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Wood Decay by Brown-Rot Fungi: Changes in Pore Structure and Cell Wall Volume

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Summary

Sweetgum *(Liquidambar styraciflua* L.) wood blocks were decayed by *Postia* (= *Poria) placenta* in soil-block cultures. Decay was terminated at various weight losses, and the pore volumes available to four low molecular weight molecules, (water, 4 Å,; glucose, 8 Å,; maltose, 10 Å; and raffinose, 128,) and three dextrans (M_r 6,000, 38 Å; 11,200, 51 Å; and 17,500, 61 Å) were determined by the solute exclusion technique (Stone and Scallan 1968b). The volume in sound (undecayed) wood that was accessible to the seven probes varied from 1.0 ml g^{-1} for the three largest to 1.35 ml g^{-1} for water. Thus, the volume in sound wood attributable to lumens, pits, and other large openings was 1.0 ml g^{-1} and that accessible to water in the cell wall was 0.35 ml g^{-1}. Of this volume, 80% was inaccessible to molecules > 12 Å in diameter. As the wood was decayed, the volume of pores in the cell wall increased steadily to 0.7 ml g^{-1} at 35% weight loss. New cell wall volume was accessible to the four low molecular weight probes but not to molecules of $M_r \geq 6{,}000$. The increase in accessible pore volume to the four smallest probes was gradual. Most of the new cell wall volume created by removal of components during decay was in the pore size range of 12 Å, to 38 Å. Within experimental error, no pores of > 38 Å, were observed in sound or decayed wood. Our results are consistent with the hypothesis that the initial depolymerization of cellulose, characteristic of brown rot, is caused by a diffusible agent. The molecular diameter of the agent is apparently in the range 12 Å, to 38 Å, and it causes erosion and thus enlargement of the pores to which it has access.

Introduction

Brown-rot fungi destroy wood by selectively degrading the hemicelluloses and cellulose without extensively changing the lignin (Cowling 1961). Cowling found that the holocellulose of brown-rotted wood is extensively depolymerized at low weight loss of total wood substance. It has been proposed (Cowling and Brown 1969; Koenigs 1974; Schmidt *et al.* 1981) that this initial depolymerization is caused by a small diffusible agent that is not an enzyme. This hypothesis was based on measurements of pore sizes in sound wood (Stone and Scallan 1965; Stone and Scallan 1968a; Kerr and Goring 1975) and on size estimates of cellulolytic enzymes (Cowling 1961; Cowling and Brown 1969; Cowling and Kirk 1976).

Previous studies examining pore sizes in wood have concerned themselves with changes that take place in the cell wall during the chemical pulping of wood. In acid chlorite pulping of white birch, for instance, the maximum pore diameter remains fairly constant throughout the delignification process (Kerr and Goring 1975). The median pore diameter begins to increase only late in delignification (> 50% removal of lignin) and then only slightly. In wood treated with alkali prior to pulping, the median pore diameter increases dramatically throughout acid chlorite delignification. In spruce wood, the maximum pore diameter is increased by both kraft and sulfite pulping, and the median pore diameter greatly increased (Stone and Scallan 1968a). These results demonstrate that chemical treatment of wood can have a substantial effect on cell wall pore volume and size distribution.

Changes in pore size and volume resulting from wood decay have not been studied. One might expect that initial attack by brown-rot fungi would open up the cell wall structure, giving access to enzymes. The objectives of the present study, therefore, were to determine the following:

(1) the pore volume in sound wood cell wall available to molecules of different size;

(2) whether attack by a brown-rot fungus, during which cellulose is depolymerized, results in a sudden increase in cell wall volume: and

(3) whether this decay opens up the pore structure of the cell wall in a manner that allows access by large molecules.

Materials and Methods

Preparation of samples

The brown-rot fungus used in this work was *Postia* (= *Poria) placenta* (Fr.) Cke. (strain Madison 698). Kiln-dried sweetgum

* The Forest Products Laboratory is maintained in cooperation with the University of Wisconsin.

(Liquidambar styraciflua L.) blocks were cross cut (2.5 by 2.5 by 0.3 cm) and incubated with the fungus for up to 4 weeks in soil-block cultures (ASTM 1971). Uninoculated control samples were incubated under identical conditions. Test and control blocks were removed on a regular basis and submerged in 0.05% sodium azide, terminating the decay process. Collected specimens were stored in 0.05% sodium azide solution. and all measurements were made before the samples were dried to determine weight losses. Extent of decay is described in terms of percentage weight loss from the original sample weight (Cowling 1961).

