Is Extracellular Hydrogen Peroxide Involved in Cellulose Degradation by Brown-Rot Fungi?

By TERRY L. HIGHLEY*

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

Contents

1. Introduction
2. Methods
3. Results and Discussion
4. Summary
References

1. Introduction

The nature of cellulose breakdown by brown-rot fungi suggests that a small metabolic product may be involved in the initial stages (E. B. COWLING and W. BROWN, 1969). Circumstantial evidence suggests that this small metabolic product may be hydrogen peroxide (J. W. KOENIGS, 1972, 1974 a, 1974 b). Hydrogen peroxide (H_2O_2) in the presence of Fe^{++} or other transition metals forms highly reactive radicals that initiate oxidative degradation of carbohydrates (B. HALLIWELL, 1978).

H. LYR (1958) was unable to detect H_2O_2 production by brown-rot fungi. J. W. KOENIGS (1972, 1974 a) reported that brown-rot fungi produced extracellular H_2O_2 in culture and in wood assayed by the aminotriazole method but the validity of this assay for determining H_2O_2 in crude filtrates from fungi has been questioned (T. L. HIGHLEY, 1981). J. W. KOENIGS (1974 a) also found the change in degree of polymerization by the H_2O_2/Fe^{++} system in pine and sweetgum to be similar to that produced by acid hydrolysis and brown rot. The alkali solubility curve of wood treated with H_2O_2/Fe^{++} nearly coincides with that of brown rot, increasing rapidly at first, then slowly.

For the H_2O_2 system to be operative, an oxidative enzyme must be produced that generates H_2O_2 by oxidation of a carbon source — such as

* The author would like to thank Dr. D. D. NICHOLAS, Forest Products Utilization Laboratory, Mississippi State University, for suggesting the *p*-nitrosodimethylaniline assay.

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

From: 14 Material und Organismen 17/3

glucose — and Fe^{++} or other transition metals must act on H_2O_2 to produce reactive radicals — e. g., · OH. The purpose of this work was to (1) examine cultures from brown-rot fungi for the presence of H_2O_2-producing oxidases and · OH radicals; (2) determine the presence or absence of H_2O_2 in brown-rotted cellulose, wood, and liquid cultures; and (3) determine if materials that influence the H_2O_2/·OH system have an effect on degradation of wood by brown-rot fungi.

2. Methods

Six brown-rot fungi were used: *Poria placenta* (Fr.) Cke., *Gloeophyllum trabeum* (Pers. ex Fr.) Murr., *Lentinus lepideus* Fr., *Coniophora puteana* (Schum. ex Fr.) Karst., *Leucogyrophana arizonica* Ginns, *L. olivascens* (Berk. et Curt.) Ginns et Weresub; and four white-rot fungi were included for comparison; *Coriolus versicolor* (L. ex Fr.) Quél., *Ganoderma applanatum* (Pers. ex Wallr.) Pat., *Phellinus ferrugineofuscus* (Karst). Bourd., and *Spongipellis unicolor* (Schw.) Murr.

The fungi were grown in stationary liquid culture, pH 5.5, with 25 ml of a basal salts solution (T. L. HIGHLEY, 1973) and with 1% carbon source. Carbon sources for the brown-rot fungi were mannose, galactose, arabinose, xylose, cellobiose, malt extract, galactomannan, or xylan. White-rot fungi were grown only on glucose media. The malt-peptone-glucose medium of J. W. KOENIGS (1972) was also used for the brown-rot fungi. Flasks were innoculated with a mycelial plug and incubated at 27° C without light for 7, 14 and 21 days. Mycelium was separated from filtrates and filtrates were assayed for H_2O_2, catalase, peroxidase, and carbohydrate oxidase. Hydroxyl (· OH) radical was determined only in glucose cultures after 21 days.

Solid cultures were 50-mg samples of cellulose (cotton, Hercules?) and hemlock sawdust (*Tsuga heterophylla* (Raf.) Sarg.), placed on pine feeder strips and decayed by the ASTM D 2017 soil-block method (ASTM, 1971) for 2, 4 and 8 weeks by *P. placenta, L. lepideus,* and *G. trabeum.* After removal, the samples were assayed for the presence of H_2O_2 and carbohydrate oxidase activity.

