

PROPERTIES OF CELLULOSE DEGRADED BY  
THE BROWN-ROT FUNGUS, POSTIA PLACENTA

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Working Group I

Biological

Problems

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the Brown-Rot Fungus, *Postia placenta*

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SUMMARY

To gain further understanding of the nature of the cellulose depolymerizing agent or agents of brown-rot fungi, brown-rotted cellulose was chemically and physically characterized. Various culture conditions, such as low nitrogen and elevated oxygen levels, did not induce degradation of cellulose by *Postia placenta* in liquid cultures. Therefore, brown-rotted cellulose for analysis was prepared by a nonliquid method using a soil-block technique. Analysis of the molecular weight distribution of the degraded cellulose indicated a fairly tight distribution centered around DP 232. From x-ray diffraction analysis it appears that there was a preferential attack on the smaller crystallites and amorphous regions of the cellulose by the fungus, confirming earlier work. Infrared spectroscopy and carboxyl determinations with methylene blue showed that carboxyl groups were present in the degraded cellulose. Uronic acids were not detected in acid hydrolysates of the brown-rotted cellulose, indicating that oxidation was not at C-6. However, several acids were separated and are currently being studied.

Key words: Wood decay, brown-rot, cellulose decomposition, *Postia placenta*

INTRODUCTION

Brown-rot fungi are the most important group of fungi that decay wood in service. They cause a very rapid reduction of strength before significant weight loss is detected. They are so destructive because they cause a rapid and extensive depolymerization (to the "limit," or crystalline, degree of polymerization (DP)) shortly after colonizing wood (Cowling, 1961). Acid hydrolysis has a similar effect on cellulose, as do several oxidants. The system producing the initial depolymerization is most likely nonenzymatic because enzymes are too large to penetrate and diffuse through wood to gain access to the cellulose. Furthermore, circumstantial evidence (Highley, 1977) suggests oxidative degradation.

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If the biochemical agent responsible for cellulose depolymerization by brown-rot fungi can be identified and then neutralized (either at the site of its synthesis or secretion or at the point of attack within the wood cell matrix), new, sharply targeted, and environmentally benign wood preservation methods could become available. To obtain a clearer picture of the nature of the biochemical agent involved in the initial depolymerization of cellulose by brown-rot fungi, we prepared large quantities of brown-rotted cellulose for detailed characterization of some of its physical and chemical properties.

## MATERIAL AND METHODS

### Culture Procedures

The brown-rot fungus, Postia placenta (Fr.) M. Lars. et Lomb. (MAD-698) [syn: Poria placenta (Fr.) Cke.] was used in this study. Liquid and nonliquid cultures were used for induction of cellulose decomposition.

1. Liquid cultures. Purified cotton cellulose (0.15 g) (Herculus,<sup>2</sup> type A-600) in 15 ml of basal solution (Highley, 1973) was subjected to decomposition by P. placenta in 125-ml Erlenmeyer flasks at (a) three oxygen levels (0.2, 0.4, and 1.0 atm) with and without the addition of 0.1% cellobiose or (b) atmospheric oxygen with 0.02% nitrogen substituted for 0.2% nitrogen in the basal solution with and without the addition of 0.1% cellobiose. Flasks were inoculated with 1 ml of mycelial suspension and incubated for up to 2 months in stationary culture. In the oxygen concentration experiments, the test flasks were flushed every 2 or 3 days for 15 min with 100 ml of the appropriate oxygen-nitrogen mixture per minute. After incubation, mycelium and cellulose were collected by filtration with suction. Mycelium was manually separated from the cellulose.

The filtrates were tested for the presence of H<sub>2</sub>O<sub>2</sub> with titanium (Wolfe, 1962) and ABTS-peroxidase (Müller, 1984). The filtrates without cellobiose were also assayed for reducing sugars (Nelson, 1944). The DP (Cowling, 1960), reducing capacity (Nelson, 1944), and weight loss of residual cellulose were determined .

