Requirements for Cellulose Degradation by a Brown-rot Fungus

By TERRY L. HIGHLEY

Forest Products Laboratory[1], Forest Service
U. S. Department of Agriculture

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1. Introduction

Brown-rot fungi readily metabolize cellulose in wood, but at least some of these fungi cannot metabolize isolated cellulose (T. L. HIGHLEY, 1973, 1975). Furthermore, enzymes isolated from many brown-rotters grown in liquid culture are inactive on insoluble cellulose (C_1-activity) but are active on soluble substrates such as CMC (C_x-activity).

T. L. HIGHLEY (1975) found that the brown-rot fungus Poria placenta was unable to utilize cellulose unless placed in contact with wood, which suggests that other wood constitutents are essential for cellulose utilization. T. NILSSON (1974 a) noted no microscopic degradation by brown-rotters of cotton fibers placed between inoculated wood pieces, but reported that after exposure no tensile strength was left in the cotton fibers, indicating attack by the brown-rot fungi. Differences in cellulose degradation by the brown-rot fungi noted in these two studies might be due to different test procedures. T. L. HIGHLEY (1975) used a soil-block method and T. NILSON (1974 a) used a vermiculite method. The studies suggest that when cultured on wood the brown-rot fungi produce a factor able to diffuse into a pure cellulose, such as cotton, and degrade it. Thus, perhaps other materials in wood such as lignin and hemicelluloses play an important role in production of a catalyst or product (hydrogen peroxide) necessary for metabolizing cellulose by brown-rot fungi.

[1] Maintained at Madison, Wis., in cooperation with the University of Wisconsin.

FOREST PRODUCTS LABORATORY (Madison, Wis. 53705)
FOREST SERVICE, U. S. DEPARTMENT OF AGRICULTURE
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T. NILSSON (1974 a) hypothesizes that because brown-rot fungi degrade cellulose in lignified substrates, the presence of lignin in some way induces the production of cellulases. He reports that lignified jute fibers are easily degraded by brown-rot fungi but delignified ones are not. Hemicelluloses could also influence cellulose utilization; S. N. BASU and S. N. GHOSE (1952) found that fungal species unable to decompose \-cellulose strongly degraded holocellulose. It is also possible that a nonprotein system such as H_2O_2/Fe (J. W. KOENIGS, 1972 a) could predispose the cellulose to conventional cellulases.

The objective of this work was to: (1) Determine the weight loss and change in degree of depolymerization (DP) produced by *P. placenta* in chemically treated cellulose and cellulose supplemented with various alternative carbohydrate sources, and (2) compare the oxidative properties of brown-rotted, acid-treated, and H_2O_2/Fe-treated cellulose.

2. Materials and Methods

Susceptibility of the cellulose to fungal attack was determined in chambers patterned after the standard ASTM soil-block procedure (AMERICAN SOCIETY FOR TESTING AND MATERIALS, 1968 a). Cellulose was conditioned at 27° C and 70 percent relative humidity before exposure to brown-rot fungus *Poria placenta* (Fr.) Cke. (Madison 698).

Measurements were made of both DP and weight loss of the following celluloses after exposure to *P. placenta* for 2, 4, 8, and 16 wk: Cellulose (50 mg) (purified cotton, Hercules; filter paper pulp, S and S; solkafloc, Brown) and sweetgum (*Liquidambar styraciflua* L.) holocellulose (100 mg). The materials were exposed in chambers on previously inoculated S and S filter paper (2.9 by 3.5 cm) or sweetgum feeder drip (0.3 by 2.9 by 3.5 cm. with the long axis in the direction of the grain).

Changes in weight loss and DP of cotton and filter paper with alternative carbohydrate sources were deterimined after exposure to *P. placenta* for 4, 8, and 12 wk on preinoculated filter paper strips. Fifty mg of cotton or filter paper were soaked for 4 h in a 0.5 % solution of glucose (Fisher), xylan (Sigma). galactomannan (Sigma), starch (Mallinckrodt), and cellobiose (NBC). In addition, cotton and filter paper were soaked in a hot and cold water extract from 2 g of sweetgum sawdust and concentrated *in vacuo* to 10 ml (AMERICAN SOCIETY FOR TESTING AND MATERIALS. 1968 b). A supplement of holo cellulose (50 mg) was also placed on preinoculated filter paper with cotton or filter paper.