Determination of pore volume

Sample wood blocks of varying extents of decay were submerged in an aqueous solution of 0.05%) sodium azide for a minimum of 2 months to insure complete saturation of the sample. Using the solute exclusion technique developed by Stone and Scallan (1968b), we determined the pore volume available to each of the probes listed in Table 1 for each test and control sample. Thus, stock solutions of the various probes of known concentration were prepared in aqueous 0.05% sodium azide. Known wet weights of test and control samples were submerged in known volumes of stock solutions for 24 hours at room temperature (−24°C)in sealed containers. Tests showed that this allowed sufficient time for the probe to penetrate the sample to the extent allowable and to establish equilibrium. Following this incubation, the solution was decanted from the sample, and the concentration of the probe was redetermined using a Model RF-600[1]) Differential Refractometer (C.N. Wood MFG. Co., Newtown, PA) at 28°C. The change in concentration of the probe, as a result of incubation with the wood block, represents the aqueous volume available to that probe in the sample. Total volume accessible to the smallest probe, water, was determined by difference in weight between the water-saturated and oven-dried samples. Inaccessible volumes in a sample were determined by subtracting the accessible volume for each probe from that for water and are reported as milliliter volume per gram decayed wood. Accessible pore volumes reported as milliliter volume per gram of original wood were calculated as follows: (ml g^{-1} decayed weight) × [(100 - % weight loss)/100].

Preparation of holocellulose

Air-dried wood samples were ground to pass a 30-mesh screen. Chlorite holocellulose was prepared using the procedure described by Browning (1967). Volumes and weights called for in the procedure were scaled in proportion to the mass of air-dried 30-mesh wood used in each preparation. The intrinsic viscosities of holocellulose solutions were determined as described by the ASTM standard (1981), no correction was made for moisture or lignin content of the air-dried samples. The degree of polymerization, DP, of chlorite holocellulose was calculated from the intrinsic viscosity accord-ing to the method used to calculate the DP for cellulose, as described in the ASTM standard (1981).

Results

We first examined the accessibility of volume in sound wood to water and the solutes of increasing molecular size. We then determined the effect of decay on these accessibilities to the various probes. The following paragraphs describe those data. For the decayed samples, we have expressed the data on the basis of the decayed weight (i.e., on the basis of the sample analyzed), as well as on the basis of the original weight (i.e., corrected for weight loss resulting from decay). The latter method provides an interpretation of changes for a given original volume of wood.

Sound wood

Volumes in sound wood that were accessible to water and to the six solutes are recorded in Table 2 and plotted in Figure 1. The volumes varied from ~1.0 ml g^{-1} for the three largest probes to 1.35 ml g^{-1} for water. From these results, it is apparent that sound wood contained ~1.0 ml g^{-1} of volume readily accessible to all of the probes. We observed that values for the accessible volumes obtained for the three high molecular weight dextrans (M_r 6,000 to 17,500) were the same within experimental error (Table 2). We attribute this volume to lumens, pits, and other large openings. Therefore, the cell wall volume was determined to be 0.35 ml g^{-1}. The data indicate that molecules larger than 12 Å were excluded from 80% of the cell wall volume of sound sweetgum wood. A plot of probe diameter as a function of accessible volume (Fig. 1) indicates that molecules larger than ~15 Å in diameter were completely excluded from the cell wall of water-swollen sound sweetgum. The cell wall volume, the accessible volumes obtained for the probes, and the

[1]) The use of trade names in this publication is for reader information and does not imply endorsement by the U.S. Department of Agriculture of any product or service.

Table 1. Molecular weights and diameters of probes[a])

Probe	Molecular weight (g mol^{-1})	M_w/M_n	Probe diameter (Å)	Reference
Water	18	1.0	4	Tanaka *et al.* 1988
Glucose	180	1.0	8	Stone and Scallan 1968a
Maltose	342	1.0	10	Stone and Scallan 1968a
Raffinose	504	1.0	12	Stone and Scallan 1968a
Dextrans				
Fluka AG	6,000	NR	38	Weimer and Weston 1985
Pharmacia T10	11,200	2.0	51	Stone and Scallan 1968a
Polysciences 15−20K	17,500	NR	61	Weimer and Weston 1985

[a]) NR, not reported. M_w is the weight-average molecular weight. M_w/M_n is the ratio between weight-average and number-average molecular weights. All data are adapted from the indicated references.

