HYDROGEN PEROXIDE AND IRON: A PROPOSED SYSTEM FOR DECOMPOSITION OF WOOD BY BROWN-ROT BASIDIOMYCETES

Jerome W. Koenigs
Research Plant Pathologist
Southeastern Forest Experiment Station
Research Triangle Park, N. C. 27709

(Received 10 January 1974)

ABSTRACT

Low concentrations of H_2O_2 and Fe^{++} caused rapid weight loss of wood of sweetgum and loblolly pine. The degree of polymerization of cellulose in treated woods decreased rapidly at low weight loss and then diminished gradually. The alkali solubility of exposed woods increased rapidly at low weight loss and was inversely related to the degree of polymerization. The H_2O_2-Fe system solubilized hemicelluloses of both woods more readily than cellulose. Lignin of sweetgum, but not pine, was changed so that it was solubilized by strong acid hydrolysis. The optimal pH for weight loss was about 3.3 for sweetgum and 3.0 for pine. Wood of both species absorbed much of the available Fe from solution.

The literature suggests that brown-rot is oxidative rather than being strictly hydrolytic. The H_2O_2-Fe^{++} system oxidizes cotton cellulose, and it essentially reproduced in wood and wood cellulose, characteristics of brown-rot basidiomycetes. These fungi produce H_2O_2 from native substrates in wood (Koenigs, 1974) and the optimal pH for degradation. Thus, it is proposed that these fungi may attack cellulose and partly decay wood via an H_2O_2-Fe^{++} system.

Additional keywords: Pinus taeda, Liquidambar styraciflua, wood decay, weight loss, cellulolysis, depolymerization, lignin solubility.

Microbial decomposition of cellulose is considered to result solely from the direct enzymatic interaction of cellulase components. These fractions have been isolated and their individual (Reese and Mandels 1971) and synergistic effects reported (Li et al. 1965; Mandels and Reese 1964; Reese and Gilligan 1954; Selby 1969; Selby and Maitland 1967; Wood 1968). Enzymatic hydrolysis usually involves random rather than endwise attack as shown by depolymerization experiments with cotton cellulose (Reese 1957; Reese et al. 1957) and delignified wood cellulose (King 1968) or its derivatives (Norkrans and Rånby 1956).

White-rot basidiomycetes, also, produce cellulase components capable of randomly attacking delignified cellulose (see references in Cowling and Brown 1969; Gasgoine and Gasgoine 1960; Highley 1973; Norkrans 1967); however, these preparations have little effect on native wood (Pew 1957). The fungi themselves depolymerize the cellulose so that at any stage of decay the bulk of the residual cellulose is not depolymerized (Cowling 1961; Kayama 1961; Kayama 1962a, b, c). This restricted depolymerization by cellulase in wood is attributed to protection by the lignin and hemicelluloses surrounding the cellulose, to the fact that capillary pores are too small to admit the cellulase molecule and to several other factors (Cowling and Brown 1969).

In contrast to white-rot basidiomycetes, brown-rot fungi lack a C_1 enzyme (Highley 1973), yet they depolymerize wood cellulose to 20% of the original chain length at only 10% weight loss (Cowling 1961; Kayama 1962a). These differences and other characteristics of the wood rotted by these two groups of basidiomycetes (see discussion in Cowling 1961) suggest that they may employ basically different cellulolytic systems. Some effects of brown-rot fungi resemble those created by acid hydrolysis or oxidative degradation, but wood cellulose is notably resistant to both weak acids

and oxidants under normal conditions (Browning 1964) and in fact, strong acids and elevated temperatures are required to achieve such degradation. However, Halliwell (1965) has demonstrated that relatively low concentrations of H_2O_2 and Fe^{++} (Fenton's reagent) in acetate buffer at an optimal pH of 4.2 rapidly decomposed cotton cellulose.

Recently, the production of extracellular H_2O_2 by wood-rotting fungi was demonstrated in culture (Koenigs 1972b) and then in wood without added nutrients (Koenigs 1974). Consequently, these recent results pointed to the possibility that at least some of these fungi might utilize this H_2O_2 to decompose cellulose. Since Halliwell (1965) had not studied intermediate effects of the H_2O_2-Fe^{++} system, such an investigation was completed with cotton cellulose (Koenigs 1972a); the H_2O_2-Fe^{++} system rapidly depolymerized cellulose at low weight loss and rapidly reduced strength in the manner of brown-rot fungi. This suggested that the system might similarly affect cellulose in wood, a possibility also alluded to by Cowling and Brown (1969). Yet, the possibilities remained that lignin and hemicellulose afforded protection or that extractives modified the reactions.

The present investigation was undertaken to ascertain (a) whether the H_2O_2-Fe^{++} system might alter cellulose in native wood similarly to cotton cellulose, (b) whether measured effects could be achieved at concentrations of reagents and conditions likely to be encountered in wood during decay, and (c) whether effects were typical of either brown- or white-rot fungi as described in the literature. Answers to these questions were needed to determine whether it was rational to advance a hypothesis that brown-rot fungi employ a H_2O_2-Fe^{++} system to decompose or partially decompose wood. Based on experimental results, the hypothesis is reasonable pending proof that brown-rot fungi oxidize cellulose.

MATERIALS AND METHODS

Wood for these experiments was taken from a 22-year-old loblolly pine (*Pinus taeda* L.) and from a 21-year-old sweetgum tree (*Liquidambar styraciflua* L.). Bark was removed and bolts were cut into discs 1-inch thick. Discs were dried for 24 h at 105 C and ground in a Wiley mill to pass a 1,000-μm screen. The fraction retained on a 250-μm screen was saved. Samples of approximately 265 mg were dried for 24 h at 65 C and weighed to the nearest 0.1 mg. Samples of each wood were placed in separate 250-ml Erlenmeyer flasks, which had been rinsed with 0.5 *M* HCl to remove traces of Fe and then washed and rinsed thoroughly with distilled water. Solutions of $FeSO_4 \cdot 7H_2O$ (0.0, 0.028, 0.11, 0.44 m*M*): H_2O_2 (0.0, 0.06, 0.25, 1.00% w/v) were prepared in 0.1 *M* acetate buffer at pH 4.2, and 100 ml was added at each concentration to each flask for 10 replicate samples of sweetgum and 8 of pine. Flasks were incubated for 3 days at room conditions (25 ± 2 C) on a reciprocating shaker at 120 cpm.

For depolymerization studies, two samples that had weight losses nearest the average for the treatment were nitrated and delignified (Alexander and Mitchell 1949) at -16 ± 2 C for 1 h and at 25 ± 1 C for 23 h in 30-ml beakers with a weight of nitrating solution proportional to the sample weight. The oven-dried delignified samples were weighed and dissolved in 100-ml acetone (Fisher, AR) with vigorous magnetic stirring for 1 h and gentle agitation for 23 h. Suspensions were centrifuged for 10 min at $20{,}000 \times g$. The pellet was recovered, oven-dried, weighed, and then the weight was subtracted from the total weight to yield the net weight of the nitrated wood dissolved in the acetone. Viscosity of the acetone solution was measured at 25 ± 0.1 C in a Cannon-Ubbelohde ASTM #75 viscosimeter at five dilutions from ⅓–⅕ of the original. The remainder of the acetone solution was precipitated in 1 liter of distilled water, the precipitate was divided into two portions, which were dissolved separately in 40 ml salicylic acid-H_2SO_4 (24 g/liter H_2SO_4) reagent overnight (Bremmer 1965) to convert NO_3^- to NO_2^-, and then assayed by the Kjeldahl procedure. Samples of sweetgum

TABLE 1. *Percentage weight loss of sweetgum and loblolly pine wood meal exposed for 3 days to hydrogen peroxide and iron in 0.1 M acetate buffer at pH 4.2 and 25 C*

H_2O_2 %	Fe^{++} mM							
	Sweetgum [a]				Loblolly pine [b]			
	0.00	0.028	0.11	0.44	0.00	0.028	0.11	0.44
	weight loss-% of original weight							
0.00	4.8	3.9	5.3	5.9	2.6	2.8	2.0	3.1
0.06	6.1	10.1	14.3	10.6	3.4	6.3	5.0	4.7
0.25	3.5	11.0	22.2	35.6	4.1	10.2	16.6	17.5
1.00	5.3	17.3	30.7	52.6	4.9	15.1	24.6	37.6

[a] Average weight loss for ten replicate samples per treatment.

