REMOVING LIGNIN FROM WOOD WITH WHITE-ROT FUNGI AND DIGESTIBILITY OF RESULTING WOOD

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ABSTRACT

Nine white-rot fungi were examined for their ability to remove lignin faster than they removed polysaccharides from aspen and from birch wood. One of the fungi was similarly examined with southern pine, Douglas-fir, and Sitka spruce. During decay most of the fungi decreased the lignin content of the aspen and the birch; that is, they removed a larger percentage of the lignin than of the polysaccharides. Lignin removal was always accompanied by removal of polysaccharides, but lignin removal did not correlate with removal of any particular component of the polysaccharides. During decay lignin was usually more selectively removed in the first few percentages of weight loss than were the polysaccharides. *Fomes ulmarius* removed lignin faster from southern pine, Sitka spruce, aspen, and birch than it did from Douglas-fir. The decayed woods with less lignin were more digestible by a mixture of polysaccharidases and by rumen fluid than were the control samples. Digestibility was inversely related to lignin content.

INTRODUCTION

White-rot fungi decompose lignin as well as cellulose and hemicelluloses in wood. The fungi of this type vary, however, in the rates at which they remove the lignin in relation to removing the polysaccharides. Some fungi remove the lignin faster than they do the carbohydrates relative to the original percentages of each. The resulting decayed wood has a lower lignin content than that of the original wood. We refer to this lowering of the lignin content as "selective removal of lignin." The ability of several white-rot fungi to remove lignin selectively was particularly indicated by Kawase (1962), who chemically analyzed naturally decayed samples collected in the forests of Hokkaido. He found several samples almost devoid of lignin. Many species of fungi were involved, but in most of the decayed samples a single species was found. Although only a few fungi have been studied, analyses of wood decayed by some white-rot fungi under controlled laboratory conditions indicate the same selective removal of lignin as in nature (Higuchi et al. 1955; Kirk and Kelman 1965).

Our objective in this work was twofold:

(1) to examine in detail the apparent capacity of some selected white-rot fungi to delignify wood;

(2) to subject samples of decayed wood to *in vitro* assays designed to evaluate the digestibility of residual carbohydrates.

In our work some of the fungi that Kawase (1962) found that decayed woods

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to low lignin contents and certain other fungi were used to decay aspen and birch wood. Results on the two wood species were compared analytically with corresponding sound woods. One of the fungi was similarly examined with three coniferous woods.

Removing lignin from wood generally increases wood digestibility by ruminants. Thus the possibility that delignifying wood by fungal decay could render wood carbohydrates more digestible to ruminants and to micoorganisms—for example, high protein-yielding yeasts—was explored in *in vitro* assays of decayed wood.

EXPERIMENTAL

Wood

Bigtooth aspen (*Populus grandidentata* Michx.) and yellow birch (*Betula alleghaniensis* Britton) were chosen for this work because of their high susceptibility to decay. Cross-grain wafers 1 by 1 by ⅛ inch were cut from single sapwood boards of the two test species. To avoid interference of extractives with subsequent lignin analysis, the wafers were thoroughly extracted in a Soxhlet apparatus, with 95% ethanol-benzene (1:2 by volume), with 95% ethanol, then washed with water and dried. Three coniferous woods, examined here, were prepared in the same manner as the aspen and birch. The moisture contents were raised to 50–100% (oven-dry basis). The wafers were sterilized by steaming at 100 C for 30 min; some of these steamed wafers were air-dried for use as controls in the analytical work.

Decay

The wafers prepared as described were allowed to decay at 27 C in soil-block chambers (ASTM 1962). Two-liter screw-cap bottles laid on their sides were used rather than the standard 8-oz bottles. Wafers in a single layer were placed in the bottles on a mycelium-covered feederstrip "floor." The nine test fungi and the reasons for selecting them are given in the following:

(1) Because they were found in decayed wood with low lignin content (Kawase 1962):

Cryptoderma yamanoi Imaz.
Fomes ulmarius (Sox. ex Fr.) Gill. (= *Rigidoporus ulmarius* (Fr.) Imaz.)
Ganoderma applanatum (Pers. ex Wallr.) Pat. (= *Elfvingia applanata* (Pers. ex Wallr.) Karst.)
Polyporus resinosus Fr. (= *Ishnoderma resinosum* (Fr.) Karst.)