To determine the ability of P. placenta to decay wood in liquid culture, hemlock sawdust was subjected to decay in the basal salts solution containing 0.02% or 0.2% nitrogen with and without the addition of 0.1% cellobiose. Using 250-ml Erlenmyer flasks, 25 ml of media and 1% sawdust were inoculated with 1 ml of mycelial

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suspension. At 4 and 8 weeks the sawdust was removed by vacuum filtration, and weight loss and removal of lignin, glucan, mannan, and xylan were determined.

2. Nonliquid cultures. Cotton cellulose was exposed to P. placenta in chambers (1-liter jars) patterned after the standard ASTM soil-block procedure (American Society for Testing and Materials, 1971). The cotton was placed in chambers on previously inoculated pine feeder strips (0.3 x 2.9 x 3.5 cm, with the long axis in the grain direction). After incubation, mycelium was separated from residual cellulose, and the cellulose was washed with 0.1 N NaOH. Microscopic examination showed that not all mycelium was removed. The DP and weight loss of the residual cellulose were determined as -before.

#### X-Ray Diffraction of Brown-Rotted Cellulose

Cotton cellulose degraded to a 60% weight loss by P. placenta in soil-block tests was examined by x-ray diffraction (Caulfield and Moore, 1974).

#### Molecular Weight Distribution of Brown-Rotted Cellulose

Molecular weight distributions of the degraded cellulose were determined by the Institute of Paper Chemistry (Appleton, WI). This was accomplished by producing cellulose tricarbonyl derivatives for gel permeation chromatography (Schroeder and Haigh, 1979). The samples were analyzed on Stryrogel columns calibrated by a dispersion-compensated universal technique (McCrackin, 1977).

#### Carboxyl Content and Uronic Acids

The carboxyl content of the cellulose was estimated by the methylene blue method (Technical Association of the Pulp and Paper Industry, 1963). Uronic acid content (oxidation at C-6) was determined after acid hydrolysis (Scott, 1979).

#### Fourier Transform Infrared Spectroscopy

The Fourier transform infrared (FTIR) spectra of control and rotted cellulose were recorded both with the diffuse reflectance methods and using KBr pellets to determine the presence of carboxyls in brown-rotted cellulose. Samples were also treated with NaBH<sub>4</sub> to determine the presence of carbonyl groups other than carboxyl.

#### High-Performance Liquid Chromatography of Acid-Hydrolyzed Cellulose

High-performance liquid chromatography (HPLC) (Bio-Rad HP x 87 - H<sup>+</sup>) was used to analyze acid hydrolysates of brown-rotted cellulose. The column was run at 70°C with 0.015 N H<sub>3</sub>PO<sub>4</sub> at

a flow rate of 0.6 ml/min. The sample preparation procedure for HPLC was as follows:

#### Acid hydrolysis

1. Primary hydrolysis -- 72% H<sub>2</sub>SO<sub>4</sub>, 30°C, 1 hr
2. Secondary hydrolysis -- 3% H<sub>2</sub>SO<sub>4</sub>, 120°C, 1 hr
3. Neutralize with Ba(OH)<sub>2</sub>
4. Evaporate and adjust to pH 8

#### Anion exchange--Dowex 1-x8 (200–400-mesh)

1. Apply sample
2. Remove glucose with warm distilled water
3. Remove acids with 0.5 N formic acid
4. Evaporate to dryness
5. Dissolve in distilled water

## RESULTS

### Liquid Cultures

Analysis of residual cellulose from P. placenta liquid cultures grown under various oxygen concentrations or at a low nitrogen concentration showed that the brown-rot fungus was unable to produce cellulose-degrading agents in the liquid cultures. P. placenta did not produce weight loss of cellulose, and DP of residual cellulose differed little from that not exposed to the fungus (Table 1). Reducing capacity of the cellulose was not increased nor were reducing sugars detected in filtrates.