Table. 2. Accessible pore volumes available to probes in sound water-swollen wood[a]

	Volume (ml g^{-1})						
					Dextrans		
Sample	Water	Glucose	Maltose	Raffinose	6K	11.2K	17.5K
1	1.34	1.26	1.15	1.07	0.97	0.94	0.97
2	1.35	1.29	1.17	1.11	0.96	1.02	0.98
3	1.36	1.30	1.16	1.04	1.00	1.05	0.99
Average	1.35	1.28	1.16	1.07	0.98	1.00	0.98
Std. Dev.[b]	0.01	0.02	0.01	0.03	0.02	0.05	0.01

[a] Physical data for each-probe are presented in Table 1. Samples 1, 2, and 3 were incubated in soil-block bottles in the absence of fungus for 1, 2, and 4 weeks, respectively.

[b] Standard deviation.

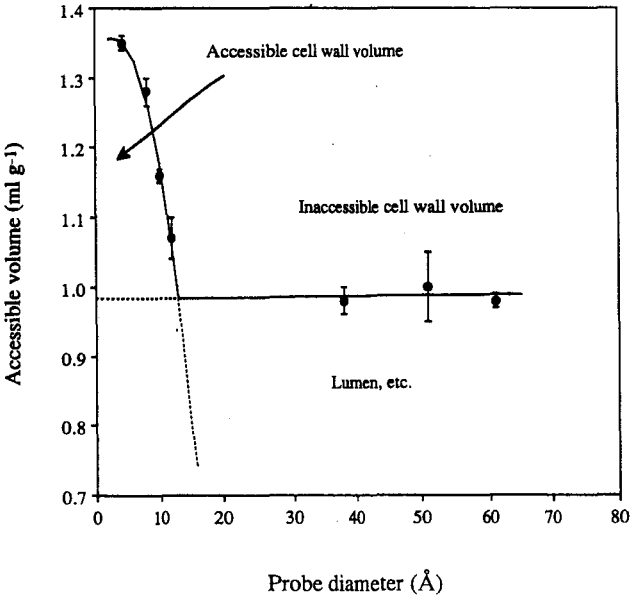

Fig. 1. Volumes available in sound sweetgum wood to solutes of various size. Values represent averages of three replicate samples; vertical lines are standard deviations.

maximum pore diameter all remained constant over 4 weeks (Table 2). That is, incubation in soil-block bottles in the absence of fungus did not affect the pore size distribution or cell wall volume in sound wood.

Decayed wood

We next determined the changes in cell wall volume during decay by a brown-rot fungus. The average DP of the holocellulose decreased gradually from 1,200 in sound wood to approximately 200 in wood decayed to 35% weight loss (Fig. 2). Results of the pore size analyses of these samples, expressed on a decayed weight basis, showed that volume became increasingly accessible to water and to all of the solutes with increasing weight loss (Fig. 3A). However, when the data are expressed on the basis of the original sound wood (Fig. 3B), it is seen that wall volume accessible to the three largest probes increased only slightly if at all during decay, whereas the volume accessible to the four smallest probes increased to a small extent. This plot also reveals that the sugars gained access to new volume during decay more rapidly than water, showing that existing pores were enlarged. The increase in accessible pore volume to the four smallest probes was gradual.

Another way to examine pore volume data based on size exclusion studies is to plot inaccessible wall volume as a function of probe diameter (i.e., wall volume accessible to water but not accessible to a given solute, plotted against solute diameter) (Stone and Scallan 1968b). This kind of plot for our data gives a family of curves for the sound and decayed samples (Fig. 4A). Plateaus in inaccessible volume are reached with the three largest probes for all samples. The plateaus represent fiber saturation points (total cell wall pore