(2) An attempt to duplicate a *Grifola* sp. associated with decayed wood with a very low lignin content (Kawase 1962). (At one time these species were classified in the genus *Grifola*.)

Polyporus berkeleyi Fr.
Polyporus giganteus Pers. ex Fr.

(3) Arbitrarily chosen:

Pleurotus ostreatus (Fr.) Quel.
Polyporus frondosus Dicks. ex Fr.

(4) Included as a comparative because this fungus removes lignin and carbohydrates simultaneously:

Polyporus versicolor L. ex Fr.

Cultures of all the test fungi were supplied by the Center for Forest Mycology Research, Forest Products Laboratory, Madison, Wis.

Rates of decay were monitored by removing wafers from test bottles at regular intervals to check weight loss. After removal, wafers were scraped free of mycelium, air-dried, equilibrated at 27 C and 70% relative humidity, and weighed. After varied periods of decay, wafers of a minimal range in weight losses were combined for analyses.

Analyses

Decayed wood samples and control samples, ground to pass a 40-mesh screen, were analyzed—without further extraction—for sulfuric acid lignin (Moore and Johnson 1967) and for total reducing sugars in acid

TABLE 1. *Lignin and carbohydrate content and digestibility of sound and decayed aspen wood*

Fungus	Approx. decay time (days)	Average weight loss (%)	Range of weight losses (%)	Lignin (%)	Loss of lignin[a] (%)	Total carbohydrates (%)	Loss in total carbohydrates[a] (%)	Polysaccharidase digestibility (%)	Rumen fluid digestibility (%)
None (control)	0	0	—	15.9	0	71.3	0	12.8	46
Cryptoderma yamanoi	133	22	18–24	9.8	51.7	69.8	23.6	42.5	—
Fomes ulmarius	77	13	10–17	11.9	34.6	73.6	10.2	34.3	64
Ganoderma applanatum	19	26	24–31	16.5	23.3	65.3	32.3	28.9	—
	71	51	46–56	18.7	42.2	61.5	57.6	34.6	46
Polyporus berkeleyi	88	21	16–23	8.5	57.8	70.5	22.0	54.2	77
P. frondosus	64	20	19–22	10.5	47.2	71.8	19.3	43.4	71
	101	50	45–53	12.8	59.8	65.0	59.4	38.7	66
P. giganteus	28	15	14–18	14.9	20.1	68.8	18.0	26.3	57
	63	47	42–51	7.5	74.9	73.6	45.3	55.2	—
	77	59	55–68	7.2	81.8	71.9	58.8	51.9	—
P. resinosus	99	14	13–15	9.8	47.1	72.3	12.6	51.7	—
P. versicolor	33	26	—	15.7	27.1	70.9	26.4	20.7	—
	46	40	—	15.4	39.6	70.6	40.6	22.0	52
	82	57	—	16.9	54.1	69.3	58.2	22.7	—

[a] Based on original amount of component.

hydrolysates. Sugars were estimated from a glucose standard curve (Moore and Johnson 1967). We refer to this total sugar analysis as a "carbohydrate" analysis. Selected samples were also analyzed for the relative amounts of glucose, xylose, and mannose in acid hydrolysates by paper chromatography (Moore and Johnson 1967). The "digestibility" of the samples by a polysaccharidase enzyme mixture was determined. A sample (< 40 mesh) was incubated with a commercial "cellulase" preparation (Onazuka SS, Kanematsu-Gosho (USA), Inc., New York) at 40 C for 96 hr; then the released reducing sugars were determined as glucose. Finally, selected samples were subjected to an *in vitro* assay with strained rumen fluid (Mellenberger et al. 1970).