Fifty mg of cotton or filter paper were also treated with various reagents to predispose them to brown-rot attack. Treatments were (1) 0.1 % and 1 % H_2O_2 + 0.44 mM $FeSO_1 \cdot 7H_2O$ for 3 days (J. W. KOENIGS, 1972 a); (2) 0.1 N oxalic acid at 22 C for 24 h; (3) mercerization with 24% NaOH at 4 C for 3 h, followed by dilution with 2 % NaOH and neutralization with acetic acid (4) swelling in phosphoric acid for 2 h (C. S. WALSETH 1952) The cellulose was exposed to *P. placenta* on preinoculated filter paper strips as above.

After incubation, mycelium was separated from residual cellulose, but microscopic examination showed that not all mycelium was removed. ROMANELLI et al. (1975) had used 1 N NaOH to digest mycelium of thermophilic fungi but this solution did not dissolve *P. placenta.* Tests were then conducted on both mycelium alone and mycelium with cellulose to determine the effect of mycelium on DP measurements and oxidative tests. Except for ion exchange capacity, it was found that the presence of some mycelium in samples would not affect results. Mycelium had ion-exchange capacity and thus the values for carboxyl content of brown-rotted cellulose may be high.

Percentage weight loss was determined by comparing conditioned (27° C and 70% relative humidity) air-dry weights before and after exposure to *P. placenta.* Average DP was measured viscosimetrically (E. B. COWLING, 1960) with cupriethylendiamine hydroxide solvent (Ecusta Paper Corporation). Brown-rotted cellulose decayed 16 wk, cellulose treated with saturated oxalic acid for 24 h, arid cellulose treated with 0.3 % and 1 % $FeSO_4 \cdot 7H_2O$ were tested for oxidation. Oxycelluloses differ considerably from one another in their properties depending on the method and extent of oxidation. There are two types - the reducing type characterized by a large number of carbonyls, and the methylene blue type characterized by a large number of carboxyls (J. T. MARSH and F. C. WOOD, 1939; T. NILSSON, 1974).

The carboxyl content or ion-exchange capacity of the cellulose was determined by the methylene blue method (AMERICAN SOCIETY FOR TESTING AND MATERIALS, 1970). Presence of carbonyls was estimated by copper number (AMERICAN SOCIETY FOR TESTING AND MATERIALS, 1968 a). Oxidation at C_6 (uronic acids) of brown-rotted cellulose was determined by Ralph Scott (FPL) by a colorimetric method (unpublished).

The reducing type of oxycellulose is characterized by a relatively large loss of weight when boiled with alkali. Solubility of cellulose in 1 % NaOH was determined by the method of COWLING (1960).

Cellulose was tested qualitatively for oxidation by immersion in $FeSO_4 \cdot 7H_2O$, followed by rinsing in water and subsequent immersion in potassium ferricyanide (J. T. MARSH and F. C. WOOD, 1939). Oxycellulose develops a deep blue color but unoxidized cellulose is only slightly colored.

The acidic nature of cellulose was confirmed by placing samples in 10% NaCl solution (J. T. MARSH and F. C. WOOD, 1939) and measuring pH. This cellulose shows an acid reaction because a certain proportion of the hydrogen ions arising from the carboxyl groups are replaced by sodium ions and are thus able to escape from the cellulose phase.

Infrared spectra were made by Martin Wesolowski (FPL) of brown-rotted cotton and cotton treated with 0.1 % H_2O_2/Fe and 1% H_2O_2/Fe to determine the introduction of carbonyls into the cellulose chain.

3. Results

Table 1 shows the weight loss and DP of cellulose and holocellulose exposed to *P. placenta* in contact with wood or filter paper. Holocellulose was the only substrate that suffered substantial decreases in DP and weight losses without wood contact. All substrates lost

weight with large decreases in DP when in contact with wood. Holocellulose was the most severely attacked. The DP of the cellulose was considerably reduced before much weight loss occurred.