Carbohydrate oxidase was assayed in filtrates of liquid and solid cultures by measuring H_2O_2 production with the *o*-dianisidine-peroxidase reagent (R. M. KERWIN and H. W. RUELIUS, 1969) or with titanium tetrachloride reagent (W. C. WOLFE, 1962). Enzyme reactions were conducted at pH 5.5 (0.05 M phosphate buffer) with either glucose, galactose, mannose, xylose, cellobiose, galactomannan (Sigma), xylan (Aldrich), or cellulose (Solk-floc SW-40) as substrates for the brown-rot fungi. Only glucose was used as a substrate for the white-rot fungi. Carbohydrate oxidase activity was determined periodically for up to 24 hours.

Catalase production in liquid cultures was determined by the method of Y. YOSHPE-PURER and Y. HENIS (1976). One ml of filtrate was incubated with 1 ml of H_2O_2 to final conc. of 0.1, 0.15, 0.2, 0.25, 0.4, and 0.5%. After 1 minute of incubation at 25° C, the reaction was stopped by adding rapidly 50 ml of

[2] The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the U.S. Department of Agriculture of any product or service to the exclusion of others that may be suitable.

2 % H_2SO_4, and the residual amount of H_2O_2 determined by titration with 0.01 N $KMnO_4$ for the 0.1 to 0.25% and 0.02 N for the 0.4 and 0.5 % dilutions Of H_2O_2.

Peroxidase activity was determined by using the *o*-dianisidine system described by the Worthington Biochemical Corporation (1972).

Presence of H_2O_2 in cultures was detected with *o*-dianisidine reagent (R. M. KERWIN and H. W. RUELIUS, 1969) and titanium reagent (W. C. WOLFE, 1962) (both able to measure H_2O_2 at 1 μg/ml) without a carbohydrate added to the reaction mixture.

Bleaching of *p*-nitrosodimethylaniline (W. BORS et al., 1979) was used to assay the presence of · OH in 21-day-old glucose filtrates. One ml of filtrate was added to 2 ml of 0.05 mM *p*-nitrosodimethylaniline and the change in absorbance measured immediately at 440 nm.

To determine if filtrates of the fungi contained materials destructive to H_2O_2, 1 ml of filtrate was incubated with 1 ml of various conc. of H_2O_2 (10 to 100 μg/ml) for 24 hours at 4° C and conc. of H_2O_2 determined.

To measure the effect of materials interfering with the H_2O_2/radical system on decay, weighed blocks of southern pine (1/4 × 3/4 × 3/4 inch, small dimension in grain direction) were impregnated under vacuum with sterile solutions of the test compounds. The blocks were tested for decay resistance by the standard ASTM D 2017 soil-block method (ASTM, 1971) and an agar-block method (W. E. ESLYN and T. L. HIGHLEY, 1976) with *P. placenta* as test fungus. The agar-block method was used to eliminate the effect of exogenous culture conditions such as metals that might generate radicals from the underlying wood feeder used in soil-block tests. After 8 or 12 weeks, blocks were removed from bottles, conditioned at 27° C and 70 % humidity, and weighed. Weight loss was used as an estimate of decay susceptibility.

3. Results and Discussion

Extracellular H_2O_2 was detected in liquid cultures of only one brown-rot fungus, *L. olivascens,* grown on various carbon sources and assayed by the *o*-dianisidine and titanium tetrachloride reagent (Table 1). No H_2O_2 was detected in sawdust or cellulose decayed by G. trabeum, P. placenta, *L. lepideus.* Filtrates, mycelium, decayed sawdust, and cellulose assayed for H_2O_2-generating carbohydrate oxidases were also negative.

Catalase and peroxidase produced extracellularly by the brown-rot fungi would destroy H_2O_2 if it were formed, but this was not the case here as neither of these enzyme activities was detected in our filtrates. J. W. KOENIGS (1972) also did not find catalase or peroxidase in filtrates of several brown-rot fungi. The possibility that other materials in fungal filtrates from brown-rot fungi might destroy H_2O_2 was eliminated when it was found that up to 24 hours' incubation of filtrates with known concentrations of H_2O_2 did not lower the concentration of H_2O_2.

For the white-rot fungi, H_2O_2 was detected with the *o*-dianisidine reagent in filtrates of all except *G. applanatum* (Table 1). H_2O_2 was not

detected with the titanium reagent, which is similar to *o*-dianisidine in sensitivity of H_2O_2 detection. The explanation for this discrepancy is only speculative: possibly *o*-dianisidine stimulates and protects the H_2O_2-producing carbohydrate oxidases during their catalytic actions.