RESULTS AND INTERPRETATION

Effect of fungi on lignin contents of aspen and birch

In agreement with the observations of Kawase (1962), *Cryptoderma yamanoi*, *Fomes ulmarius* (*Rigidoporus ulmarius*), and *Polyporus resinosus* (*Ishnoderma resinosum*) removed the lignin faster than the carbohydrates (total sugars) on a relative basis from both aspen and birch woods (Tables 1 and 2). For example, at a 14% total weight loss of the aspen, *P. resinosus* removed 47% of the lignin and 13% of the carbohydrates; at an 11% total weight loss of the birch, the fungus removed 34 and 13%, respectively. With these decayed samples and with most of the others in Tables 1 and 2, the total per cent loss in weight does not adequately reflect the per cent losses in lignin and carbohydrates. This discrepancy is caused by the presence of materials in the decayed samples that did not analyze as lignin or as carbohydrates. *Pleurotus ostreatus* (examined only with birch), *Polyporus berkeleyi*, and *P. frondosus* also removed lignin selectively. *Ganoderma applanatum* removed carbohydrates somewhat faster than lignin from the aspen, but rates were roughly proportional to the occurrence of these components in the birch. *Polyporus giganteus* removed lignin faster than carbohydrates from aspen, but removed carbohydrates faster than lignin from birch. *P. versicolor* removed the lignin and carbohydrates from aspen at the

TABLE 2. *Lignin and carbohydrate content and digestibility of sound and decayed birch wood*

Fungus	Approx. decay time (days)	Average weight loss (%)	Range of weight losses (%)	Lignin (%)	Loss of lignin[a] (%)	Total carbohydrates (%)	Loss in total carbohydrates[a] (%)	Polysaccharidase digestibility (%)	Rumen fluid digestibility (%)
None (control)	0	0	—	21.2	0	66.8	0	5.6	20
Cryptoderma yamanoi	83	15	13–18	11.1	55.7	58.8	25.1	44.7	—
	125	38	37–38	10.5	69.3	69.3	35.8	40.9	—
Fomes ulmarius	64	15	14–16	14.7	41.1	69.8	11.2	34.8	57
Ganoderma applanatum	34	17	15–20	21.1	17.4	63.4	20.8	14.4	29
	64	32	30–33	20.0	35.9	63.0	35.9	24.5	41
	125	62	58–67	19.2	65.6	60.0	65.9	40.7	—
Pleurotus ostreatus	82	33	31–36	15.8	50.1	66.2	33.7	27.4	—
Polyporus berkeleyi	44	8	6–10	16.2	29.7	66.4	8.6	32.5	54
	69	22	19–26	16.0	41.1	64.1	25.2	38.1	60
	111	39	37–40	12.8	63.2	64.1	41.5	38.5	—
	125	47	44–48	16.2	59.5	60.7	51.9	41.5	—
P. frondosus	44	14	12–16	14.7	40.4	66.8	14.1	36.9	63
	70	41	36–47	14.7	59.1	65.1	42.5	37.8	—
	125	63	53–74	19.1	66.7	58.0	67.9	32.2	—
P. giganteus	125	33	30–36	24.4	22.9	58.1	41.8	5.1	—
P. resinosus	40	11	9–14	15.7	34.1	65.7	12.5	32.4	57
	111	22	18–24	15.4	43.3	64.6	24.6	34.7	57
	111	26	22–34	14.0	51.1	63.9	29.3	27.2	—
P. versicolor	41	21	20–22	18.7	30.2	68.0	19.6	25.3	—
	72	32	31–33	19.2	38.4	64.7	34.1	24.8	—
	72	36	34–38	20.6	37.8	65.2	37.6	21.6	—

[a] Based on original amount of component.

same relative rates, but removed the lignin faster from birch in early stages of decay.

Individual fungi varied in the selectivity of removing lignin with stage of decay; a fungus that selectively removed the lignin in the first few percentages of weight loss did not continue to remove lignin selectively until only carbohydrate was left (Table 3). The variation in the relative rates of removing lignin and carbohydrates with the stage of decay was not examined for all the fungi that at some stage selectively removed lignin. With those examined, however, the lignin generally was removed most selectively in the first few percentages of weight loss (Table 3).

Relationship between lignin removal and removal of carbohydrate components

Although carbohydrates were always removed with lignin, removal of the lignin did not correlate with removal of any single component of the polysaccharides. The

TABLE 3. *Variation of ratio of lignin loss to carbohydrate loss with extent of decay*

Fungus	Weight loss interval (Per cent of original weight)	Ratio of lignin loss to carbohydrate loss[a] (%)
	ASPEN	
Polyporus frondosus	0–20	2.4
	20–50	0.4
P. giganteus	0–15	1.1
	15–47	2.0
	47–59	0.5
	BIRCH	
Cryptoderma yamanoi	0–11	2.2
	11–22	1.3
Polyporus berkeleyi	0–8	4.2
	8–22	0.7
	22–39	1.4
	39–47	0
P. frondosus	0–14	2.9
	14–41	0.7
	41–63	0.3
P. resinosus	0–11	3.1
	11–22	0.8

[a] Per cent loss of original lignin divided by per cent loss of original carbohydrates.

