Biomimetic studies of wood decay:
Simulating the effect of low molecular weight compounds and fungal enzymes

by
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ABSTRACT

The effect of $FeCl_3$ (Fe^{3+}), hydrogen peroxide (H_2O_2), a low molecular weight compound (2,3-Dihydroxybenzoic acid), and oxalic acid on wood were tested in a study designed to mimic wood degradation by brown rot fungi. Previous studies suggest that these components are involved in the early stages of brown rot decay where they catalyze the formation of hydroxyl radicals through the Fenton reaction or related mechanisms. However, the separate and combined effects of these individual chemical components on wood have not been thoroughly investigated.

Tests were conducted in Erlenmeyer flasks containing 50 ml of 40 mM acetate buffer (pH 4.5), white pine wood powder and varying combinations of the chemicals previously mentioned. Changes in cellulose crystallinity were analyzed by X-ray diffraction using a θ-2θ scan. Findings suggest that iron, H_2O_2, chelators and oxalic acid may affect over all crystallinity and crystallite size. Understanding how these chemicals modify wood will facilitate characterization of the mechanisms involved in wood biodegradation by brown rot fungi.

INTRODUCTION

Brown rot fungi are the most destructive wood decay fungi, causing severe damage to in-service wood. For this reason they have been studied extensively. Still, the exact mechanisms involved are not fully understood. A widely accepted non-enzymatic oxidative mechanism involved in decay is the Fenton reaction, in which Fe^{3+} is reduced to Fe^{2+} in the presence of H_2O_2, resulting in the production of highly reactive hydroxyl radicals (Koenings et al. 1974, Hyde and Wood 1997). As strong oxidants, these radicals catalyze the degradation of cellulose, hemicellulose, and to some extent lignin, by random attack on the polymers. It has been proposed that this process is enhanced by the production of low molecular weight compounds such as DHBA, which bind, solubilize and reduce Fe^{3+}, enhancing the reaction and increasing decay (Goodell et al. 1997).

Biomimetic studies have been used to investigate and clarify the mechanisms involved in brown rot decay on components extracted from wood. Previous studies have shown that chelators, iron, hydrogen peroxide, and pH are major factors affecting cellulose degradation (Arantes and Milagres 2006, Jellison et al. 1991, Xu and Goodell 2001). These studies looked at wood components such as CMC, Avicel, and Birch xylan, and used viscosity models and a quantification of reducing sugars to monitor changes (Xu and Goodell 2001, Arantes and Milagres 2006),

X-ray diffraction (XRD) has been used to determine, among other things, the average width of the cellulose microcrystals and the percent of crystalline cellulose within wood. An interference pattern is created when x-rays encounter a regularly spaced matrix, and can be used to examine the changes in the crystalline cellulose in wood during degradation (Andersson et al 2003, Howell et al. 2007, Thygesen et al. 2005). Jellison et al. (1991) used X-ray diffraction for detecting biomimetic changes in wood, and found that crystallinity decreased significantly when wood powder was treated with iron, H_2O_2, and partially purified low molecular weight compounds extracted from *Gloeophyllum trabeum*. The effects of varying levels of this low molecular weight compound were not tested.

The purpose of this study was to establish the feasibility of using x-ray diffraction as a tool for observing minute structural changes in wood, to observe the effects of the Fenton reaction on the structure of wood, to test the effects of varying levels of low molecular weight compounds and enzymes on this system, and to examine the role of oxalic acid in the degradative process.

MATERIAL AND METHODS

All biomimetic tests were conducted in 250mL conical flasks containing 50mL of 40mM acetate buffer (pH 4.5). Iron was added to the solution first, followed by addition of oxalic acid, 2,3- DHBA, 1g white pine wood powder ground to pass through a 20-mesh screen, and finally H_2O_2. Flasks were covered with aluminum foil to prevent light from affecting the reaction and were incubated on a rotary shaker for 7 days at room temperature. There were five replicates for each treatment (n = 5).

Effect of DHBA to iron ratio

All flasks contained a standard solution of 0.5 mM $FeCl_3$ and 80 mM H_2O_2. The 2,3-DHBA was added in a 1:30, 1:65, 1:100 or 1:135 iron to 2,3-DHBA ratio.

Effect of commercial enzymes and crude extract

All flasks contained 0.5 mM $FeCl_3$, 80 mM H_2O_2, and a 1:65 ratio of 2,3-DHBA to iron. Flasks were treated with cellulase from *Trichoderma viride* (Sigma Chemical Co., St. Louis, MO) in low (0.0145g/mL) and high (0.145g/mL) concentrations, or concentrated crude extract in low (1.5mL) and high (15mL) concentrations from liquid cultures of *Coniophora puteana* grown in malt extract broth (Sigma Chemical Co., St. Louis, MO).