Table 1. H_2O_2-producing oxidase and peroxidase activity in fungal filtrates from *glucose* cultures after 14 days.

Fungus	H_2O_2-producing oxidase		Peroxidase[c] ($\Delta A_{460\,nm}$)
	Dianisidine[a] ($\Delta A_{460\,nm}$)	Titanium[b] ($\Delta A_{460\,nm}$)	
Brown-rot			
L. olivascens	0.22	0.14	0
White-rot			
C. versicolor	0.31	0	0.03
S. unicolor	0.42	0	0.39
P. ferrugineofuscus ..	0.015	0	0.09
G. applanatum	0	0	0

a) Change in absorbance/min. of the oxidation of *o*-dianisidine + glucose by filtrates.
b) Filtrates were incubated with 1 % glucose for 10 min. and titanium reagent added.
c) Change in absorbance/min. of the oxidation of *o*-dianisidine + 0.003 % H_2O_2 by filtrates.

Extracellular H_2O_2-generating carbohydrate oxidases are reported in several mold fungi as well as some white-rot fungi (G. AVIGAD et al., 1962; J. M. GANCEDO et al., 1967; T. NAKAMATSU et al., 1975). *Aspergillus niger* and *Penicillium purpurogenum* produce extracellular glucose oxidase (J. M. GANCEDO et al., 1967; T. NAKAMATSU et al., 1975). The white-rot fungus *Polyporus circinatus* produces extracellular galactose oxidase which is also active on the polymer galactomannan (G. A. AVIGAD et ai., 1962). *Sporotrichum pulverulentum* produces extracellular cellobiose oxidase but H_2O_2 was not detected as a product of enzyme activity (A. R. AYERS et al., 1978). The function of this enzyme is regulatory to prevent end-product inhibition of cellulase by simple sugars (A. R. AYERS et al., 1978). Cellulose breakdown by many brown-rot fungi, however, is not inhibited by breakdown end-products (T. L. HIGHLEY, 1973). It is only logical that many white-rot fungi produce extracellular H_2O_2 as most of these fungi produce extracellular peroxidase, which requires H_2O_2 to oxidize phenolic substrates.

To the author's knowledge, extracellular H_2O_2-producing oxidases have not, with the exception of J. W. KOENIGS (1972), been reported in filtrates of brown-rot fungi. We found significant H_2O_2-generating oxidase production by only one brown-rot fungus, *L. olivascens*. Alcohol

oxidase is found in several brown-rot fungi and it generates H_2O_2, but this enzyme is intracellular (R. M. KERWIN and H. W. RUELIUS, 1969). J. W. KOENIGS (1972) was only able to detect the presence of extracellular H_2O_2 in filtrates of brown-rot fungi with the aminotriazole assay; he was unsuccessful with the titanium reagent. T. L. HIGHLEY (1981) found the aminotriazole assay for H_2O_2 to be invalid for determining H_2O_2 in crude filtrates from wood decay fungi because of materials in the filtrates that apparently interfere with the assay. Thus, apparently many brown-rot fungi do not produce detectable amounts of extracellular H_2O_2.

Despite the failure to detect measurable amounts of H_2O_2 in most filtrates from brown-rot fungi, filtrates cultured on glucose bleached *p*-nitrosodimethylaniline, suggesting that ·OH radicals are present in filtrates (Table 2). Filtrates from the white-rot fungi also bleached *p*-nitrosodimethylanaline. Bleaching of *p*-nitrosodimethylaniline, however, is not specific to ·OH radicals, as organic radicals and easily auto-oxidizable compounds can produce the same reactions (W. BORS et al., 1979). H_2O_2 was not detected in filtrates of most of the brown-rot fungi and, therefore, these other reagents might cause the (bleaching reaction. Another possibility is that ·OH radicals produced intracellularly may "leak" out of fungal cells.

Table 2. Bleaching of *p*-nitrosodimethylaniline by filtrates of brown- and white-rot fungi[a].

Fungus	Bleaching[b] ($\Delta A_{440\,nm}$)
Brown-rot	
G. trabeum	1.35
L. lepideus	0.96
L. arizonica	1.35
L. olivascens	1.31
C. puteana	1.37
P. placenta	1.35
White-rot	
C. versicolor	1.20
S. unicolor	1.06
P. ferrugineofuscus	1.35
G. applanatum	1.28

a) Carbon source was 1 % glucose.
b) Initial absorbance was 1.5. The change in absorbance was determined immediately upon addition of filtrate to p-nitrosodimethylaniline.