[b] Average weight loss for eight replicates per treatment.

were wrapped in tissue paper and placed directly in the acid while those of pine were thoroughly teased apart before wrapping to permit rapid dissolution. Intrinsic viscosity (η) was determined graphically by plotting reduced viscosity over the concentration of holocellulose usually of the higher three or four dilutions and extrapolating to zero concentration (Cowling 1961). The degree of polymerization (DP) was calculated using a K value of 94 (for acetone from Alexander and Mitchell 1949) in the formula $DP = K[\eta]$. The DP was then corrected for average percentage of N in the sample (Fig. 3 in Lindsley and Frank 1953).

Triplicate samples were tested for solubility in 1% w/v alkali using essentially the method of Cowling (1960), except for the smaller sample size and proportionally lesser volumes of reagents in this study. Extracted samples were harvested on tared, fritted-glass filter crucibles, dried at 50 C for 3 h and weighed.

Iron concentration was determined on duplicate samples exposed to the H_2O_2-Fe treatments, ashed for 8 h at 450 C and dissolved overnight in 0.4 *M* HCl-0.2% w/v La_2O_3. For wood high in Fe, it has since been found necessary for accuracy to dissolve the sample in 0.5-ml concentrated HCl and then to dilute to 0.4 *M* with 0.2% w/v aqueous La_2O_3. The solutions were assayed by atomic absorption spectrometry at appropriate dilutions and the concentration was determined graphically by comparison with values for standards from 0.0–20.0 μg Fe^{++}/ml.

Lignin, total sugars, and hemicellulose sugars were assayed by personnel, whose assistance is gratefully acknowledged, at the Forest Products Laboratory, Madison, WI. Single samples of both woods exposed to each combination of H_2O_2 and Fe^{++} were reground to pass an 80-mesh screen and then analyzed for each component (Moore and Johnson 1967), using the modified procedure for lignin in wood.

The effect of pH on weight loss by H_2O_2 and Fe^{++} was studied at pH 3.0 and 3.5 in 0.1 *M* acetate-HCl buffer and at pH 4.0, 4.2, 4.5, and 5.0 in the acetate buffer used in the other studies. Five sweetgum samples were exposed under standard conditions at each pH to 0.25% w/v H_2O_2 and 0.028 m*M* Fe^{++} and to buffers with neither H_2O_2 nor Fe^{++}; five pine samples were similarly treated with and without 1.0% w/v H_2O_2 and 0.028 m*M* Fe^{++}. In another test the same number of samples of wood meal of each species was exposed under standard conditions to solutions without Fe^{++} and without and with 1% H_2O_2. Samples were harvested and weight losses determined.

TABLE 2. *Solubility in 1% sodium hydroxide of sweetgum and loblolly pine wood meal exposed for 3 days to hydrogen peroxide and iron in 0.1 M acetate buffer at pH 4.2 and 25 C*[a]

H_2O_2 %	Fe^{++} mM							
	Sweetgum				Loblolly pine [b]			
	0.00	0.028	0.11	0.44	0.00	0.028	0.11	0.44
	weight loss-% of H_2O_2-Fe^{++} treated wood solubilized by 1% NaOH							
0.00	27.5	24.1	26.0	25.6	27.6	25.1	25.0	25.8
0.06	29.8	28.7	43.7	43.3	24.8	36.0	38.7	31.4
0.25	26.2	37.2	52.5	62.4	24.7	40.2	48.7	51.0
1.00	25.9	49.4	57.7	62.6	26.7	40.9	47.1	49.8
	weight loss-% of original weight solubilized by H_2O_2-Fe^{++} and 1% NaOH							
0.00	29.6	26.4	30.3	30.8	30.5	26.9	27.6	27.8
0.06	33.6	35.6	51.8	50.2	28.9	41.4	43.7	38.4
0.25	29.4	44.4	65.3	75.8	27.8	47.6	59.2	63.1
1.00	29.5	57.8	73.9	80.2	30.2	50.5	63.6	70.7

[a]Average weight loss of three replicates for samples exposed in the same tests as in Table 1.

[b]Average weight loss of three replicates.

RESULTS

Weight loss

Neither H_2O_2 nor Fe^{++} alone caused much weight loss of sweetgum wood (Table 1). The lowest concentrations of Fe^{++} and H_2O_2 decreased the weight approximately 5%. Weight loss generally increased with increasing concentration of H_2O_2 at each concentration of Fe^{++} and with increasing concentration of Fe^{++} at 0.25 and 1.00% but not at 0.06% H_2O_2. At 0.11 m*M* Fe^{++} and 0.25% H_2O_2 and higher concentrations of both reagents, weight losses became appreciable, increasing from 22.2% to 52.6% at the maximum concentrations tested.

Weight losses in two experiments with loblolly pine were less than in sweetgum (Table 1) little weight loss being apparent below 0.25% H_2O_2 and 0.028 m*M* Fe^{++} in one test and 0.11 m*M* Fe^{++} in the other. Maximum weight loss for the two tests averaged 38.6%.

Alkali solubility

At zero concentrations of either H_2O_2 or Fe^{++}, increasing the concentration of the alternate component had little effect on the solubility of either sweetgum or loblolly pine in 1% NaOH (Table 2). As concentrations of both reagents were increased, the solubility of the residue increased for both species in a manner similar to weight losses due to H_2O_2-Fe^{++}. Alkali solubility was greater at 0.06% H_2O_2 = 0.028 m*M* Fe^{++} for pine than sweetgum. With sweetgum, a maximum of about 62.6% of the H_2O_2-Fe^{++} exposed wood was soluble in 1% alkali; with pine, the figure was 51.0%. Expressed as a percentage of the original weight solubilized by H_2O_2-Fe and 1% NaOH, a maximum of 80.2% of the sweetgum and 70.7% of the pine sample was dissolved by the two systems at the highest concentration of H_2O_2-Fe^{++}.

Depolymerization

In sweetgum, increasing concentrations of Fe^{++} at 0% H_2O_2 and increasing concentrations of H_2O_2 without added Fe^{++} appear to have slightly lowered DP (Table 3). At low concentrations of both reagents, depolymerization initially increased rapidly. With

TABLE 3. *Depolymerization of sweetgum and loblolly pine wood meal exposed for 3 days to hydrogen peroxide and iron in 0.1 M acetate buffer at pH 4.2 and 25 C*[a]

H_2O_2 %	Fe^{++} mM							
	Sweetgum				Loblolly pine [b]			
	0.00	0.028	0.11	0.44	0.00	0.028	0.11	0.44
0.00	3080	2710	2595	2520	2715	2845	2490	2635
0.06	2860	1690	1435	1360	2775 [c]	2020	2065	1830
0.25	2980	1560	995	785	2515	1995	1465 [c]	1575
1.00	2675	1470	750	480	2700	2075	1570 [c]	1280

[a] Average number of glucosyl molecules per cellulose molecule for two replicate samples per treatment. Samples exposed in the same test as in Table 1. Values corrected for % N content.