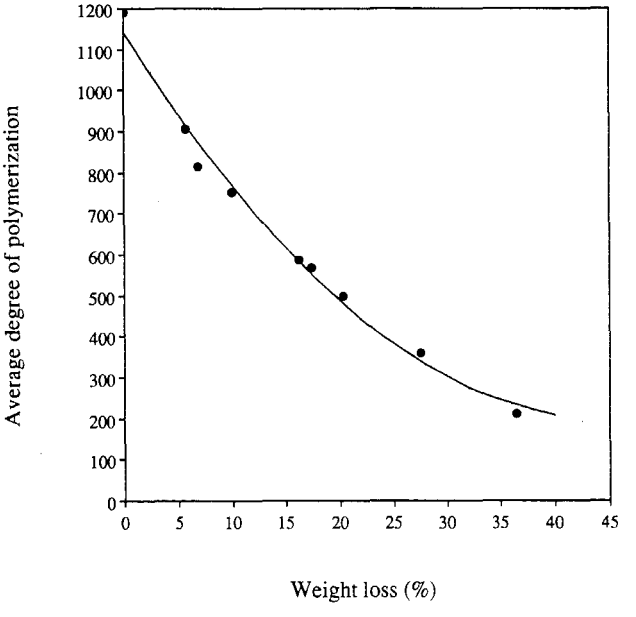

Fig. 2. Changes in average degree of polymerization of chlorite hollocellulose from sweetgum wood as a function of weight loss.

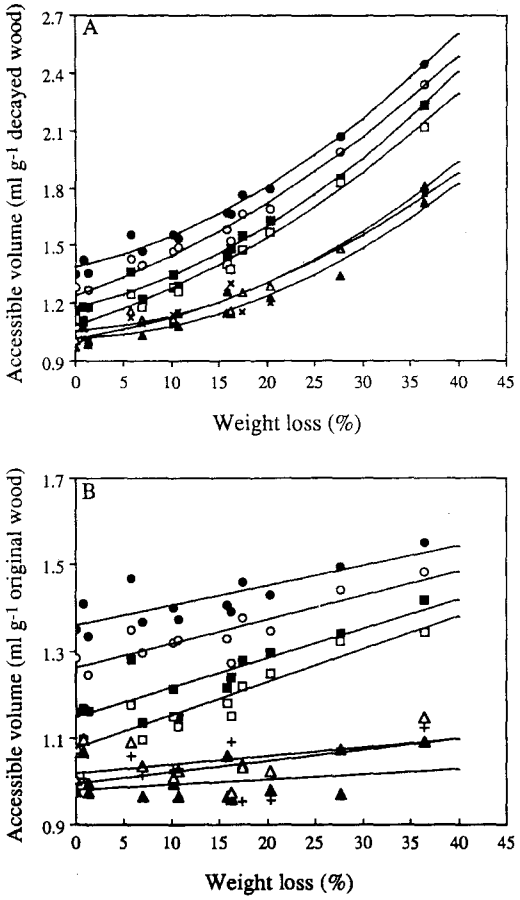

Fig. 3. Panel A. Accessible pore volumes available to various probes expressed on a ml g^{-1} decayed wood basis for sweetgum wood decayed to weight losses: water (˜), Glucose (™) maltose (¢), raffinose (£), M_r 6,000 dextran (p), M_r 11,200 dextran (r), and M_r 17,500 dextran (×). The lines represent least squares best fits.

Panel B. Accessible pore volumes available to various probes expressed on a ml g^{-1} sound wood basis for sweetgum wood decayed to weight losses: water (˜), Glucose (™) maltose (¢), raffinose (£), M_r 6,000 dextran (p), M_r 11,200 dextran (r), and M_r 17,500 dextran (×). The lines represent least squares best fits.

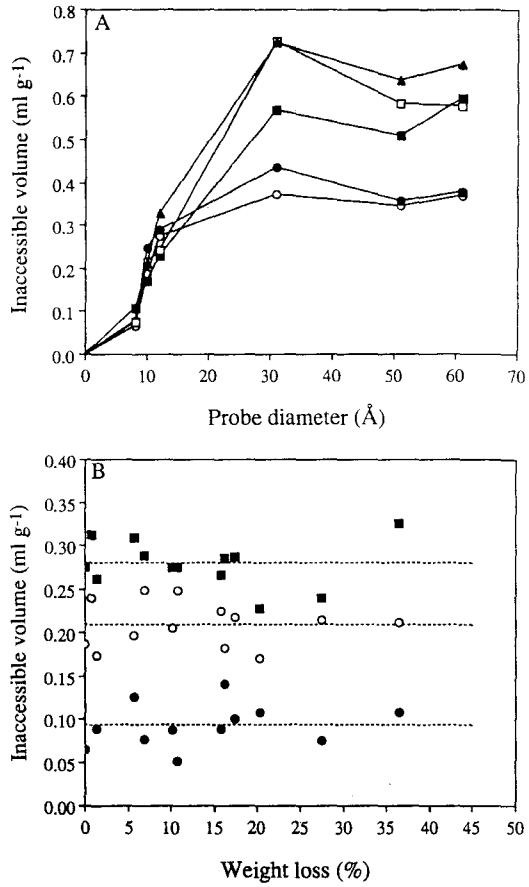

Fig. 4. Panel A. Inaccessible volumes in sound and decayed sweetgum wood for solutes of various diameter for wood decayed to various weight loss: 0% (˜), 6.9% (™), 20.3% (¢), 27.6% (£), and 35% (p). Not all of our data is shown.