Depolymerization of cellulose by H_2O_2 is due to the formation of highly reactive radicals such as ·OH, which rapidly attack and destroy

carbohydrates. The metal-catalyzed Haber-Weiss reaction has been suggested to be the mechanism by which · OH is formed in biochemical systems (B. HALLIWELL, 1978). The net reaction is:

$$\cdot O_2 + H_2O_2 \xrightarrow[\text{metals}]{\text{transition}} O_2 + \cdot OH + OH^-$$

Materials that quench . OH radicals, therefore, should prevent cellulose breakdown by this system. Chelators may inhibit or stimulate radical formation from H_2O_2 depending on the nature of the chelator. For example, B. HALLIWELL (1978) found the chelator bathophenanthroline sulphonate inhibitory to · OH formation, but ethylenediaminetetraacetic acid enhances the reaction.

The chelators and quenching agents did not affect decay by *P. placenta* (Table 3). However, if the · OH exists as an · OH-analogous complex, it may not react with . OH quenching agents (W. BORS et al., 1979). Information on additional radical-quenching agents and their effect on cellulose degradation by brown-rot fungi should be sought.

Although the evidence questions the involvement of extracellular H_2O_2 in cellulose degradation by brown-rot fungi, the nature of brown-rot attack still rules out cellulose degradation by the conventional synergistic action of exo- and endo-cellulases. Exo-cellulase activity is not present in filtrates of many brown-rot fungi (T. L. HIGHLEY, 1973). Predisposition of the cellulose substrate to endo-cellulase attack most likely takes place via a nonenzymatic mechanism as proposed by E. B. COWLING (1969) and J. W. KOENIGS (1972).

Carbohydrate oxidases that do not produce H_2O_2 but generate radicals, such as the superoxide radical · O_2- or strongly oxidizing species resembling · OH, could be involved in cellulose degradation by brown-rot fungi. K.-E. ERIKSSON (1981) recently found that cellobiose oxidase from *Sporotrichum pulverulentum* produces · O_2-. Furthermore, he found that · O_2- is produced both intra- and extracellularly by several wood-rotting fungi. He proposes that radicals of this type might be involved in the primary attack on crystalline cellulose. In a previous study (T. L. HIGHLEY, 1977), cellulose degraded by the brown-rot fungus P. placenta had oxidized characteristics, suggesting that radicals could be involved in oxidative degradation of cellulose by this fungus. Because brown-rot fungi reportedly produce intracellular H_2O_2 (R. M. KERWIN and H. W. RUELIUS, 1969), · OH radicals or · OH-analogus complexes may "leak" out of the cell or be released during autolysis, and oxidatively degrade cellulose. Future mechanistic studies of cellulose decomposition by brown-rot fungi should concentrate on the role radicals may play in this degradative process.

Table 3. Decay of southern pine by *Poria placenta* in the presence of materials influencing peroxide/radical levels.

Compound	Concentration	Percent weight loss	
		Soil-block[a)]	Agar-block[b)]
Control		64.5	61.0
Chelators			
Dipyridyl	0.1 %	58.5	52.0
	0.05 %	63.0	—
Phenanthroline	10^{-2} M	61.0	63.0
Sodium gluconate	0.5 %	62.5	62.5
	1.0 %	65.5	47.0
Bathophenanthroline-disulfonic acid (BPS)	10^{-5} M	63.0	—
	10^{-4} M	64.0	57.0
Norepinephrine	10^{-2} M	63.0	62.5
Diethyl dithiocarbamate	10^{-2} M	65.0	6.0
Radical- and peroxide-quenching agents			
KI	10^{-2} M	59.0	41.0
$KMnO_4$	10^{-2} M	64.0	56.0
Sodium pyruvate	1.0 %	62.0	63.5
Thiourea	10^{-2} M	65.0	62.5
	10^{-1} M	1.5	0.0
Mannitol	1.0 %	62.5	53.0
	0.5 %	64.0	3.0
Na formate	10^{-2} M	64.0	26.0
	10^{-1} M	63.5	—
Na benzoate	10^{-1} M	63.0	22.0
Na metabisulfite	10^{-1} M	62.0	60.0
Formic acid	0.5 %	65.0	—
Peroxidase stabilizers			
Acetanilide	10^{-3} M	65.0	65.0
	10^{-2} M	61.0	—
Protein-synthesizing inhibitors			
Chloroamphenicol	10^{-4} M	63.0	—
Fluorouracil	10^{-4} M	5.3	—

a) 8 weeks.
b) 12 weeks.