[b] Excessive variation in nitrogen contents of three samples (footnote 3) was believed to be caused by incomplete conversion of organic to inorganic nitrogen due to the compactness of some samples. This resulted in correction factors believed to be more variable than depolymerization values prior to correction.

[c] Nitrogen corrections are for one replication based on the average value (12.86%) for all samples rather than the measured value for the individual; see footnote 2.

increasing concentrations of either reagent when the other is held constant, the rate of depolymerization increased more slowly. In pine, there appeared to be no trend in DP's at zero concentrations of each reagent; otherwise, depolymerization progressed in a generally similar manner to sweetgum. In sweetgum, the DP at the highest concentration of H_2O_2-Fe^{++} is 16% of the original, but for pine, DP has been lowered to 47%.

An analysis of variance of the individual values for DP's in Table 3 indicated highly significant effects of Fe^{++}, H_2O_2 and species on DP and highly significant interactions of Fe^{++} with H_2O_2, of Fe^{++} with species and H_2O_2 with species. The response of DP vs. the concentration of H_2O_2 without added Fe and of DP vs. the concentration of Fe^{++} without added H_2O_2 was subjected to linear regression. The regressions indicated that DP decreased linearly and possibly curvilinearly with increasing concentrations of H_2O_2 for sweetgum, but neither relationship held in the case of pine. DP decreased linearly with increasing concentration of Fe^{++} for sweetgum but not pine; there was no evidence of a curvilinear trend for either species.

Lignin content

The H_2O_2-Fe^{++} treatment markedly increased the solubility of lignin of sweetgum during subsequent acid hydrolysis (Table 4). At the highest concentration of H_2O_2 and Fe^{++}, nearly 74% of the lignin was rendered soluble. In pine, there seems to be little solubilizing effect in any treatment and, actually, lignin content appears to have increased slightly for both H_2O_2 and Fe^{++} in most treatments.

Noncellulosic polysaccharides

Analyses for individual sugars (Table 5) reveal a more rapid depletion of hemicellulosic sugars of both woods than of cellulose with the H_2O_2-Fe^{++} treatment. For brevity, data for only the treatments without Fe^{++} and with 0.44 m*M* Fe^{++} are presented. Data for other concentrations were intermediate. In comparison to controls exposed only to buffer, total sugars (both cellulose and noncellulosic polysaccharides) of sweetgum decreased 3% at 1% H_2O_2 and 0.028 m*M* Fe^{++} and increased 3% at 1% H_2O_2 and 0.44 m*M* Fe^{++} while glucose (cellulose) increased 7 and 24% at similar concen-

TABLE 4. *Klason lignin in sweetgum and loblolly pine exposed for 3 days to hydrogen peroxide and iron in 0.1 M acetate buffer at pH 4.2 and 25 C*[a]

H_2O_2 %	Fe^{++} mM							
	Sweetgum				Loblolly pine			
	0.00	0.028	0.11	0.44	0.00	0.028	0.11	0.44
	Acid insoluble residue-% of H_2O_2-Fe^{++} treated wood submitted for analysis							
0.00	18.14	-	18.53	17.89	28.99	29.54	29.09	-
0.06	17.50	16.87	17.32	17.24	29.24	29.10	29.30	29.29
0.25	18.43	16.7	14.90	13.22	29.29	29.18	29.86	29.69
1.00	17.85	13.55	14.03	8.39	28.94	29.15	28.34	28.35

[a] Values are residual weights after acid hydrolysis for Klason lignin determinations (Moore and Johnson 1967) based on a single replication performed by personnel at the USDA Forest Products Laboratory, Madison, WI.

trations. However, respective amounts for other sugars dropped: xylose, 10 and 36%; mannose, 24 and 72%; galactose, 73 and –8%; and arabinose, 30 and 78%. Similar calculations for loblolly pine at the same concentrations of reagents showed an increase of 0 and decrease of 4% in total sugars and 4 and 11% for glucose. Again, values for most other sugars declined: xylose, 11 and 22%; mannose, 9 and 25%; galactose, 6 and 27%. Arabinose increased 2 and 4%.

TABLE 5. *Percentage of total sugars and individual sugars in wood of sweetgum and loblolly pine exposed for 3 days to hydrogen peroxide and iron in 0.1 M acetate buffer at pH 4.2 and 25 C*

H_2O_2 %	Fe^{++} mM											
	0.00						0.44					
	Total sugar	Glu [a]	Xyl	Man	Gal	Ara	Total sugar	Glu	Xyl	Man	Gal	Ara
	Sweetgum - % of sample weight or of total sugars [b]											
0.00	66.67	62.64	31.42	3.60	0.89	1.45	65.02	63.79	31.19	3.27	0.65	1.10
0.06	66.21	63.06	31.78	3.46	0.74	0.96	64.23	65.06	30.51	2.97	0.51	0.95
0.25	66.76	63.07	31.00	3.57	0.73	1.63	66.47	74.33	23.28	1.39	0.34	0.66
1.00	66.92	62.93	31.38	3.73	0.84	1.12	68.86	77.61	20.02	1.10	0.96	0.31
	Loblolly pine - % of sample weight or of total sugars [b]											
0.00	61.31	66.53	10.67	15.50	5.15	2.15	-	-	-	-	-	-
0.06	61.14	66.32	10.40	15.60	5.57	2.11	60.80	66.54	10.49	15.59	5.24	2.14
0.25	61.86	67.31	10.33	15.14	5.35	1.87	58.97	73.59	8.66	12.34	3.75	1.66
1.00	61.67	67.93	10.00	15.22	4.92	1.93	59.16	74.00	8.30	11.70	3.77	2.23

[a] Abbreviations: Glu = glucose, Xyl = xylose, Man = mannose, Gal = galactose, Ara = arabinose

[b] Data for total sugars (total carbohydrates) are expressed as percentage of the weight of a single sample presented for analysis for each treatment. Individual sugars are presented as percentage of total sugars.

Analyses (Moore and Johnson 1967) conducted by personnel at USDA Forest Products Laboratory, Madison, WI.