Hydrogen peroxide was not detected in any of the cultures with either the titanium reagent or the ABTS-peroxidase reagent.

Degrading agents also were not produced in liquid cultures with hemlock sawdust, because no weight loss and no loss of cell wall constituents were found.

### Nonliquid Cultures

X-ray diffractometer traces of the undegraded cotton cellulose control and the brown-rotted cellulose showed no marked differences. The patterns are clearly indicative of cellulose I (native cellulose). These patterns can be used to provide measures of crystallinity index (Segal et al., 1959) and crystallinity size (Caulfield, 1971) (Table 2). The differences

Table 1.--Effect of oxygen concentration on degree of polymerization of cellulose by P. placenta grown in liquid media

oxygen concentration (atm)	Carbon source	Degree of polymerization			
		Exposed to <u>P. placenta</u>		Control	
		4 weeks	8 weeks	4 weeks	8 weeks
0.2	Cellulose	1,604	1,400	1,946	1,872
	Cellulose + 0.1% cellobiose	1,408	1,240	1,842	1,858
0.4	Cellulose	1,720	1,824	1,432	1,872
	Cellulose + 0.1% cellobiose	1,590	1,230	1,840	1,952
1.0	Cellulose	1,812	1,468	1,618	1,666
	Cellulose + 0.1% cellobiose	1,740	1,146	1,858	1,840

Table 2.--X-ray diffraction of brown-rotted cellulose

	Crystallinity (%)	Crystallite size (Å)
Brown-rotted cellulose	82.7	54
Control cellulose	76.3	60

are not large, but it appears that the brown-rotted cellulose is more crystalline and has larger crystallites. This may result from a preferential attack on the smaller crystallites and amorphous regions of the cellulose.

Molecular weight determinations by HPLC of carbanilate derivatives gave a symmetrical DP distribution with a fairly tight distribution around DP 232 (Fig. 1). Thus, random cleavage of all the cellulose must have occurred in the amorphous regions, not just at the fiber surfaces. The number average molecular weight was 188 and the weight average 335. This is close enough to a 1:2 ratio to be consistent with random cleavage.