4. Summary

Only one brown-rot fungus was found to produce significant quantities of extracellular H_2O_2. Several white-rot fungi, however, produced extracellular H_2O_2. Peroxidase or catalase that might destroy H_2O_2 were not found in brown-rot culture filtrates. Nor did culture filtrates from brown-rot fungi incubated with known quantities of H_2O_2 destroy it. Furthermore, compounds affecting the H_2O_2/. OH system did not affect degradation of wood by the brown-rot fungus *Poria placenta.* Thus, the results question the involvement of extracellularly produced H_2O_2 in cellulose depolymerization by brown-rot fungi. Both brown-rot and white-rot fungi released · OH radicals or . OH-analogous radicals into culture filtrates. The possible involvement of radicals in cellulose degradation by brown-rot fungi is discussed.

Zusammenfassung

Ist extracelluläres Wasserstoffperoxid am Celluloseabbau durch Braunfäulepilze beteiligt?

Unter den Braunfäulepilzen produzierte nur einer bedeutende Mengen an extracellulärem H_2O_2. Mehrere Weißfäulepilze produzierten jedoch extracelluläres H_2O_2. Peroxidase oder Katalase, die H_2O_2 zerstören könnten, wurden in Kulturfiltraten von Braunfäulepilzen nicht gefunden. Ebensowenig wurde H_2O_2 von Kulturfiltraten aus Braunfäulepilzen zerstört, die mit bekannten Mengen an H_2O_2 angesetzt wurden, noch beeinflußten Verbindungen, die das H_2O_2/· OH-System angriffen, den Holzabbau durch den Braunfaulepilz *Poria placenta.* Die Ergebnisse lassen es daher zweifelhaft erscheinen, daß extracelluläres H_2O_2 beim Celluloseabbau durch Braunfäulepilze beteiligt ist. Sowohl Braun- als auch Weißfäulepilze geben · OH-Radikale oder · OH-analoge Radikale in die Kulturfiltrate ab. Die mögliche Beteiligung von Radikalen beim Celluloseabbau durch Braunfäulepilze wird besprochen.

Résumé

Le peroxyde d'hydrogène extracellulaire est-il impliqué dans la dégradation de la cellulose par les champignons de pourriture brune?

On a trouvé seulement un champignon de pourriture brune produisant des quantités significatives de H_2O_2 extracellulaire. Cependant, plusieurs champignons de pourriture blanche produisaient de 1'H_2O_2 extracellulaire. On n'a pas trouvé dans les filtrats de culture de pourriture brune de peroxydase ou de catalase pouvant détruire H_2O_2. I1 n'y a pas non plus de destruction par les filtrats de culture de pourriture brune incubés avec des quantités connues de H_2O_2. De plus, des composes affectant le système H_2O_2/OH n'affectaient pas la degradation du bois par le champignon de pourriture brune *Poria placenta.* Donc, les résultats remettant en question la participation de H_2O_2 produit etracellulaire, dans la dépolymérisation de la cellulose par les champignons de pourriture brune. A la fois les champignons de pourriture brune et blanche libéraient dans leurs filtrats de culture des radicaux OH ou des radicaux analogues OH. On discute de l'implication possible de radicaux dans la degradation de la cellulose par les champignons de poiurriture brune.

Resumen

Participa el peróxido de hidrógeno extracelular en la degradaciôn de celulosa por hongos de la pudrición parda?

Entre los hongos causantes de la pudrición parda, uno sólo resultó producir cantidades significantes de H_2O_2 extracelular. En cambio, varios hongos de la pudrición blanca produjeron H_2O_2 extracelular. En filtrados de cultivos de hongos de la pudrición parda no se encontraron peroxidasa o catalasa que pudieran destruir el H_2O_2. Este último no fue destruido tampoco por filtrados de cultivos de hongos de la pudrición parda incubados con cantidades conocidas de H_2O_2. Además, compuestos que atacaban al sistema $H2O_2/ \cdot OH$, no influyeron en la degradación de la madera por el hongo de la pudrición parda, *Poria placenta*. Estos resultados ponen en duda, por tanto, la participación de H_2O_2 extracelular en la depolimerización de celulosa por hongos de la pudrición parda. Tanto los hongos de la pudrición parda como los de la blanca despiden radicales $\cdot OH$ o análogos radicales $\cdot OH$ en los filtrados de cultivo. Se procede a discutir la posible participatión de radicales en la degradacion de celulosa por hongos causantes de la pudrición parda.