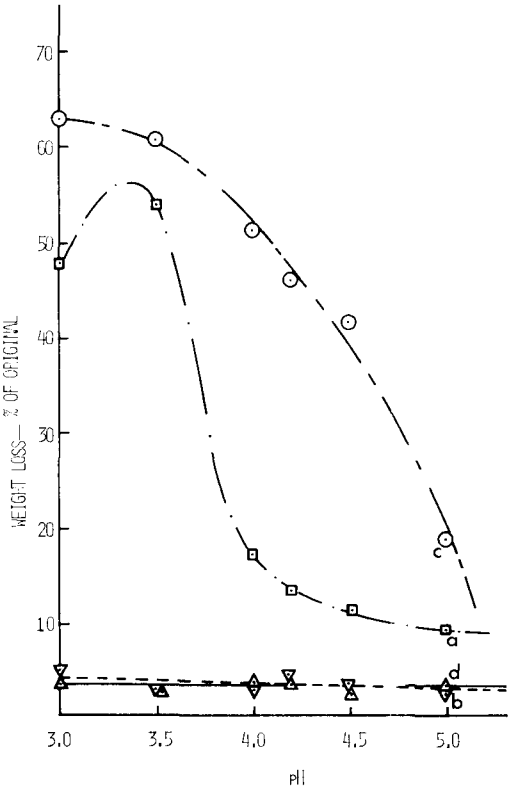

FIG. 1. Effect of pH on weight loss of woods of sweetgum and loblolly pine exposed to H_2O_2-Fe^{++} solutions in 0.1 *M* acetate buffer for 3 days at room conditions on a reciprocal shaker at 120 cpm. Sweetgum incubated in 0.25% H_2O_2 with 0.028 m*M* Fe^{++} (a) or 1% H_2O_2 alone (b); pine incubated in 1% H_2O_2 with 0.028 m*M* Fe^{++} (c) or with no Fe^{++} (d). Each point is the average of three replicates. Experiments with and without Fe^{++} were conducted at different times. However, in both tests each wood was incubated in 0.1 *M* acetate buffer only at each pH; the average weight loss for this treatment for both woods in both tests at all pH's was within ± 0.5% of the average for the woods incubated in 1% H_2O_2 without Fe^{++}.

Effect of pH on solubilization

The optimal pH found for solubilization of sweetgum by H_2O_2-Fe^{++} was approximately 3.5 (Fig. 1); at this pH solubilization was ca. 4 times greater than at 4.2, the pH at which the rest of the studies were conducted. With pine, a pH optimum was not clearly established partially because of the nearly complete removal (ca. 95%) of carbohydrate at pH 3.0. Solubilization of pine wood was 1.3 times greater at pH 3.0 than 4.2. Buffer alone and buffer with 1% H_2O_2 each solubilized only about 3.2 ± 1% of sweetgum and pine at all pH's.

Adsorption of Fe by wood

Both sweetgum and pine woods avidly adsorb Fe from solution, the amounts increasing more rapidly with increasing Fe^{++} concentration at the lower concentrations of H_2O_2 (Table 6). Adsorption of Fe at any particular concentration of Fe decreases with increasing concentration of H_2O_2; this decrease is only partially offset by correction for weight losses. Subsequent assays conducted on samples dissolved first in concentrated HCl and then diluted to 0.4 *M* HCl with 0.2% aqueous La_2O_3 revealed that the highest concentrations of Fe reported in Table 6 may be as low as ⅓ of the actual values.

DISCUSSION

Effects of the H_2O_2-Fe^{++} system on wood of sweetgum and loblolly pine

In these experiments, combinations of H_2O_2 and Fe^{++} decreased the weight of wood of sweetgum and loblolly pine under conditions of temperature and pH that normally exist or develop in wood during decay. Considering the brief duration and the mild conditions of exposure, weight losses seem quite impressive. Weight losses of wood were greater than reported for sawdust by Halliwell (1965), possibly because of species differences. Normal weight losses of 2.5–6.0% in the control treatments under these conditions are probably due to removal of water-soluble components and other extractives. Unusually high weight losses and other effects in individual samples without added Fe^{++} may be due to traces of Fe^{++} (Table 6, footnotes 2 and 3) from grinding or chance contamination.

The decided increase in alkali solubility at even the lowest concentration of H_2O_2-Fe^{++} for treated sweetgum and pine indicates that some of the cellulose molecules had been shortened to fragments of <200

TABLE 6. *Adsorption of iron by sweetgum and loblolly pine exposed for 3 days to hydrogen peroxide and iron in 0.1 M acetate buffer at pH 4.2 and 25 C*[a]

H_2O_2 %	Fe^{++} mM							
	Sweetgum				Loblolly pine [c]			
	0.00	0.028	0.11	0.44	0.00	0.028	0.11	0.44
0.00	19.0	220	575	1379	14.0(2)	225(4)	543(4)	2464(2)
0.06	30.2 [b]	160	253	1702	14.8(4)	163(3)	540(4)	2110(3)
0.25	12.9	140	281	464	15.3(3)	178(2)	330(4)	1187(3)
1.00	12.9	81	104	159	10.7(3)	131(3)	184(1)	212(2)

[a] Average in µg/g for two replicate samples from a single test for sweetgum and for the number of samples in parentheses from two experiments for loblolly pine. Subsequent tests have shown that the highest concentrations of Fe may be only about 1/3 of actual values reported here.

[b] Average of two other samples in each of two tests was 17.4 and 21.6 µg/g.

[c] Value for unsoaked pine was 11.8 µg/g.

glucosyl units. At the highest concentration of H_2O_2-Fe^{++}, in addition to all of the cellulose having been extensively depolymerized in sweetgum, approximately 10% of the sweetgum lignin was attacked sufficiently to be solubilized by 1% NaOH. The solubility of residual pine cellulose in 1% NaOH was also markedly increased. At the highest concentration of reagents, probably only pine cellulose was solubilized since the lignin was insufficiently affected to have become more soluble in acid (Table 4).

Depolymerization sensitively indicated chemical attack as is attested by the 43% reduction in chain length of cellulose in sweetgum and the 26% reduction in pine at 0.028 m*M* Fe^{++} and 0.06% H_2O_2. The rapid leveling-off of the rate of depolymerization, as H_2O_2 concentration increased at 0.028 m*M* Fe^{++} for both species, was probably due in part to oxidation of Fe^{++} in solution and to the increasing competition for H_2O_2-Fe^{++} in reactions leading to destruction of the logarithmically increasing number of shorter chain-length molecules that were formed. At low concentrations of H_2O_2, the decreased rate of depolymerization with increasing concentration of Fe^{++} may be caused by adsorption of Fe by the woods (Table 6). The increases in weight loss and alkali solubility in these treatments confirm a continued attack producing short-chain components.

Iron alone depolymerized cellulose in sweetgum but not pine in this study; this depolymerization may have resulted from formation of small amounts of H_2O_2 by light during exposure. Iron alone depolymerized pine cellulose during prolonged exposure but did not affect the compression strength of Scots pine (*Pinus sylvestris* L.), indicating that pine lignin was not depolymerized (Marian and Wissing 1960a, b).

The standard deviation of the DP as estimated from the residual variation was large (160.0) and is believed caused by relatively large correction factors for N content incurred from variation in solubilization of samples during conversion of NO_3^- to NO_2^-.

The pH optima near 3.3 and 3.0 for weight loss of sweetgum and pine, respectively, contrast sharply with that for cotton cellulose (pH 4.2; Halliwell 1965). This may be particularly fortuitous since these pH's are typical in wood decayed by brown-rot fungi and should favor solubilization of Fe and its reduction.