The effect of carbohydrate supplements on degradation of cotton and filter paper by *P. placenta* is shown in Table 2. Cellulose supplemented with holocellulose was rapidly depolymerized and metabolized. As with cellulose placed on wood, there was considerable decrease in DP before weight loss occurred. Except with xylan, the DP of cellulose was considerably reduced with the other carbohydrate supplements, but the cellulose was metabolized more slowly than the holocellulose. Cellulose treated with cold and hot water extracts from sweetgum was not depolymerized or utilized by *P. placenta*.

Cellulose swollen with phosphoric acid was rapidly depolymerized and metabolized by *P. placenta* (100% wt loss, 8 wk). Cellulose treated with 1 % H_2O_2/Fe was also metabolized, but not as rapidly (cotton 60 % wt loss, 12 wk; Alter paper 70 % wt loss, 12 wk). The treatment with 1 % H_2O_2/Fe extensively depolymerized the cellulose before exposure to the fungus (see table 3). Swelling with saturated oxalic acid and 24 % NaOH did not permit utilization of cellulose by *P. placenta,* nor did treatment with the 0.1 % $H2O_2$/Fe solution.

Oxidative properties of three types of cellulose samples were compared (Table 3): cellulose exposed to *P. placenta* for 16 wk, cellulose treated with H_2O_2/Fe, and cellulose treated with saturated oxalic acid. The copper number of brown-rotted cellulose increased substantially as did the carboxyl content. The carbonyl content of brown-rotted cellulose is probably high, as testing with mycelium showed it to have ion-exchange capacity. Uronic acid content of brown-rotted cellulose was not increased. The remaining tests indicated that brown-rotted cellulose was oxidized. Values for copper number, ion-exchange capacity, and solubility in 1 % NaOH boil for brown-rotted cellulose were similar to the cellulose treated with 1 % H_2O_2/Fe but different from values for cellulose treated with 0.3 % H_2O_2/Fe and with oxalic acid.

Finally, infrared spectra of the brown-rotted cellulose showed that carbonyl groups had been introduced. Spectra of mycelium also indicated carbonyl groups. However, mycelial spectra did not correspond to spectra of brown-rotted cellulose indicating that the carbonyl groups in the brown-rotted cellulose were not due to mycelium. The brown-rotted cellulose spectra were similar to the spectra of the cellulose treated with 1 % H_2O_2/Fe.

Table 1: Weight losses and depolymerization (DP) of cellulose exposed to brown-rot fungus *Poria placenta*

Substrate a	Wood contact								Filter-paper contact							
	2 Weeks		4 Weeks		8 Weeks		16 Weeks		2 Weeks		4 Weeks		8 Weeks		16 Weeks	
	Wt. loss	DP	Wt. loss	DP	Wt. loss	DP	Wt. loss	DP	Wt. loss	DP	Wt. loss	DP	Wt. loss	DP	Wt. loss	DP
	%		%		%		%		%		%		%		%	
Holocellulose (sweetgum)	45	< 196 b)	93	< 196 b)	95	< 196 b)	100	—	45	520	80	210	95	< 196 b)	100	—
Cotton	0	1200	10	660	20	312	76	170	0	2340	0	2120	0	2330	0	2078
Solka-floc	10	450	42	350	60	186	100	—	0	900	0	749	0	985	0	860
Filter-paper	0	670	6	242	40	285	80	< 196 b)	0	1056	0	1003	0	920	0	912

a) DP of unexposed substrate: Gum holocellulose — 1386; cotton — 2340; solka-floc — 1000; and S and S filter-paper — 1100. — b) Flow in viscosimeter too fast to determine the minimum DP with the concentration of substrate used.