Fig. 1: Flasks containing wood powder and various treatments.

Effects of varying levels of oxalic acid

All flasks contained 0.5 mM $FeCl_3$, 80 mM H_2O_2, and a 1:65 ratio of 2,3-DHBA to iron. Oxalic acid was added in concentrations of 0.1mM, 1mM, 10mM or 30mM.

Processing and X-Ray Diffraction Analysis

Following incubation, the flask contents were filtered through a Whatman filter no. 1 (Whatman, England), and washed twice with 50 ml dH_2O. Filter papers containing wood powder were dried at 95°C for 24 hours and weighed. The wood powders were pressed manually using a Dake Press (Dake, Grand Falls, MI, USA) into cylindrical wafers 2 cm in diameter and approximately 0.4 cm thick.

Wood wafers were scanned by X-ray diffraction, and the crystallite width and sample crystallinity were determined as outlined by Howell et al. (2007).

RESULTS

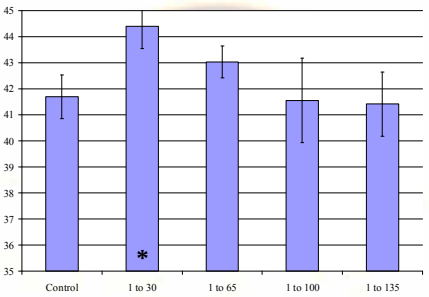

Figure 2: Effect of varying DHBA to iron ratios on the percent crystallinity in the wood powder. The 1:30 ratio treatment was statistically significant (*) from the control (P = 0.001) on the P > 0.05 level, causing a increase in percent crystallinity. No differences were observed between the control treatment containing only acetate buffer and the treatment with lower iron to DHBA ratios.

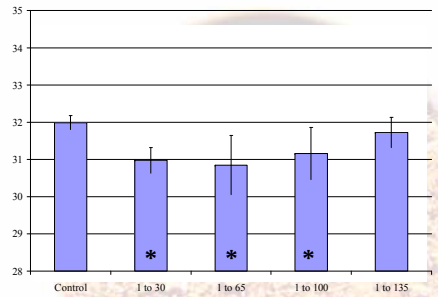

Figure 3: The graph shows the effect of various DHBA to iron ratios on the crystallite width in the wood powder. The treatments with ratios of 1:30, 1:65, and 1:100 showed a significant reduction in crystallite width relative to the control (P = 0.007, 0.003, and 0.024. respectively).

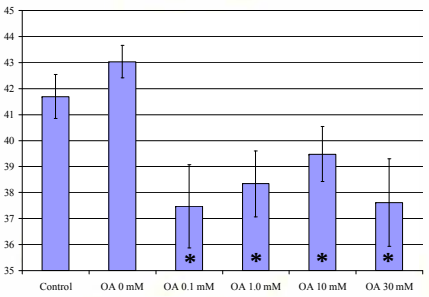

Figure 4: All treatments containing oxalic acid yielded significant effects on the percent crystallinity of the wood powder. No significant differences were found between the control with buffer and the 0 mM oxalic acid with $FeCl_3$, H_2O_2, and DHBA. All treatments showed a P-value of 0.00.

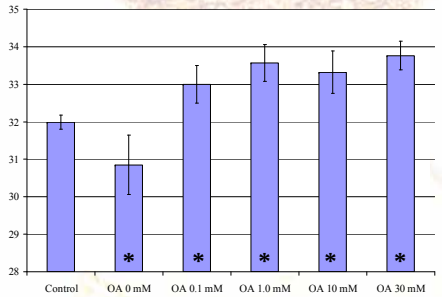

Figure 5: Treatment with $FeCl_3$, H_2O_2, and DHBA cause a significantly decrease in the crystallite width (P = 0.002), however, the addition of oxalic acid to this system significant increased the crystallite width of the cellulose crystals over both the treatment with 0 mM OA (P < 0.006) and the buffer control (P = 0.0).

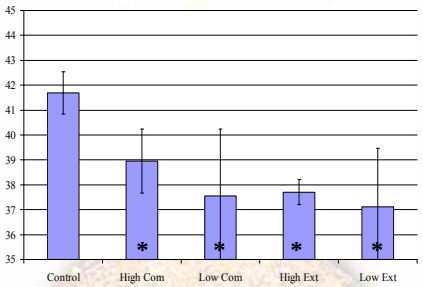

Figure 6: The addition of commercial enzymes (P_{high} = 0.023, P_{low} = 0.001) and fungal crude extract (P = 0.001) significantly decreased the percent crystallinity of the cellulose portion of the wood powder.