Panel B. Changes in inaccessible pore volume in sweetgum wood for three low molecular weight probes as a function of weight loss; glucose (˜), maltose (™), and raffinose (¢). The dotted lines represent the average values for inaccessible volume for each probe.

volumes) for the samples. Thus, it can be seen that the cell wall volume increased from 0.35 ml g^{-1} in sound wood to 0.7 ml g^{-1} in wood decayed to 35% weight 1oss. The three largest probes did not gain entry to the cell walls despite the decay. These results indicate that the maximum pore diameter in our sound and decayed samples never exceeded 38 Å. Plots of inaccessible volume as a function of weight loss for the three sugar probes show no change on decay (Fig. 4B). Inaccessible pore volume did not increase for molecules ≤ 12 Å in diameter during decay. Thus, newly created pore volume accessible to water is also accessible to the three sugars. In the case of the three largest probes, those ≥ 38 Å in diameter, inaccessible volume for each of those increased as the wood decayed (Fig. 4A), indicating that these molecules are excluded from newly created cell wall volume. It follows that any new pore volume created by brown-rot decay results from either enlargement of existing pores that are ≥ 12 Å in diameter or from the creation of new pores between 12 Å and 38 Å in diameter.

Discussion

The purpose of this study was to examine changes in cell wall volume in wood during decay by a brown-rot fungus. We hoped that the information gained would lend insight into the mechanisms of cellulose depolymerization employed by this group of fungi and point to the size of the putative depolymerizing agent. Changes in the pore structure of wood during decay processes have apparently never been examined. The results of such a study may provide answers to some intriguing questions regarding the accessibility of degradative proteins to their respective substrates.

The sweetgum wood that we used had been dried, and its maximum pore diameter was expected to be small compared to previously studied woods. Stone and

Scallan (1968b) showed that the pore size and volume of wood pulp fibers decrease on drying, and that they do not increase again on re-wetting. Therefore, our finding Of a relatively small maximum pore size of ~15 Å in kiln-dried sound wood (Fig. 1) is not surprising. In addition, a cell wall volume of 0.35 ml g^{-1} (Table 2) is in good agreement with other work. Tarkow *et al.* (1966) found that molecules 18 Å to 20 Å in diameter were completely excluded from the cell walls of never-dried sitka spruce wood and that water gained access to ~0.4 ml of wall volume per gram of wood. Molecules of ≥ 45 Å diameter were excluded from the cell walls of never-dried black spruce wood, whose volume was slightly more than 0.4 ml g^{-1} (Stone and Scallan 1968a). Kerr and Goring (1975) found a cell wall volume of 0.5 ml g^{-1} and a maximum pore diameter of ~100 Å in white birch wood. Their report did not indicate whether the wood samples had ever been dried, but we suspect that they had not been. Grethlein (1985) found for mixed hardwood (90% birch, 10% maple) a cell wall volume of 0.5 ml g^{-1} and a maximum pore diamter of about 100 Å. Again, we suspect that never-dried wood was used. It is perhaps fortuitous that we used kiln-dried wood in the present study, because it revealed (discussion follows) the apparently small size of the cellulose-depolymerizing agent. Green wood would not have provided that insight.

This study focused on changes taking place in the cell wall during a period of decay in which a brown-rot agent penetrates the cell walls of wood, cleaving virtually all of the cellulose in the amorphous regions of the microfibrils. At 35% weight loss, the cell wall volume doubled to approximately 0.7 ml g^{-1} (Fig. 4). This increase in cell wall volume may be attributed to two effects: (1) the creation of new openings in the cell wall or erosion of pre-existing pores in the range of 12 Å to 38 Å and (2) the swelling of the cell wall as a result of fungal action. The data obtained in this study indicate that molecules ≥ 38 Å in diameter were excluded from the walls of sound sweetgum wood, as well as from the walls of the wood after it had been decoyed to various losses in total weight. In addition, molecules of ≤ 12 Å diameter gained access to all newly created cell wall volume. In our decayed samples. the cellulose was depolymerized to its "limit" DP of 200 (Fig. 2). Therefore, it is presumably pores of diameter 12 Å to 38 Å through which the cellulose-de-Polymerizing agent must pass to gain access to cellulose cleavage sites. From this, we may conclude that the cellulose-depolymerizing agent of *P. placenta* must be between 12 Å and 38 Å in diameter.