Table 2. Weight losses and DP of celluloses supplemented with carbohydrates over filter-paper inoculated with *Poria placenta.*

Carbohydrate supplement	Filter-paper						Cotton					
	4 Weeks		8 Weeks		12 Weeks		4 Weeks		8 Weeks		12 Weeks	
	Wt. loss	DP	Wt. loss	DP	Wt. loss	DP	Wt. loss	DP	Wt. loss	DP	Wt. loss	DP
	%		%		%		%		%		%	
None	0	1258	0	1089	0	1150	0	2576	0	2264	0	2250
Xylan	0	998	0	1044	0	1100	0	2028	0	2264	0	2176
Mannan	0	414	16	242	74	< 196 a)	0	286	8	276	64	286
Starch	0	750	0	216	74	< 196 a)	0	1812	5	350	40	295
Cellobiose	0	466	20	383	80	< 196 a)	0	460	16	304	70	252
Glucose	0	1258	10	238	80	< 196 a)	0	750	10	322	64	286
Hot water extract	0	1258	0	1100	0	1200	0	2176	0	2200	0	2250
Cold water extract	0	1258	0	1150	0	1100	0	2200	0	2300	0	2276
Holocellulose	0	670	30	285	60	< 196 a)	0	250	50	302	60	360

a) Flow in viscosimeter too fast to determine the minimum DP with the concentration of cellulose used.

Table 3: Comparative properties of brown-rotted, oxalic-acid-treated, and H_2O_2/Fe treated cellulose.

Properties	Cellulosic materials									
	Cellulose untreated		Brown-rot cellulose		Saturated oxalic acid cellulose		1% H_2O_2 Fe cellulose		0.3% H_2O_2 Fe cellulose	
	Cotton	Filter-paper	Cotton	Filter-paper	Cotton	Filter-paper	Cotton	Filter-paper	Cotton	Filter-paper
Copper number a)	0	0.67	7.5	6.9	1.4	1.4	5.6	2.6	3.4	2.7
Degree of polymerization	2300	1050	150	198	1272	620	180	390	920	780
Wt. loss with 1% NaOH boil (%) of treated sample	2	0	31	34	3	0	32	34	9	15
Ferrous sulfate — potassium ferricyanide test	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
pH in 10% NaCl	5.9	5.9	3.2	3.8	1.9	1.7	4.2	3.9	5.4	5.3
Ion exchange b)	0.80	0.74	10.0	6.7	1.0	0.80	5.0	5.0	0.80	0.91

a) g of copper reduced per 100 g of cellulose. — b) mM of carboxyl per 100 g of cellulose.

4. Discussion

The brown-rot fungus, *P. placenta,* as previously reported (T. L. HIGHLEY, 1975), was unable to utilize isolated cellulose or to affect its DP. Sweetgum holocellulose, however, was rapidly depolymerized and metabolized without wood or any additional carbohydrate supplemental nutrients present. Likewise, cellulose exposed with most other carbohydrate supplements in the absence of wood was depolymerized and metabolized by *P. placenta,* but was metabolized much more slowly than with holocellulose or cellulose in contact with wood. Xylan is utilized by *P. placenta* in wood (E.B. COWLING, 1964) and xylanases are produced in culture (T. L. HIGHLEY, 1976) but cellulose was not utilized by *P. placenta* in the presence of xylan. Why this occurred was not established in this study but possibly xylan was broken down to nonutilizable material during autoclaving or xylan by itself may not induce the system required by *P. placenta* for cellulose utilization.

P. placenta was unable to metabolize isolated cellulose supplemented with glucose in a prior study (T. L. HIGHLEY, 1975) but was able to do so here. Different results may be due to different test conditions. In the previous study, cellulose strips were soaked in glucose solution and placed directly over soil in bottles. Under these conditions, the cellulose may have been too wet for utilization by *P. placenta.* I have observed that in agar-block tests *P. placenta* will not degrade wood that has been excessively wetted due to contact with the agar or to capillary movement of water from the agar to the wood.

T. NILSSON (1974 b) also found that, several soft-rot fungi did not degrade cotton in a liquid medium, but did so if culture conditions were such that the moisture of the cotton fiber was not excessively high. Oxidation may be difficult at very high moisture contents because aeration is necessary for oxidation. It would be interesting to determine the degradation of cellulose by brown-rot fungi at different moisture contents.