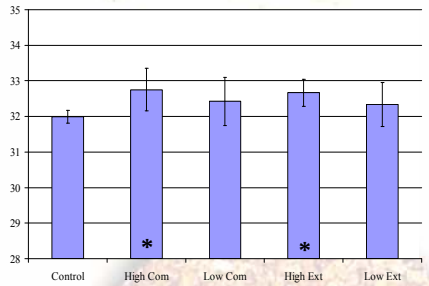

Figure 7: A significant increase in crystallite width was found with the addition of large amounts of commercial enzyme and fungal crude extract (P_{com} = 0.028 and P_{ext} = 0.020, respectively).

DISCUSSION

This study was designed to clarify the role of low molecular weight compounds and enzymes on the biodeterioration of wood and establish the feasibility of using x-ray diffraction for monitoring minute changes in wood composition. This study showed that the addition of varying amounts of 2,3-DHBA, oxalic acid and both commercial enzymes and crude fungal extract caused changes in wood structure detectable by x-ray diffraction. These results confirmed earlier reports characterizing changes in wood structures due to the Fenton system and low molecular weight compounds, and the observation of these changes using x-ray diffraction. (Koenings 1972, Jellison et al. 1991, Rätto et al. 1997, Xu and Goodell 2002).

Treatment with DHBA

Lower ratios of DHBA to iron were found to be more effective at decreasing the percent crystallinity and increasing the crystallite width (Fig. 2 and Fig. 3). This optimum supports the results of Xu and Goodell (2002) who found that a ratio of 1:30 was most effective in reducing the degree of polymerization (DP) in crystalline cellulose (Avicel) after 24 hours, though a ratio of 1 to 100 was found to favor reactions over a 48 hour period. The difference in optimum ratio between these results and those of Xu and Goodell (2002) could be attributed to a difference in substrate, as this study uses ground wood powder rather than Avicel, or differences in analytical methods. Further study to determine the optimum ratio for wood modification is currently underway using reduced DHBA to iron ratios.

Effects of varying levels of oxalic acid

All levels of oxalic acid treatments caused a significant decrease in percent crystallinity (Fig. 4) and a significant increase in crystallite width (Fig. 5). The increase in the crystallite width from oxalic acid treatments supports the theory of intracrystalline swelling caused by strong acids. Swelling results from penetration and enlargement of the amorphous and crystalline regions of the microfibril resulting in a change in the x-ray pattern (Guiness and Shafizadeh 1991).

Oxalic acid, the strongest of the organic acids, is known to play multiple roles in wood degradation by brown rot fungi. These results may be indicative of another role for oxalic acid in the degradation process, as crystalline swelling would facilitate penetration of low molecular weight compounds into the wood cell wall, aiding the degradation of these structures.

Increasing the amount of oxalic acid from 1 mM to 30 mM did not result in a significant increase in crystallite width. This may be due to the fact that there are a finite amount of crystals to be affected and that these crystals can only be swollen to a certain size, ~ 33.8 ± 0.4 (Fig. 5). Treating wood with 0.1mM oxalic acid as in this set up is not sufficient to react with all available crystals, as indicated by a significant difference between the results from the 1 mM and 30 mM oxalic acid (P = 0.028). The sudden decrease in percent crystallinity at 30 mM oxalic acid may be a failure of the hydrogen bonds responsible for crystalline structure (Fig. 4).

Effect of commercial enzymes and fungal crude extract

A depolymerization of both the crystalline and amorphous portions of the cellulose chains would result in an overall increase in the amorphous fraction. This which could explain the significant decrease seen in the percent crystallinity of wood powder treated with commercial enzymes and fungal crude extract (Fig. 6). The crude extract may also affect hemicelluloses within the wood as more than just enzymes are present in the filtrate. The modification of the hemicelluloses may make more cellulose available for depolymerization, partially explaining the slightly lower, though not significantly different, percent crystallinity values observed in the crude extract treatments compared to the commercial enzyme treatments.

Only high levels of crude extract and commercial enzyme appeared to affect the crystallite width (Fig. 7). This may be due to the larger effect of greater concentrations of enzyme. The higher amounts of enzymes appear to be less effective in decreasing percent crystallinity may be indicative of greater removal of free amorphous portions of the wood during the washing process. This removal of amorphous material would result in an increase in crystallinity, making the overall decrease in percent crystallinity relatively less compared to the reduced enzyme treatment. However, it should be noted that for both percent crystallinity and crystallite width there were no significant differences between the high and low treatments.

SUMMARY

The results from this study showed that X-ray diffraction could provide an effective, non-destructive tool for the characterization of biomimetic studies. It was found that percent crystallinity was significantly increased and crystallite width was significantly decreased by addition of Fe^{3+}, H_2O_2, and DHBA at ratios of 1:30 DHBA to iron. It was also found that the addition of oxalic acid or enzymes along with Fe^{3+} and H_2O_2 significantly decreased the crystallinity and increased the crystallite width. These results may show some of the effects each of these components has on crystalline cellulose during non-enzymatic brown rot decay.

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