References

American Society for Testing and Materials: Standard method for accelerated laboratory test of natural decay resistance of woods. ASTM Desig. D 2017. Philadelphia, Pa., 1971.

Avigad, G., Amaral, D., Asensio, C., and Horecker, B. L.: The D-galactose oxidase of *Polyporus circinatus*. J. Biol. Chem. *237* (1962) 2736 - 2743.

Ayers, A. R., Ayers, S. B., and Eriksson, K.-E.: Cellobiose oxidase, purification and partial characterization of a hemoprotein from *Sporotrichum pulverulentum*. Eur. J. Biochem. 90 (1978) 171 - 181.

Bors, W., Michel, C., and Saran, M.: On the nature of biochemically generated hydroxyl radicals. Studies using the bleaching of *p*-nitrosodimethylaniline as a direct assay method. Eur. J. Biochem. *95* (1979) 621 - 627.

Cowling, E. B., and Brown, W.: Structural features of cellulose materials in relation to enzymatic hydrolysis. In: Hajny, G. J., and Reese, E. T. (Eds.): Cellulases and their applications. Adv. Chem. Ser. *95* (1969) 152 - 187.

Eriksson, K.-E.: Microbial degradation of cellulose and lignin. Proc. Internat. Symp. Wood Pulping Chemistry [Stockholm, June 1981] *3* (1981) 60 - 65.

Eslyn, W. E., and Highley, T. L.: Decay resistance and susceptibility of sapwood of fifteen tree species. Phytopathology *66* (1976) 1010 - 1017.

Gancedo, J. M., Gancedo, C., and Asensio, C.: Widespread occurrence of galactose oxidase and glucose oxidase in fungi. Arch. Biochem. Biophys. *119* (1967) 558 - 590.

Halliwell, B.: Superoxide-dependent formation of hydroxyl radicals in the presence of iron salts. FEBS Lett. *96* (1978) 238 - 242.

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

— Requirements for cellulose degradation by a brown-rot fungus. Material u. Organismen *12* (1977) 25 - 36.

— Degradation of cellulose by *Poria placenta* in the presence of compounds that affect hydrogen peroxide. Material u. Organismen *15* (1980) 82 - 90.

— Catalase-aminotriazole assay — an invalid method for measurement of hydrogen peroxide by wood-decay fungi. Appl. Environ. Microbiol. *42* (1982) 925 - 927.

KERWIN, R. M. and RUELIUS, H. W.: Production of alcohol oxidase by several basidiomycetes. Appl. Microbiol. *17* (1969) 347 - 351.

KOENIGS, J. W.: Production of extracellular hydrogen peroxide and peroxidase by wood-rotting fungi. Phytopathology *62* (1972) 100 - 110.

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

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

LYR, H.: Über den Nachweis von Oxydasen und Peroxydasen bei höheren Pilzen und die Bedeutung dieser Enzyme für die Bavendamm-Reaktion. Planta *50* (1958) 359 - 370.

NAKAMATSU, T., AKAMATSU, T., MIYAJIMA, and SHIIO, I.: Microbial production of glucose oxidase. Agric. Biol. Chem. *39* (1975) 1803 - 1811.

RUELIUS, H. W., KERWIN, R. M., and JANSSEN, R. W.: Carbohydrate oxidase, a novel enzyme from *Polyporus obtusus*. I. Isolation and purification. Biochim. Biophys. Acta *167* (1968) 493 - 500.

WOLFE, W. C.: Spectrophotometric determination of hydroperoxide diethylether. Anal. Chem. *34* (1962) 1328 - 1330.

Worthington Biochemical Corp.: Worthington Enzyme Manual. Freehold, New Jersey, 1972, 216.

YOSHPE-PURER, Y., and HENIS, Y.: Factors affecting catalase level and sensitivity to hydrogen peroxide in *Escherichia coli*. Appl. Environ. Microbiol. *32* (1976) 465 - 469.

Address of the author:

Dr. TERRY L. HIGHLEY

Forest Products Laboratory
Forest Service
US. Department of Agriculture
P.O. Box 5130
Madison, Wisconsin, 53705
U.S.A.