Lignin has been proposed by T. NILSSON (1974 a) as an inducer of enzymes required by brown-rot fungi to metabolize cellulose. However, lignin is evidently not necessary for utilization of cellulose by *P. placenta* because holocellulose was rapidly metabolized as was isolated cellulose with alternative carbohydrates. S. N. BASU and S. N. GHOSE (1952) also found that several fungi unable to decompose a-cellulose could strongly degrade holocellulose. Thus, the presence of other carbohydrates, such as the hemicelluloses, is apparently essential for the metabolism of cellulose by the brown-rotter *P. placenta.*

Most fungi (Ascomycetes, Fungi Imperfecti, and white-rot) have very little effect on DP of cellulose even at very large weight loss (E. B. COWLING, 1960; G. A. GREATHOUSE, 1950; C. S. WALSETH, 1950). Cellulases from such fungi apparently hydrolyze crystallites only at the surface with rapid and complete destruction of each chain. This would be accomplished by two cellulases acting synergistically (C_x-C_1 concept) (C. HALLIWELL and M. GRIFFIN, 1973).

Brown-rot fungi, however, drastically reduce the DP of cellulose even at very low weight loss, and the decomposition products are readily soluble in alkali (E. B. COWLING, 1961). For example, in this study, the DP of cotton was reduced to about one-half of the original without detectable weight loss. This type of attack on cellulose is similar to acid hydrolysis, were at only 6 % weight loss the DP of cellulose was reduced from 1,385 to 105 (C. S. WALSETH, 1952). Thus, the brown-rot fungi must produce an agent which penetrates the cellulose structure and breaks down the glucosidic bonds and which cannot easily diffuse from the cellulose structure. To have this effect, an agent smaller than any known cellulase would be required. No isolated cellulase has been able to duplicate the effect of brown-rot fungi on cellulose. Brown-rot fungi, therefore, may employ an alternative mechanism of cellulose breakdown.

J. W. KOENIGS (1972 b, 1974 b) found that brown-rot fungi produce H_2O_2 in culture and in wood, and presented evidence that an H_2O_2/Fe system may be employed by brown-rot fungi to depolymerize cellulose. He found the change in DP by H_2O_2/Fe in pine and sweetgum similar to that produced by acid hydrolysis and brown-rot. The alkali solubility curve of wood treated with H_2O_2/Fe nearly fits that of brown-rotters, increasing rapidly at first, then slowly. This differs from the curve for acid-hydrolyzed cellulose which is constant with increasing weight loss.

An H_2O_2/Fe system oxidizes cellulose (G. L. MOODY, 1964; N. I. NIKITIN, 1962). Primary OH groups (C_6) are oxidized first by H_2O_2 to aldehydes and carboxyls, then secondary OH's at C_2 and C_3. This results in scission of the pyranose ring to form aldehydes first, then carboxyl groups. Subsequently, the carboxyl groups apparently catalyze the hydrolysis of the cellulose chain resulting in depolymerization with an accompanying loss of carboxyl (C. F. DAVIDSON and H. A. STANDING, 1951). Thus, if brown-rot fungi employ this system to depolymerize cellulose, degradation products should be oxidized.

Several different tests on cellulose degraded by brown-rot indicated degradation was oxidative. Studies by J. G. BOSWELL (1938) and by M. W. BRAY and T. M. ANDREWS (1924) also imply that brown-rotters oxidize

cellulose. My results indicate that brown-rotted cellulose and 1 % H_2O_2/Fe have many similar oxidative properties. However, comparison of results from cellulose treated with H_2O_2/Fe and brown-rotted cellulose are difficult to interpret. In particular, the ratio of Fe lo H_2O_2 and the pH during treatment affect the amount of intramolecular oxidation and the nature of resulting oxidized groups (V. I. IVANOV et al., 1953).