Greater weight losses and alkali solubilities, and lesser DP's for sweetgum than pine at the same concentrations of reagents

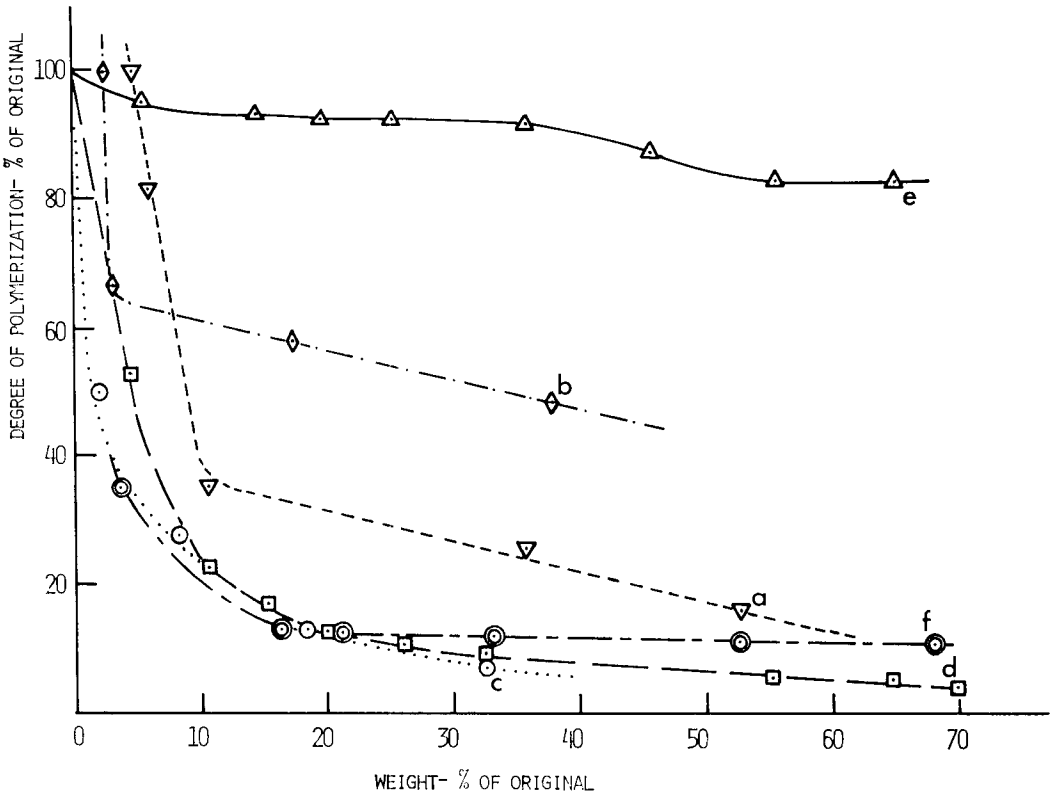

FIG. 2. Relationship between weight loss and the degree of polymerization caused by various treatments: a—sweetgum and b—loblolly pine (Tables 1 and 3), and c—cotton cellulose (Koenigs 1972a) each exposed for 3 days at room conditions with 0.44 m*M* Fe^{++} and 0–1% H_2O_2 in 0.1 *M* sodium acetate buffer pH 4.2 and expressed as % of control without H_2O_2 and Fe^{++}; d—sweetgum decayed to various weight losses by the brown-rot fungus, *Poria monticola* Murr. (Cowling 1961); e—sweetgum decayed to various weight losses by the white-rot fungus, *Polyporus versicolor* L. ex. Fr. (Cowling 1961); f—western hemlock pulp hydrolyzed at 100 C in various concentrations of HCl to various weight losses (from original data published by Millett et al. 1954).

suggest that the cellulose of sweetgum is of a lower degree of crystallinity than that of pine. Values for crystallinity for hardwoods and conifers apparently have not been measured, but Hawley et al. (1928) note that the percentage of cellulose hydrolyzable only with difficulty is greater in softwoods than in hardwoods, which indicates that the degree of crystallinity may be greater in softwoods. Millett et al. (1954) have shown that the DP of this same cellulosic fraction is lower for sweetgum than for southern pine sulfate pulp. Alternative explanations are that sweetgum cellulose is less well protected by lignin than is pine cellulose because of qualitative differences in the lignins (Sarkanen and Ludwig 1971) or their different spatial arrangement within the wall. Lignin is nearly uniformly dispersed through the S1, S2, and S3 layers of the secondary cell wall of tracheids of southern pine (Wilcox 1968), which might tend to make the cellulose of all layers about equally resistant; in sweetgum, lignification is lowest in S2 and greatest in S3.

Although natural lignin is normally resistant to acid hydrolysis (Browning 1964), exposure of sweetgum to H_2O_2-Fe^{++} greatly enhanced the subsequent solubility of the lignin to strong (Table 4), but not mild acid hydrolysis (Table 5). Pine lignin, on the other hand, was not similarly af-

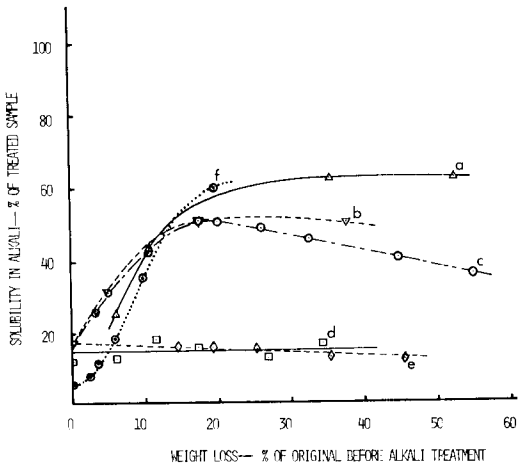

FIG. 3. Relationship between weight loss of wood caused by various treatments and solubility in NaOH. Weight loss on abscissa equals weight of volatile and cold water fractions solubilized by the H_2O_2-Fe^{++}, the fungi or acid hydrolysis; total weight loss on ordinate equals the weight of the treated sample solubilized in 1% NaOH for substrates other than cotton (= 18% NaOH) @ 100 C for 1 h and expressed as a percentage of the original weight of the treated sample: a—sweetgum and b—loblolly pine treated for 3 days at room conditions with 0.44 m*M* Fe^{++} and 0–1% H_2O_2 in 0.1 *M* acetate buffer pH 4.2 (Tables 1 and 2); c—sweetgum decayed to various weight losses by the brown-rot fungus, *Poria monticola* (Cowling 1961); d—Sitka spruce hydrolyzed at 100 C in various concentrations of HCl to various weight losses (Hawley and Campbell 1927; e—sweetgum decayed to various weight losses by the white-rot fungus, *Polyporus versicolor* (Cowling 1961); f—cotton cellulose treated for 3 days at room conditions with 0.44 m*M* Fe and 0–1% H_2O_2 and then with 18% NaOH (Koenigs 1972a).

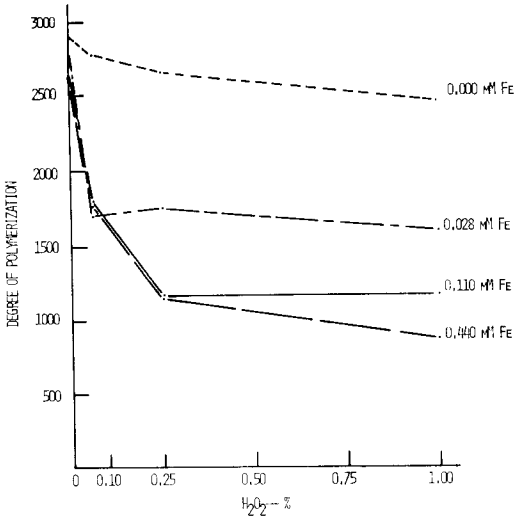

FIG. 4. Relationship between the degree of polymerization of wood cellulose from loblolly pine and sweetgum wood meal exposed in 0.1 *M* acetate buffer at pH 4.2 with various concentrations of H_2O_2 and Fe^{++} for 3 days at room conditions. Each point represents the average for two samples of each species; s = 160.

fected by any treatment except possibly at the highest concentration of reagents. The greater solubilization of hardwood than of pine lignin is in line with the lower redox potential of the former.