TABLE 4. *Lignin, glucan, mannan, and xylan in sound and decayed birch wood*[a]

Fungus	Weight loss (%)	Lignin (%)	Glucan (%)	Mannan (%)	Xylan (%)	Difference[b] (%)
None (control)	0	21.50 (0)	39.18 (0)	2.39 (0)	26.62 (0)	10.29 (0)
Fomes ulmarius	15	14.67 (41.59)	44.36 (3.06)	2.34 (15.93)	22.22 (28.53)	16.39
Ganoderma applanatum	17	21.07 (18.17)	33.65 (28.27)	2.08 (27.03)	27.56 (13.52)	15.62
	32	19.95 (36.90)	35.68 (38.07)	1.76 (49.80)	25.20 (35.61)	17.40
Polyporus berkeleyi	8	16.15 (30.89)	35.95 (15.58)	2.37 (8.45)	27.89 (3.58)	17.62
	22	15.97 (42.06)	34.65 (31.01)	2.04 (33.35)	27.21 (20.25)	20.12
	39	12.88 (63.15)	35.73 (43.90)	1.90 (50.97)	26.16 (39.54)	23.31
P. resinosus	11	15.73 (34.88)	36.48 (17.12)	2.00 (25.38)	26.90 (10.03)	18.87
	22	15.44 (43.98)	35.09 (30.12)	2.24 (26.72)	27.08 (20.63)	20.13
P. versicolor	21	18.72 (31.21)	39.85 (19.64)	2.24 (25.63)	25.42 (25.54)	13.75
	36	20.60 (38.96)	37.85 (38.89)	1.71 (54.25)	25.45 (39.08)	14.64

[a] Values in parentheses are percent loss.

[b] Difference = [100% − (% lignin + glucan + mannan + xylan)].

ratios of the individual sugars in acid hydrolysates of some of the birch samples were analyzed, and the total reducing sugars as glucose in the hydrolysates were determined. From these the per cent of *glucan*, *xylan*, and *mannan* was calculated, and the per cent loss of each was determined (Table 4). At a 15% weight loss, *Fomes ulmarius* removed only 3% of the glucan, but removed 41% of the lignin and 28% of the xylan, indicative that removal of a substantial amount of glucan is not associated with removal of lignin by this fungus. *Polyporus berkeleyi*, however, at an 8% weight loss, removed only 3% of the xylan but 31% of the lignin and 15% of the glucan, indicative that removal of a substantial amount of xylan is not associated with removal of lignin by this fungus. Similarly, no relationship was seen between mannan removal and lignin removal when all of the fungi were considered.

In Table 4, the per cent of material in the samples not acounted for by the glucan, xylan, mannan, and lignin is also given. The decayed wood had from 14 to 23% of this type of material, whereas the sound wood had 10%. In sound wood, this "not accounted for" material is mainly uronic acid, acetyl, acid-soluble lignin, and inorganic constituents. In the decayed wood, this material may be augmented by fungus products (excluding those hydrolyzable to glucose, xylose, or mannose), acid-soluble degraded lignin, etc.

Wood digestibility increased by decay

The polysaccharidase digestibility of the aspen and the birch was increased by all of the test fungi except *Polyporus giganteus* on birch (Tables 1 and 2). The susceptibility of the woods to enzymatic depolymerization by a mixture of polysaccharidases was inversely related to lignin content; thus the fungi most effective in selectively removing lignin generally caused the largest increase in digestibility (Tables 1 and 2).