Thus, the evidence is strong that brown-rot fungi, contrary to most other groups of fungi, employ an oxidative mechanism to break down cellulose in wood. Although the infrared spectra of brown-rotted and H_2O_2/Fe cellulose are similar, it is still uncertain that this catalyst system involves H_2O_2 and Fe. The reaction products should be identified to gain further insight into the mechanism of brown-rot attack. This is riot easy, however, because in the later stages of oxidation the reaction products become so numerous that identification of primary reaction products is almost impossible (N. I. NIKITIN, 1962). Further studies of the degradation products by infrared absorption spectra should provide valuable information on the mechanism of cellulose degradation by brown-rot fungi.

5. Summary

The brown-rot fungus, Poria placenta, was unable to metabolize isolated cellulose but effectively degraded holocellulose. Cellulose was depolymerized when supplemented with various carbohydrates but was utilized more slowly than holocellulose. Thus, the presence of other carbohydrates such as hemicelluloses apparently is essential for the utilization of cellulose by *P. placenta.* The properties of the brown-rot degraded cellulose indicated that it was oxidized.

Zusammenfassung

Voraussetzungen für den Cellulose-Abbau durch einen Braunfäule-Pilz

Der Braunfäule-Pilz *Poria placenta* konnte isolierte Cellulose nicht angreifen, baute aber Holocellulose stark ab. Cellulose wurde depolymerisiert, wenn sie durch verschiedene Kohlenhydrate ergänzt wurde, aber sie wurde langsamer verwertet als Holocellulose. Die Gegenwart anderer Kohlenhydrate - wie Hemicellulosen - scheint für die Verwendbarkeit der Cellulose durch *P. placenta* notwendig zu sein. Die Eigenschaften durch Braunfäule abgebauter Cellulose sprechen dafür, daß sie beim Abbau oxydiert wird.

Résumé

Conditions requises pour la dégradation de la cellulose par un champignon de pourriture brune

Le champignon de pourriture brune *Poria placenta* était incapable de métaboliser la cellulose isolée mais dégradait effectivement l'holocellulose. La cellulose était dépolymerisée lorsque complementée avec divers hydrates de

carbone mais était utilisée plus lentement que I'holocellulose. Ainsi, la présence d'autres hydrates de carbone tels que hémicelluloses est apparemment essentielle pour l'utilisation de la cellulose par *P. placenta* Les propriétés de la cellulose degradée de pourriture molle indique qu'elle est oxydée.

Resumen

Condiciones previas para la degradacón de celulosa por un hongo causante de la pudrición parda

Poria placenta, un hongo causante de la pudrición parda, fue incapaz de atacar celulosa aislada, causando, en cambio, una eficaz degradación de la holocelulosa. La celulosa fue despolimerizada añadiéndosele varios hidratos de carbono, siendo, sin embargo, aprovechada más despacio que la holocelulosa. Por tanto, parece ser necesaria la presencia de otros hidratos de carbono, tal como hemicelulosas, a fin de que *P. placenta* pueda aprovechar la celulosa. Las propiedades de la celulosa degradada por la pudrición parda indican una oxidación en el proceso de su degradación.

References

American Society for Testing and Materials: Standard method of test for copper of paper and paperboard. ASTM Desig. D 9919 - 49. In: Annual ASTM Standards, 1968 a, Philadelphia, Pa.

— Standard method of test for water solubility of wood. ASTM Desig. D 1110 - 56. In: Annual ASTM Standards, 1968 b, Philadelphia, Pa.

— Standard method of test for carboxyl content of cellulose. ASTM Desig. D 1926. In: Annual ASTM Standards, 1970, Philadelphia, Pa.

— Standard method of test for accelerated laboratory test of natural decay resistance of woods. ASTM Desig. D 2017. In: Annual ASTM Standards, 1971, Philadelphia, Pa.

Barton-Wright, E. C., and J. G. Boswell: LX. The biochemistry of dry-rot in wood. II. An investigation of the products of decay of spruce wood rotted by *Merulius lacrymans*. Biochem. J. *25* (1931) 494 - 506.

Basu, S. N., and S. N. Ghose: Fungal decomposition of jute fibre and cellulose. IV. The action of some physical and chemical agencies on subsequent fungal decomposition of jute. J. Textile Inst. *43* (1952) 355 - 361.