The agent might actually be considerably smaller than 38 Å. Plots of probe diameter as a function of accessible wall volume for the various samples give a family of curves (Fig. 5; the wall volume data are based on

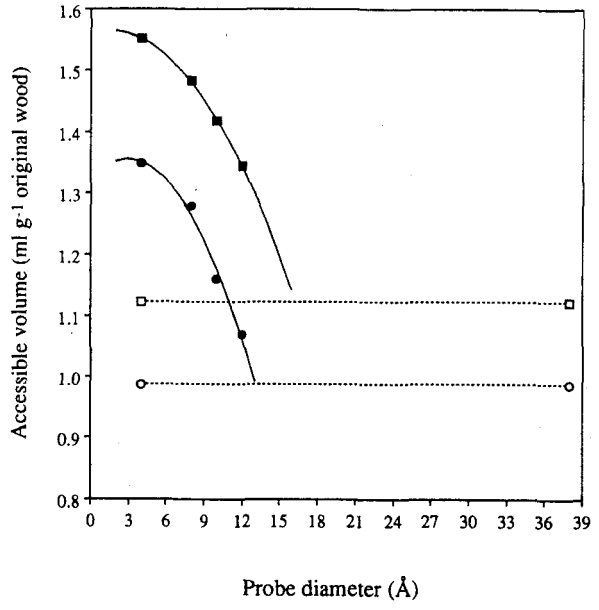

Fig. 5. Plots of accessible volume for the four low molecular weight probes in sound wood (˜) and wood decayed to 35% weight loss (¢). The dotted lines represent the average of the accessible volumes for the three high molecular weight probes in sound wood (™) and wood decayed to 35% weight loss (£).

the original weight of the wood, Fig. 2B). We have calculated the best fit lines for the data and extrapolated those curves through intersections with the base lines, which represent the accessible volumes of the three dextrans in sound or decayed wood. The intersects should give us estimates of the maximum pore size in the various samples. These plots suggest that the cell walls of the wood decayed to a 35% weight loss can be penetrated only by molecules of approximately 20 Å in diameter and smaller. Thus, our data suggest that the cellulose-depolymerizing agent of Postia placenta may actually be smaller than 20 Å in diameter.

Although our results reveal something about the size of the depolymerizing agent, they do not disclose its nature. The agent could be a transition metal chelate that works in conjunction with peroxide as mentioned in the introduction, or it could be a small enzyme or some other type of molecule. The smallest known protein for which we have reliable data, ribonuclease, has a molecular weight of ~13,000 and a diameter of ~30 Å (Tyn and Gusek 1990). A few small cellulolytic proteins, M_r 11.000 to 13,000, have been reported (Bisarin and Ghose 1981). However, small cellulases have not been detected in *P. placenta* (Highley 1975; Highley and Wolter 1982; Green *et al.* 1989).

Several recent studies have examined the penetrability of wood decay enzymes into the cell wall by the use of transmission electron microscopy and immunogold labeling (Srebotnik *et al.* 1988: Daniel *et al.* 1989: Blanchette *et al.* 1989). Those studies have concluded that lignin peroxidase (M_r ~42,000) is incapable of

penetrating the walls of sound wood. The areas of the walls that have been heavily decayed by white-rot fungi, which degrade lignin and hemicelluloses somewhat selectively, were penetrated by the enzyme (Blanchette *et al.* 1989). This suggests that biological lignin removal, like alkaline delignification in pulping, opens up the walls, whereas our study indicates that cellulose and hemicellulose removal by brown-rot fungi does not.

Our results do not seem to be in accord with the recent report of Agosin and coworkers (1989), who found an increase in rumen digestibility of pine sawdust after brief exposure to the brown-rot fungus *Gloeophyllum trabeum.* They concluded that initial attack by the fungus caused the cell walls to become more susceptible to the polysaccharidases of the rumen microflora. Our results suggest that changes other than increases in accessible cell wall volume are responsible for this observation. It is possible that the wood structure was mechanically disrupted during treatment in the rumen, because the initial depolymerization of cellulose by brown-rot fungi causes wood to become friable (Cowling 1961).

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