Comparison of the effects of H_2O_2-Fe^{++} on wood cellulose and cotton cellulose

The H_2O_2-Fe^{++} system did indeed affect the wood cellulose in a manner similar to cotton cellulose (Koenigs 1972a). Although the DP of celluloses from all sources decreased rapidly at weight losses < ca. 10% (Fig. 2), at higher weight losses there was proportionally less effect on the DP of pine cellulose than for that of cotton and sweetgum. The proportional reduction in DP for the latter two substrates appears to be nearly the same at 70% weight loss.

The solubility in 18% NaOH of treated cotton cellulose at a given weight loss is slightly less than for either sweetgum or pine in 1% NaOH (Fig. 3). This may be accounted for by the greater amount of hemicellulose in wood (32–36%) than cotton (ca. 1%), and the greater solubility of hemicellulose than cellulose in alkali but in part reflects the preferential attack of H_2O_2-Fe^{++} on hemicellulose (Table 5). In wood, attack seems to have resulted in the increased formation of a water-soluble fraction, since weight losses were greater at the same and lower concentrations of reagents than for cotton. Although the effects of treatment on solubility might in part be accounted for by the differences in the crystalline and lattice features and the hemicellulosic content, they occurred despite any protection by lignin. The spatial arrangement of lignin at the anatomical or even at

molecular level may channel reagents to points of initial attack and restrict subsequent cleavages to sites near the initial cleavage, thus producing proportionally more short water-soluble segments.

The chemical nature of H_2O_2-Fe^{++} attack on cellulose and wood

The H_2O_2-Fe^{++} system is fundamentally nonspecific in its action on cellulose. Effects are multiple, and sometimes sequential. In cellulose the system is believed to initiate a free-radical chain-reaction that ultimately hydrolyzes glucosidic bonds, although intermediate reactions would indicate that oxidation had taken place (see Moody 1964). H_2O_2 and the H_2O_2-Fe^{++} system oxidize cotton cellulose and do so more vigorously under acidic than under neutral conditions (Ivanov et al. 1953; Moody 1964). H_2O_2 alone first oxidizes primary OH groups (at C_6) to aldehydes and carboxyls, and secondary OH's at C_2 and C_3 to ketones; subsequent oxidation at this position cleaves the pyranose ring forming first aldehyde and then carboxyl groups. Fe^{++} catalyzes oxidation but particularly speeds reactions leading to early ring cleavage and early appearance of nonuronic carboxyls. These reactions theoretically would be expected to increase the susceptibility of nearby glucosidic linkages to scisson (see Sharples 1971). The DP of acidic oxidized cellulose does in fact decrease concomitantly with a decrease in carboxyl content of the residual cellulose and with the appearance of soluble acidic products, suggesting that acid-catalyzed hydrolysis of glucosidic bonds had occurred (Davidson and Standing 1951; Sharples 1971). In lignin, H_2O_2 produces mostly the same qualitative changes of demethylation and an increase in hydroxyl and carboxyl groups as O_2, but does so more rapidly (Katuščák et al. 1971). Worth noting here is that a biochemist explaining these multiple effects (and those of brown-rot) would invoke the participation of numerous enzymes. In the inorganic system they are achieved simply by alterations in the ratio of H_2O_2 to Fe^{++}.

The changes in the DP caused by H_2O_2-Fe^{++} in pine and sweetgum cellulose here (Table 3) are similar to those produced by acid hydrolysis (see McBurney 1954) with perhaps but a slight difference. Hydrolysis rapidly depolymerizes heterogeneous cotton and wood celluloses to about 10% weight loss (see references in Millett et al. 1954). Cotton cellulose is not depolymerized further, but this trend is less distinct for wood cellulose (Immergut and Rånby 1956). The H_2O_2-Fe^{++} system results in the same rapid initial depolymerization up to about 10% weight loss; however, depolymerization continues slowly over a broad range of weight losses (Fig. 2).

The difference in the relationship between the alkali solubility vs. weight loss of acid hydrolyzed and of H_2O_2-Fe^{++} treated wood is striking. The alkali solubility of acid hydrolyzed wood is essentially constant with increasing weight loss (Hawley and Campbell 1927) while that of H_2O_2-Fe^{++} treated wood and cotton cellulose increases rapidly at first and then more slowly (Fig. 3). The effects on alkali solubility suggest that H_2O_2-Fe^{++} acts on both wood and cotton celluloses differently than does acid hydrolysis.

Chemical nature of brown-rotted wood

Brown-rot fungi are generally believed to employ an enzyme that hydrolyzes cellulose or at least produces effects similar to acid hydrolysis (Campbell 1952; Cowling 1958; Cowling and Brown 1969; Gasgoine and Gasgoine 1960; Levi 1964). These conclusions apparently were based on: (a) the preferential attack by brown-rot fungi on hemicellulose rather than cellulose (Barton-Wright and Boswell 1931; Boswell 1938); (b) the similarity in DP-weight loss curves for acid hydrolyzed cellulose (Hawley and Campbell 1927; Millett et al. 1954) with that in wood attacked by brown-rot fungi (Boswell 1938; Cowling 1961; Kayama 1962c) and (c) the production of acidic conditions by these fungi in wood (Birkinshaw et al. 1940; Cowling 1961; Rabanus 1939; Seifert 1962). In general studies such as this one, the DP-weight loss

curve seems unreliable for differentiating between acid hydrolyzed and oxidized cellulose for reasons mentioned earlier. On the other hand, if brown-rotted wood resulted from hydrolysis, its solubility in alkali (Apenitis et al. 1951; Bray 1924; Bray and Andrews 1924; Campbell and Booth 1929; Cowling 1961; Findlay 1932; Kennedy 1958; Richards 1962) might be expected to coincide more closely with that of acid hydrolyzed wood over a wide range of weight losses, a possibility noted by Hawley and Campbell (1927). Instead, its solubility curve nearly matches that of wood treated with H_2O_2-Fe^{++} (Fig. 3). This suggests that brown-rotted wood may have been attacked by an H_2O_2-Fe^{++} system.

Furthermore, there is some direct evidence that brown-rotted wood or cellulose is oxidized. This includes high Cu-numbers of groundwood pulp (Bray and Staidl 1922; Bray and Andrews 1924), the presence of uronic carboxyls and aldehydes in cellulose (Barton-Wright and Boswell 1931) and carboxyls in the alkali-soluble fraction of wood and cellulose (Boswell 1938). Oxidative changes in lignin by brown-rot fungi include an increase in O content (Barton-Wright and Boswell 1931; Enkvist et al. 1954; Kirk 1971), demethylation (see Kirk 1971) accompanied by an increase in phenolic hydroxyls (Kirk et al. 1970), an inferred increase in carboxyls (Enkvist et al. 1954; Leopold 1951; Kirk 1971), and an increased Cu-number of lignin (Kirk 1971) or of lignin-extracts (Apenitis et al. 1951; Cowling 1961). Although the literature strongly suggests that brown-rot fungi oxidize lignin and probably the cellulose in wood, it seems necessary to settle this point definitively for cellulose.

GENERAL DISCUSSION

Calculations from experimental data (Koenigs 1974) indicate that brown-rot fungi produce sufficient H_2O_2 from native substrates in sweetgum wood in 3 days theoretically to reduce the DP to 25% of the original. This figure is within 2% of that observed for sweetgum decayed by a brown-rot fungus to a 10% weight loss (from Cowling 1961) or within 10% observed for the H_2O_2-Fe^{++} system in sweetgum at the same weight loss (Fig. 2). Other calculations reveal that to solubilize 5% of sweetgum cellulose (= 10% total weight loss) with a DP = 1600, 245 mg of H_2O_2 would be required. This is surprisingly close to the 240 mg supplied in the 100 ml of 0.06% solution, which gave a weight loss of 10.1% (Table 1). Although a fungus could not produce 245 mg H_2O_2 while causing a weight loss of only 5%, much lesser amounts may alter the substrate sufficiently to enhance its susceptibility to other cellulase components (Koenigs 1972a) that brown-rot fungi do produce (Highley 1973).