Boswell, J. G.: XXX. The biochemistry of dry-rot in wood. III. An investigation of the products of the decay of pine wood rotted by *Merulius lacrymans*. Biochem. J. *32* (1938) 218 - 229.

Bray, M. W., and T. M. Andrews: Chemical changes of groundwood during decay. Ind. Eng. Chem. *16* (1924) 137 - 139.

Cowling, E. B.: Methods for chemical analysis of decayed wood. U. S. Dep. Agric., For. Serv. FPL Rep. 2177, 1960, 24 p.

— Comparative biochemistry of the decay of sweetgum by white-rot and brown-rot fungi. U. S. Dep. Agric., For. Serv. Tech. Bull. 1258, 1961, 79 p.

Greathouse, G. A.: Microbiological degradation of cellulose. Textile Res. J. *20* (1950) 4, 227 - 238.

HALLIWELL, G., and M. GRIFFIN: The nature and mode of action of the cellulolytic component of *Trichoderma koningii* on native cellulose. Biochem. J. *135* (1973) 587 - 594.

HIGHLEY, T. L.: Influence of carbon source on cellulase activity of white-rot and brown-rot fungi. Wood and Fib. *5* (1973) 1, 50 - 58.

— Can wood-rot fungi degrade cellulose without other wood constituents? For. Prod. J. *25* (1975) 7, 38 - 39.

— Hemicellulases of white- and brown-rot fungi in relation to host preferences. Material u. Organismen *11* (1976) 1, 33 - 46.

IVANOV, V. I., E. D. KAVENZNEVA, and Z. I. KUZNETSOVA: Chemical changes produced in the cellulose macromolecule by oxidizing agents. Communcation 8. Chemical changes in cellulose by oxidation with hydrogen peroxide. Div. Chem. Sci. Acad. USSR Bull. No. 2 (Engl. Trans.) (1953) 341 - 350.

KOENIGS, J. W.: Effects of hydrogen peroxide on cellulose and its susceptibility to cellulase. Material u. Organismen *7* (1972 a) 133 -147.

— Effects of extracellular hydrogen peroxide and peroxidase by wood-rotting fungi. Phytopath. *62* (1972 b) 100 - 110.

— Hydrogen peroxide and iron: A proposed system for decomposition of wood by brown-rot basidiomycetes. Wood and Fib. *6* (1974 a) 1, 66 - 79.

— Production of hydrogen peroxide by wood-decaying fungi in wood and its correlation with weight loss, depolymerization and pH changes. Arch. Mikrobiol. *99* (1974 b) 129 - 145.

MARSH, J. T., and F. C. WOOD: An introduction to the chemistry of cellulose. Inc., New York, N. Y. (Van Nostrand Co.) 1939, 431 p.

MOODY, G. J.: The action of hydrogen peroxide on carbohydrates and related compounds. Adv. Carbohydr. Chem. *19* (1964) 149 - 179.

NIKITIN, N. I.: [The chemistry of cellulose and wood.] Israel Program for Sci. Transl. 1966, New York (Daniel Davey and Co., Inc.) (1962) 691 p.

NILSSON, T.: Comparative study in the cellulolytic activity of white-rot and brown-rot fungi. Material u. Organismen *9* (1974 a) 3, 173 - 198.

— The degradation of cellulose and the production of cellulase, xylanase, mannanase, and amylase by wood-attacking microfungi. Stud. For. Suec. (1974 b), N. 114.

ROMANELLI, R. A., C. W. HOUSTON, and S. M. BARNETT: Studies on thermophilic cellulolytic fungi. Appl. Microbiol. *30* (1975) 2, 276 - 288.

WALSETH, C. S.: Occurrence of cellulases in enzyme preparations from microorganisms. Tappi *35* (1952) 5, 228 - 238.

Address of the author:

Dr. TERRY L. HIGHLEY
Forest Products Laboratory
P.O. Box 5130
Madison, Wisconsin 53705
U. S. A.