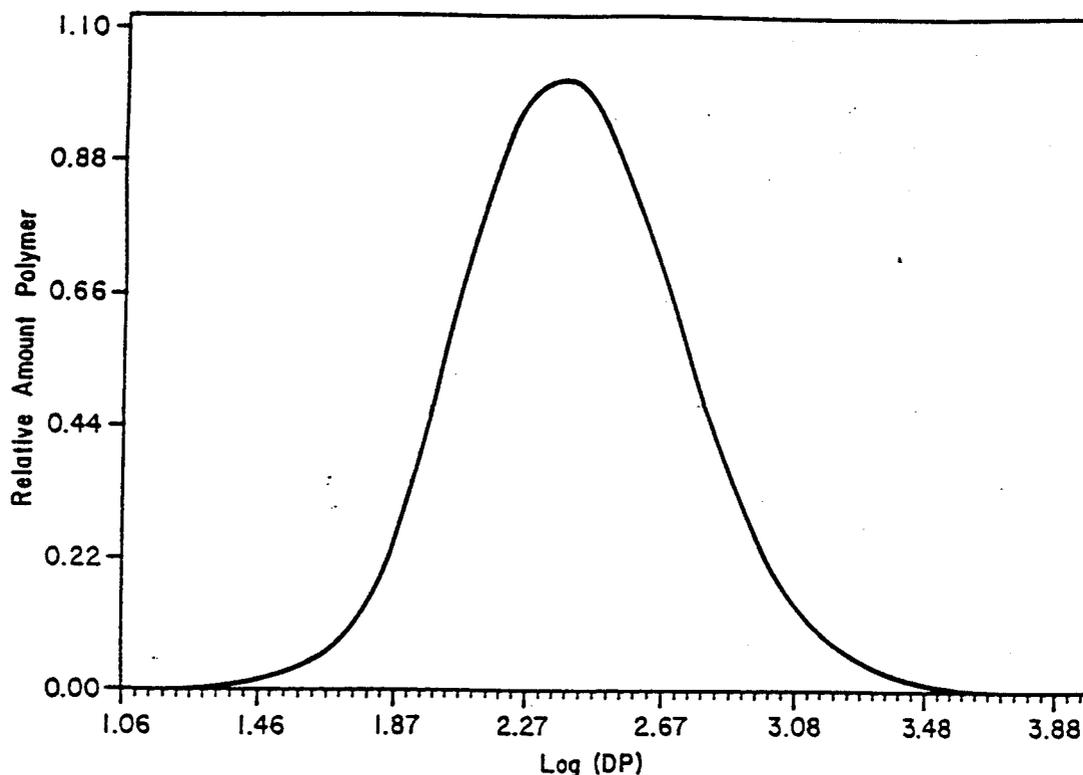


Figure 1. Molecular weight distribution of brown-rotted cellulose.

Undegraded cellulose did not show carbonyl bands with FTIR diffuse reflectance. Carbonyls were present in the brown-rotted cellulose (Fig. 2). When reduced with  $\text{NaBH}_4$ , carbonyls were still present, but in lower concentrations. This result suggests that acidic groups were present in the brown-rotted cellulose. The KBr Pellet was too opaque for quantitative determinations, and diffuse reflectance was not quantitative.

No uronic acids were detected in acid hydrolysates, showing that the acid groups that were present were not on C-6 (i.e., they had to be on the chain ends).

The presence of acid groups in the brown-rotted cellulose was verified by the methylene blue method. The NaOH-washed brown-rotted cellulose was found to contain 0.468% mole carboxyls per mole of brown-rotted cellulose, or about 1 carboxyl for 214 glucose units.