At 0.028 m*M*, the quantity of Fe^{++} added/100 ml of buffer and available per gram of wood was 282 μg or about 15–20 times the amount usually found in wood (15–19 μg/g; Table 6). However, at low concentrations of H_2O_2 (0.06%), concentrations of Fe^{++} much lower than 0.028 m*M* will still have a large effect on DP (Fig. 4) in the present experimental system, and more so at pH's near 3. Fungal acids may solubilize Fe and stimulate weight loss (Fig. 3). Acid production, or more probably, physiological events leading to it, are strongly implicated in the regulation of H_2O_2 as a cellulolytic agent in *Lenzites trabea* Pers. ex Fr. Isolates with high decay capacity (25–50% weight loss in 1 month) lower the pH of sweetgum to 3.2–3.5, whereas those of low decay capacity (<3.1% weight loss) *raise* the pH of the wood above normal (Koenigs 1973). Devising a better experimental system for exploring the role of Fe and investigating microbial metabolic systems for solubilizing and sequestering Fe are likely to answer quantitative questions about Fe.

Conventional cellulases are incapable of attacking cellulose in intact native wood (Pew 1957). A major obstacle may be that the diameter of the enzymes [30 pm (Table 5 in Cowling and Brown 1969)] probably excludes them from most capillary pores [$\bar{x}$ diameter = 15 pm (Stone et al. 1969)] in cellulose. Free OH radicals generated by

the H_2O_2-Fe^{++} system should easily gain access.

In this study H_2O_2-Fe^{++} created effects in wood and wood cellulose similar to brown-rot fungi. These results suggest that some brown-rot fungi may derive at least part of their cellulolytic ability via an H_2O_2-Fe^{++} system. How much cellulolysis results from the system is uncertain. It can decompose the substrate to CO_2 and H_2O (Halliwell 1965) and enzymes (Highley 1973) may act on partially degraded material (Koenigs 1972a). If the effects of the H_2O_2-Fe^{++} system and brown-rot fungi on cellulose, hemicellulose, and lignin are oxidative and complimentary in other respects, the system as a comprehensive mechanism explaining wood decay by these fungi gains a simplicity that makes it particularly attractive for future investigation.

REFERENCES

ALEXANDER, W. J., AND R. I. MITCHELL. 1949. Rapid measurement of cellulose viscosity by the nitration method. Anal. Chem. 21:1497–1500.

APENITIS, A., H. ERDTMAN, AND B. LEOPOLD. 1951. Studies on lignin. Part V. The decay of spruce wood by brown-rotting fungi. Sven. Kem. Tidskr. 63:195–207.

BARTON-WRIGHT, E. G., AND J. G. BOSWELL. 1931. 60. The biochemistry of dry-rot in wood. II. An investigation of the products of decay of spruce wood rotted by *Merulius lacrymans*. Biochem. J. 25:494–506.

BIRKINSHAW, J. H., W. P. K. FINDLAY, AND R. A. WEBB. 1940. Biochemistry of wood-rotting fungi. II. A study of the acids produced by *Coniophora cerebella* Pers. Biochemistry 34: 906–916.

BOSWELL, J. G. 1938. 30. 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. 32:218–229.

BRAY, M. W. 1924. Decay of wood and groundwood pulp. Relation of loss in weight to chemical properties. Paper Trade J. 78:58–60.

BRAY, M. W., AND T. M. ANDREWS. 1924. Chemical changes of groundwood during decay. Ind. Eng. Chem. 16:137–139.

BRAY, M. W., AND J. A. STAIDL. 1922. The chemical changes involved during infection and decay of wood and wood pulp. Ind. Eng. Chem. 14:35–40.

BREMMER, J. M. 1965. Total nitrogen. Pages 1149–1178 *in* C. A. Black, ed. Methods of soil analysis. Part 2. Chemical and microbiological properties.

BROWNING, B. L. 1964. The composition and chemical reactions of wood. Pages 57–101 *in* B. L. Browning, ed. The chemistry of wood. Interscience Publ., New York.

CAMPBELL, W. G. 1952. The biological decomposition of wood. Pages 1061–1116 *in* L. E. Wise and E. C. Jahn, eds. Wood chemistry. 2nd ed., v. 2, Rheinhold Publ. Corp., New York.

CAMPBELL, W. G., AND J. BOOTH. 1929. LCIV. The effect of partial decay on the alkali solubility of wood. Biochem. J. 23:566–572.

COWLING, E. B. 1958. A review of literature on the enzymatic degradation of cellulose and wood. U.S.D.A., For. Prod. Lab. Rep. 2116, 26 pp.

COWLING, E. B. 1960. Methods for chemical analysis of decayed wood. U.S.D.A., For. Prod. Lab. Rep. 2177, 24 pp.

COWLING, E. B. 1961. Comparative biochemistry of the decay of sweetgum sapwood by white-rot and brown-rot fungi. U.S.D.A., For. Serv. Tech. Bull. 1258, 79 pp.

COWLING, E. B., AND W. BROWN. 1969. Structural features of cellulosic materials in relation to enzymatic hydrolysis. Pages 152–187 *in* G. J. Hajny and E. T. Reese, eds. Cellulases and their applications. Adv. Chem. Ser. 95.

DAVIDSON, G. F., AND H. A. STANDING. 1951. Auto-hydrolysis of acidic oxycelluloses. J. Text. Inst. Trans. 42:T141–T144.

ENKVIST, T., E. SOLIN, AND U. MAUNULA. 1954. Studies on pine wood decayed by brown rot. Pap. Puu 36:65–69, 86.

FINDLAY, W. P. K. 1932. A study of *Paxillus panuoides* Fr. and its effects upon wood. Ann. Appl. Biol. 19:331–350.

GASCOINE, J. A., AND M. M. GASCOINE. 1960. Biological degradation of cellulose. Butterworths, London. 264 pp.

HALLIWELL, G. 1965. Catalytic decomposition of cellulose under biological conditions. Biochem. J. 95:35–40.

HAWLEY, L. F., AND W. G. CAMPBELL. 1927. Effect of partial hydrolysis on the alkali solubility of wood. Ind. Eng. Chem. 19:742–744.

HAWLEY, L. F., L. C. FLECK, AND C. A. RICHARDS. 1928. The effect of decay on the chemical composition of wood. Ind. Eng. Chem. 20: 504–507.

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

IMMERGUT, E. A., AND B. G. RÅNBY. 1956. Heterogeneous acid hydrolysis of native cellulose fibers. Ind. Eng. Chem. 48:1183–1189.

IVANOV, V. I., E. D. KAVERZNEVA, AND Z. I. KUZNETSOVA. 1953. Chemical changes pro-

duced in the cellulose macromolecule by oxidizing agents. Communication 8. Chemical changes in cellulose produced by oxidation with hydrogen peroxide. Div. Chem. Sci., Acad. Sci. USSR Bull. No. 2 (Engl. Trans.): 341–350.

KATUŠČÁK, S., K. HORSKÝ, AND MAHDALIK. 1971. Oxidation of lignin with oxygen and peroxides. Pap. Puu 54:197–202.

