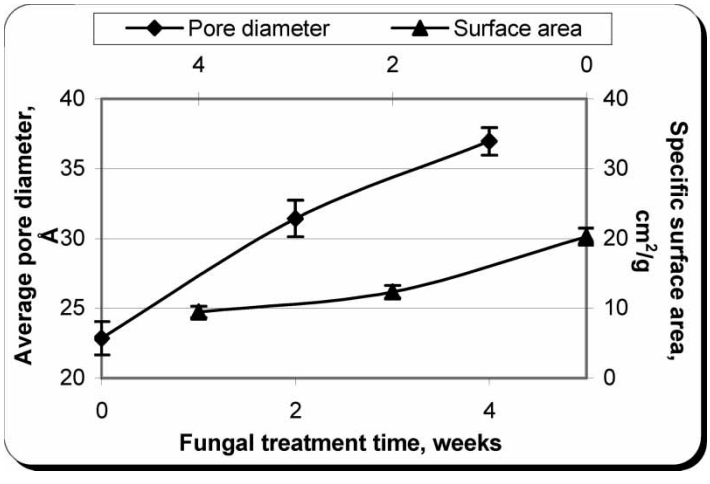

Figure 4. Average pore diameter and surface area of loblolly pine as a function of treatment time with *C. subvermispora*.

Similar losses were reported by Fischer et al.[12] who found that *C. subvermispora* and *O. piliferum* (a commercial depitching fungus) lowered the resin content of loblolly pine by 18–27% in 2 weeks and 33–35% in 4 weeks. These results demonstrate that although *C. subvermispora* is not a specific depitching agent, it has the ability of colonizing and degrading the resinous substances in wood.

Although the lignin content of the chips was not very sensitive to the fungal treatment in the first two 2 weeks where there was no statistical difference with the untreated chips, a significant decrease (8%) in the Klason lignin content was observed after 4 weeks treatment. Studies of wood and lignin degradation by fungi are very complex due to several factors. One of these is the nature of the lignin polymer which, unlike many other biopolymers, does not contain repeating units joined by bonds that are readily cleaved. With a complex material such as the lignin macromolecule in a wood matrix, it is possible to have important changes in the wood physical properties without having the same extent of changes in chemical properties of the component macromolecules.

There was no statistically significant change in the lignin phenolic hydroxyl groups after 2 weeks of fungal treatment. However, after 4 weeks of incubation time there was a minimum of 14% increase in the phenolic hydroxyl content caused by microbial attack. The formation of new phenolic hydroxyl groups in degraded lignin produced by microbial attack could be due to demethylation of methoxyl groups and the cleavage of β-aryl ether structures.[13] However, the analysis used in this study would not detect demethylation of guaiacyl units since the method cannot detect the resultant catechol-type units.

The untreated (control) loblolly pine had 0.2% acid-soluble lignin, a value in agreement with the literature for softwoods.[14] The higher acid-soluble lignin contents at increasing incubation times are an indication of the increased amount of lignin degradation products that are soluble in 3% H_2SO_4.

After 2 weeks incubation the solubility of material in the chips in 1% NaOH increased 30% relative to the control chips. This is considerably lower than the solubility gain of 84% found previously at the same treatment time.[15] This result could indicate that in the current study the carbohydrates were altered to a lesser extent than in the previous work. The greater carbohydrate preservation can be beneficial for the pulp viscosity after the pulping process. A steady degradation was noted and after 4 weeks of treatment the solubility had increased by 63%. That was a clear indication that more material was solubilized as the incubation time proceeded up to 4 weeks. It could also indicate that the fungus had opened up the cell wall structure, allowing greater access to the 1% NaOH.

The total amount of carbohydrates in the wood as a function of incubation time is reported in Table 1. The carbohydrate content of the chips, expressed as individual glycans as a function of the fungal treatment, is reported in Table 2. The values were calculated in terms of percentage dry weight of

Table 2. Carbohydrate composition for the control and fungally-treated loblolly pine based on the untreated wood

Sugar	Control	2 weeks	4 weeks
Glucose	44.8	42.8	43.0
Mannose	10.6	10.0	9.8
Xylose	7.8	7.6	7.0
Galactose	2.0	2.0	1.6
Arabinose	1.5	1.6	0.9
Total	66.7	64.0	62.3

the original sample. Comparing the lignin and carbohydrate data for degradation of loblolly pine by *C. subvermispora* the lignin degradation at 2 weeks (2% loss) was accompanied by a similar loss of glucose (2%). There were no significant differences between the control and 2-week fungal treatment for the rest of the carbohydrates. At 4 weeks incubation, the lignin underwent further deterioration while the glucose attack remained minimal.

The sugar composition for the untreated loblolly pine is in good agreement with similar analyses using other methods of detection.[16] It appears that the lignin, physically and to some extent chemically, serves as an effective barrier to the enzymatic degradation of polysaccharides.[17] Other researchers[18,19] have shown that at least one of the polysaccharides in wood is degraded simultaneously with lignin. In the present work similar conclusions can be drawn regarding glucose after 2 weeks of incubation. There were other carbohydrate losses at 4 weeks of fungal treatment (xylose, galactose, and arabinose).

CONCLUSIONS

The chemical analyses clearly indicate the delignification ability of *C. subvermispora* and its higher selectivity for lignin compared to other wood components. In contrast, in attack of wood by Basidiomycetes white-rot fungi all wood components are modified to some extent. Another interesting degradation feature is the ability of *C. subvermispora* to increase the lignin phenolic hydroxyl groups and acid solubility. The modifications of the physical and chemical properties of the wood by *C. subvermispora* are expected to influence the kraft pulping process and the properties of the resulting pulps

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