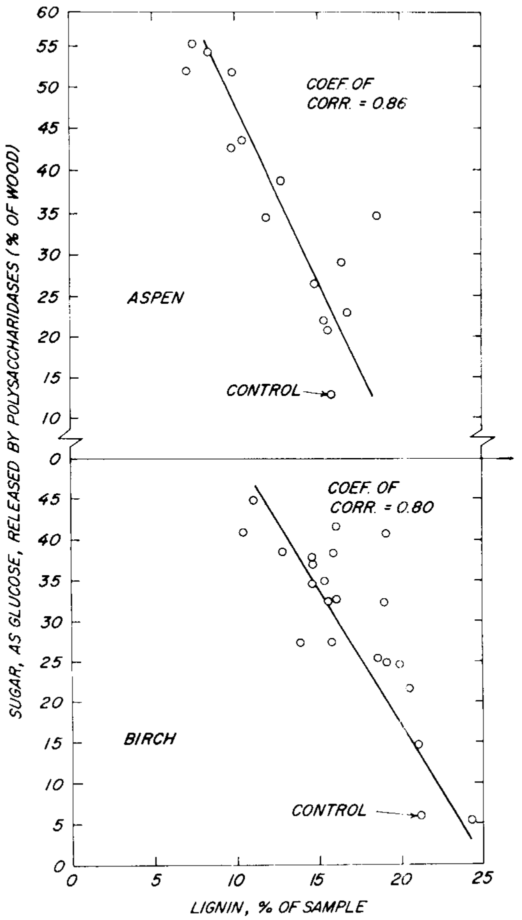

FIG. 1. Relationship of polysaccharidase digestibility to lignin content.

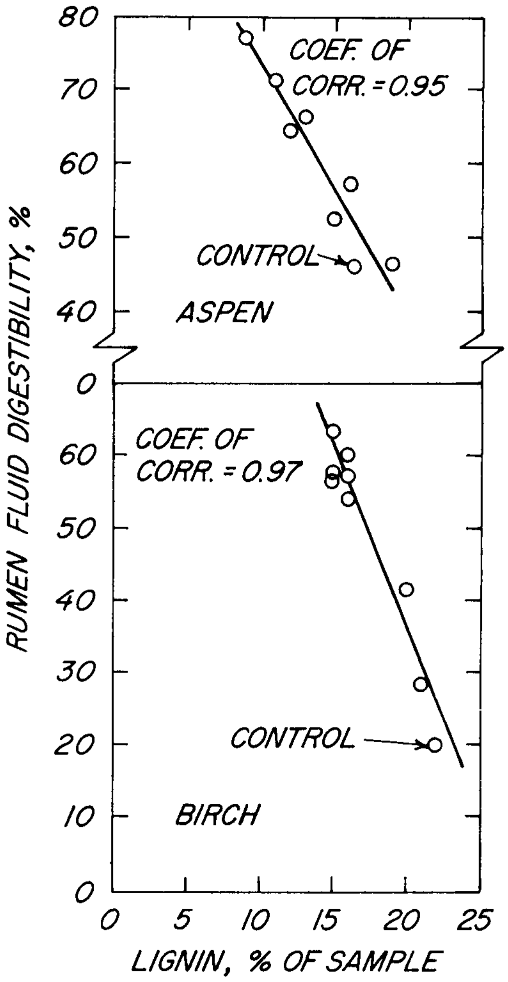

FIG. 2. Relationship of digestibility by rumen fluid to lignin content.

Decreases in lignin content were associated with substantial increases in digestibility. Birch wood decayed by *P. giganteus* had a higher lignin content than the control wood, and this decayed wood was no more digestible than the control. Lignin content is plotted against polysaccharidase digestibility of the samples in Fig. 1; regression analyses of these data gave correlation coefficients of 0.86 and 0.80 for aspen and birch, respectively.

Results of the rumen fluid assay of digestibility were similar to those of the polysaccharidase assay (Tables 1 and 2), although the percentage of digestibility with the rumen fluid was almost invariably greater, in part due to the measurement of total solubles rather than only the reducing sugars. Inverse correlations (0.95 and 0.97) were very high between lignin contents of the aspen and the birch and their rumen fluid digestibilities (Fig. 2). The relatively high digestibilities of the control aspen and birch were probably caused by the steam treatment that was used to sterilize the samples (Bender et al. 1970). In addition, the control aspen wood was unusually low in lignin content; thus this wood would be expected to be unusually high in digestibility.

TABLE 5. *Lignin and carbohydrate contents and digestibility of three conifer woods before and after decay by* Fomes ulmarius

Species	Decay time (weeks)	Average weight loss (%)	Range of weight losses (%)	Lignin (%)	Loss of lignin[a] (%)	Total carbohydrates (%)	Loss of carbohydrates[a] (%)	Polysaccharidase digestibility (%)
Southern pine	0	0	—	30.0	0	61.4	0	3.7
	37	20.5	18–23	23.3	38.3	66.1	14.5	24.7
Douglas-fir	0	0	—	33.1	0	58.6	0	2.6
	42	16.5	15–18	31.4	20.8	59.1	15.9	5.5
Sitka spruce	0	0	—	29.4	0	64.1	0	4.3
	37	11.4	10–13	22.2	33.0	66.5	8.1	31.1

[a] Based on original amount of component.