KAYAMA, T. 1961. Chemical studies on decayed wood. I. The chemical composition of decayed wood and some properties of resultant pulps. J. Jap. Wood Res. Soc. 7:161–166.

KAYAMA, T. 1962a. Chemical studies on decayed wood as a raw material for pulp. II. Classification of decay types. J. Jap. Wood Res. Soc. 8:29–31.

KAYAMA, T. 1962b. Chemical studies on decayed wood as a raw material for pulp. III. Chemical changes of wood carbohydrates during decay. J. Jap. Wood Res. Soc. 8:32–37.

KAYAMA, T. 1962c. Chemical studies on decayed wood as a raw material for pulp. V. Progressive changes in degree of polymerization of decayed wood cellulose and effect of decay on degree of polymerization and crystalline region of pulp from decayed wood. J. Jap. Wood Res. Soc. 8:197–203.

KENNEDY, R. W. 1958. Strength retention in wood decayed to small losses. For. Prod. J. 8:208–314.

KING, N. J. 1968. The degradation of wood cell components by the extracellular enzymes of *Coniophora cerebella*. Pages 558–564 *in* A. H. Walters and J. J. Elphick, eds., Biodeterioration of materials; microbiological and allied aspects. Elsevier Publ. Co., New York.

KIRK, T. K. 1971. Effects of microorganisms on lignin. Ann. Rev. Phytopathol. 9:185–210.

KIRK, T. K., S. LARSSON, AND G. E. MIKSCHE. 1970. Aromatic hydroxylation resulting from attack of lignin by a brown-rot fungus. Acta Chem. Scand. 24:1470–1472.

KOENIGS, J. W. 1972a. Effects of hydrogen peroxide on cellulose and its susceptibility to cellulase. Mater. Org. 7:133–147.

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

KOENIGS, J. W. 1973. Physiological characteristics of high- and low-decay capacity isolates of *Lenzites trabea* in relation to an H_2O_2-Fe mechanism of cellulolysis in wood. 2nd Int. Congr. Plant Pathol. Abstr. Pap. (No. 0966).

KOENIGS, J. W. 1974. Production of hydrogen peroxide by wood-rotting fungi in wood and its correlation with weight loss, depolymerization and pH changes. Arch. Mikrobiol. 99:129–145.

LEOPOLD, B. 1951. Studies on lignin. VIII. Nitrobenzene oxidation and sulphonation of wood decayed by brown-rotting fungi. Sven. Kem. Tidskr. 63:260–271.

LEVI, M. P. 1964. The fungal degradation of wood. J. Inst. Wood Sci. 12:56–66.

LI, L. H., R. M. FLORA, AND K. W. KING. 1965. Individual roles of cellulase components derived from *Trichoderma viride*. Arch. Biochem. Biophys. 111:439–447.

LINDSLEY, C. H., AND M. B. FRANK. 1953. Intrinsic viscosity of nitrocellulose related to degree of nitration. Ind. Eng. Chem. 45: 2491–2497.

MANDELS, M., AND E. T. REESE. 1964. Fungal cellulases and the microbial decomposition of cellulosic fabric. Rev. Ind. Microbiol. 5:5–20.

MARIAN, J. E., AND A. WISSING. 1960a. The chemical and mechanical deterioration of wood in contact with iron. Part I. Mechanical deterioration. Sven. Papperstidn. 63: 47–57.

MARIAN, J. E., AND A. WISSING. 1960b. The chemical and mechanical deterioration of wood in contact with iron. Part II. Chemical decomposition. Sven. Papperstidn. 63:98–106.

MCBURNEY, L. F. 1954. C. Degradation of cellulose. Pages 99–196 *in* E. Ott, H. M. Spurlin, and M. W. Grafflin, eds. Cellulose and cellulose derivatives. Part 1. Interscience Publ., Inc., New York.

MILLETT, M. A., W. E. MOORE, AND J. F. SAEMAN. 1954. Preparation and properties of hydrocelluloses. Ind. Eng. Chem. 46:1493–1497.

MOODY, G. J. 1964. The action of hydrogen peroxide on carbohydrates and related compounds. Adv. Carbohydrate Chem. 19:149–179.

MOORE, W. B., AND D. B. JOHNSON. 1967. Procedures for the chemical analysis of wood and wood products. U.S.D.A. For. Prod. Lab., Madison, Wis., not paginated.

NORKRANS, B. 1967. Cellulose and cellulolysis. Adv. Appl. Microbiol. 9:91–130.

NORKRANS, B., AND B. G. RÅNBY. 1956. Studies of the enzymatic degradation of cellulose. Physiol. Plant. 9:198–211.

PEW, J. C. 1957. Properties of powdered wood and isolation of lignin by cellulolytic enzymes. Tappi 40:553–558.

RABANUS, A. 1939. Über die Säuer-Produktion von Pilzen und deren Einfluss auf die Wirkung von Holzschutzmitteln. Mitt. Fachaussch. Holzfragen Ver. Deut. Ingen. Deut. Forstver. 23:77–89.

REESE, E. T. 1957. Biological degradation of cellulose derivatives. Ind. Eng. Chem. 49: 89–93.

REESE, E. T., AND W. GILLIGAN. 1954. The swelling factor in cellulose hydrolysis. Text. Res. J. 24:663–669.

REESE, E. T., AND M. MANDELS. 1971. Enzymatic degradation. Pages 1079–1094 *in* N. M. Bikales and L. Segal, eds. Cellulose and

cellulose derivatives. v. 5. Part 5. Wiley-Interscience Publ., New York.

REESE, E. T., L. SEGAL, AND V. W. TRIPP. 1957. The effect of cellulase on the degree of polymerization of cellulose and hydrocellulose. Text. Res. J. 27:626–632.

RICHARDS, D. B. 1962. Chemical changes in decaying wood. For. Sci. 8:277–282.

SARKANEN, K. V., AND C. H. LUDWIG. 1971. Lignins: occurrence, formation, structure, and reactions. John Wiley and Sons, New York. 916 pp.

SEIFERT, T. 1962. Die chemische Veränderung der Holzzellwand-Komponenten unter dem Einfluss pflanzlicher und tierischer Schädlinge. 1. Mitteilung Abbau von *Pinus sylvestris* L. durch *Coniophora cerebella* Pers. Holzforschung 16:102–113.

SELBY, K. B. 1969. The purification and properties of the C_1-component of the cellulase complex. Pages 34–52 *in* G. J. Hajny and E. T. Reese, eds. Cellulases and their applications. Adv. Chem. Ser. 95.

SELBY, K. B., AND C. C. MAITLAND. 1967. The cellulase of *Trichoderma viride*. Biochem. J. 104:716–724.

SHARPLES, A. 1971. Degradation of cellulose and its derivatives. Pages 991–1006 *in* N. M. Bikales and L. Segal, eds. Cellulose and cellulose derivatives, v. 5, Part 5. Wiley-Interscience Publ., New York.

STONE, J. E., A. M. SCALLAN, E. DONEFER, AND E. AHLGREN. 1969. Digestibility as a simple function of a molecule of similar size to a cellulase enzyme. Adv. Chem. 95:219–241.

WILCOX, W. W. 1968. Changes in wood microstructure through progressive stages of decay. U. S. For. Serv. Res. Pap. FPL 70, 46 pp.

WOOD, T. M. 1968. Cellulolytic enzyme system of *Trichoderma koningii*. Biochem. J. 109: 217–227.