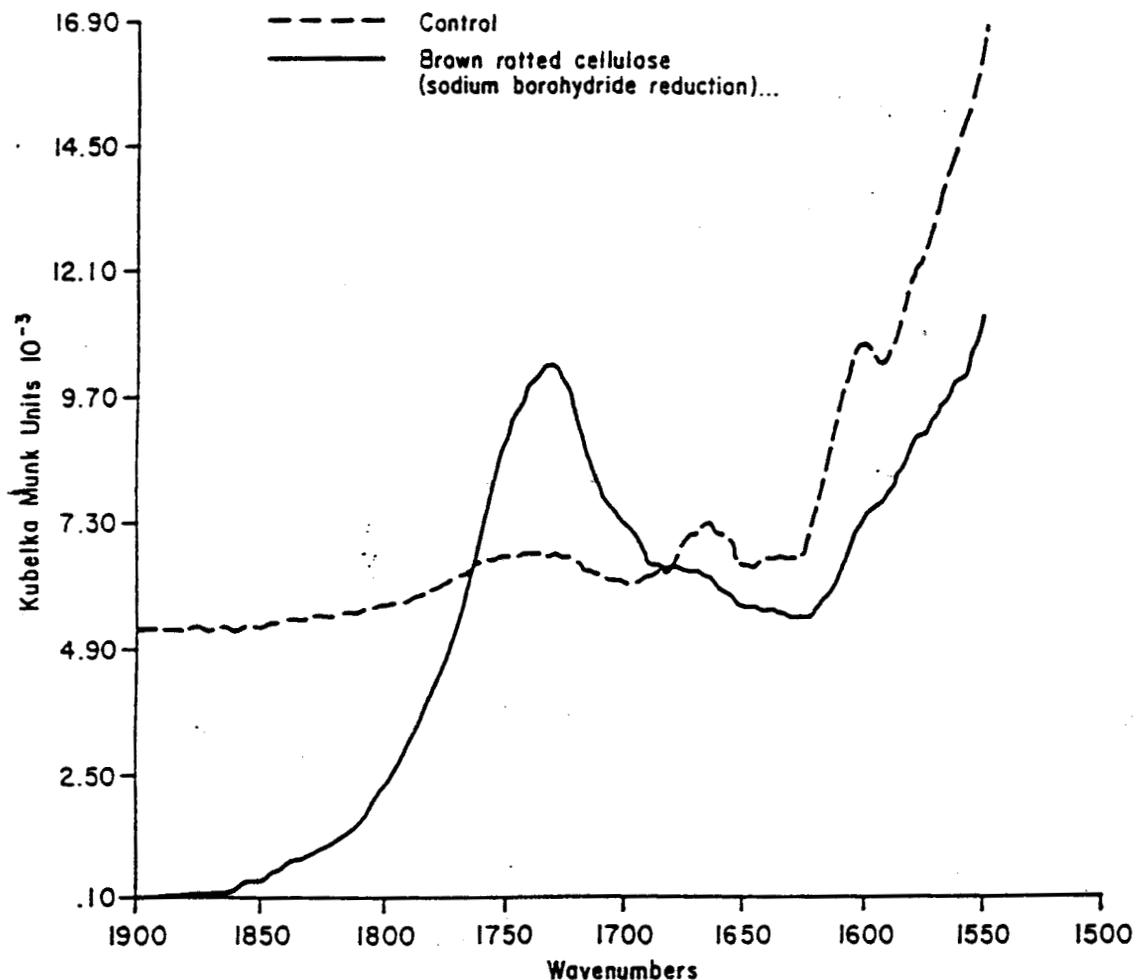


Figure 2, Fourier transform infrared spectra of undegraded cellulose (---) and brown-rotted cellulose treated with sodium borohydride (—.)

The HPLC analysis of acids recovered in hydrolysates of the brown-rotted cellulose yielded several peaks. the identification of which is underway.

#### DISCUSSION

The results confirm earlier observations that brown-rot fungi preferentially attack the amorphous areas of cellulose, leaving a highly crystalline oxidized residue. Carboxyl groups were found in the cellulose molecule after brown-rot degradation.

We are currently using gas chromatography and mass spectroscopy to identify the acids recovered after hydrolysis of the brown-rotted cellulose.

The mechanisms may differ, but the effects produced by brown-rot fungi on cellulose are similar to those of potassium superoxide (Thompson and Corbett, 1985). Both rapidly decrease the DP of cellulose to about 200 and show symmetrical molecular weight distributions, which indicate random cleavage of all the cellulose, not just the fiber surfaces.' The ratio of number

average DP to weight average DP of both is about 1:2, which is also consistent with random cleavage. Since both types of degradation reach a limit DP similar to that obtained after acid hydrolysis, degradation must proceed through the amorphous regions. The small size of intermolecular spaces makes penetration by reactants difficult. However, small molecules, such as oxygen radicals, can penetrate. The rapidity of the degradation compared With acid hydrolysis makes it likely that cellulose-metal complexes and oxidation reactions by oxygen radicals are involved in cellulose depolymerization by brown-rot fungi.

We felt that elevated oxygen levels or low nitrogen levels might induce formation of oxygen radicals and subsequent degradation of cellulose by P. placenta in liquid culture. However, cellulose degradation was not observed in liquid culture nor was H<sub>2</sub>O<sub>2</sub> detected in culture filtrates. Also, wood in liquid culture was not degraded by P. placenta. In a previous study (Highley, 1987) when the chromogen for detection of H<sub>2</sub>O<sub>2</sub> was placed into the culture medium (biochemical trap), H<sub>2</sub>O<sub>2</sub> was detected in P. placenta cultures. Thus, in an aqueous system it is possible that the oxygen radicals are quickly destroyed or are much less reactive.

The chemical reactions involved in cellulose degradation by brown-rot fungi are as yet unknown, but by identifying the cleaved end residues in brown-rotted cellulose, it should be possible to speculate about the nature of the degrading agent.

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