Selective removal of lignin from conifer woods

Kawase (1962) reported finding very low lignin contents in several naturally decayed woods of conifers as well as in decayed woods of broad-leaved trees. In our work, *Fomes ulmarius* was examined for selective removal of lignin from the wood of three conifers, southern pine (probably *Pinus taeda* L.), Sitka spruce (*Picea sitchensis* (Bona.) Carr), and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). As with aspen and birch, this fungus selectively removed lignin from the southern pine wood and from the Sitka spruce wood; polysaccharidase digestibility of both specimens greatly increased with the removal of the lignin (Table 5). However, lignin was removed only slightly faster than carbohydrates (on a relative basis) from the Douglas-fir, and its polysaccharidase digestibility was increased only slightly. The reason for this difference with Douglas-fir is not known, but it does serve to emphasize that the wood species as well as the fungus can greatly influence the selectivity of lignin removal. With all three conifers, rate of decay was very slow (Table 5), as it often is with white-rot fungi on conifers.

DISCUSSION

Low lignin content of aspen wood

Aspen wood generally contains more lignin (about 18–19%) than was in the sample used here (15.9%) (Johnson et al. 1961). Tension wood can have a markedly lower lignin content than that of normal wood of the same species (Meier 1965). (Unpublished data, however, obtained at this laboratory indicate that this is not always true.) Examination of the wood revealed tension wood in the test samples. Nevertheless, the chemical differences in the controls and in the decayed samples are in accord with those found with birch; therefore the presence of tension wood does not affect the interpretation of results.

Removal of lignin and polysaccharides

The data in Table 4 show that lignin removal did not correlate with removal of any single component of the polysaccharides, although some polysaccharide was always removed with the lignin. A considerable amount of lignin could be removed by *Fomes ulmarius* with removal of only a small amount of glucan, and by *Polyporus berkeleyi* with removal of only a small amount of xylan. Perhaps other species of fungi or individual isolates can be found that will remove lignin without removing either glucan or xylan. It is possible, however, that lignin cannot be removed if either glucan or xylan is not removed. It has been suggested that catabolism of carbohydrates may provide energy for the breakdown of lignin (Cowling 1961). It is also possible that the wood structure limits the amount of one component that is accessible to the degrading enzymes without degradation of another component. Our data do not include the extent of degradation of the polysaccharides by the fungi—only the amounts removed. Thus, degradation of the polysaccharides may be necessary before lignin can be attacked, although removal of the polysaccharide components may not be necessary. Futher study is required to clarify the relationship between degradation of lignin and polysaccharides.

Improving digestibility of wood

Because wood is 65 to 80% combined polysaccharides, it is potentially a rich source of food carbohydrate. However, the intimate association of the carbohydrates with the lignin renders most wood practically indigestible; consequently for several years work has been conducted at the U. S. Forest Products Laboratory on chemical treatments to make wood more digestible (Millett et al. 1970). It is known from this type of study that in general the more lignin removed from wood, the more digestible is the residual material. Results here with fungus-delignified woods agree with this (Figs. 1 and 2).

Practical use of white-rot fungi

Our results show that some white-rot fungi are effective in removing lignin faster than polysaccharides from wood. However, delignifying wood by these test fungi under the conditions used is relatively slow. For practical use of white-rot fungi—for example, to improve the digestibility of wood—it would be desirable to speed the process. Apparently very little research has been directed either at optimizing the conditions for decaying wood by white-rot fungi or for large-scale decaying of wood. Optimizing factors such as aeration, moisture, temperature, and source and amount of nutrient nitrogen should enhance the rate of decay. In addition, it may be possible to find conditions that would improve the selectivity of lignin removal. It may also be possible to find better fungus species or better isolates than were used, or to obtain desirable mutants, as is common in industrial microbiology. Efforts at the Forest Products Laboratory include a search for other types of organisms that will act more rapidly, and perhaps more selectively.

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