LIGNIN DEGRADATION CAPABILITIES OF *PLEUROTUS OSTREATUS, LENTINULA EDODES* AND *PHANEROCHAETE CHRYSOSPORIUM*

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ABSTRACT

Two edible mushroom species (*Pleurotus ostreatus* and *Lentinula edodes*) and one known ligninase-producing species (*Phanerochaete chrysosporium*) were evaluated for their selective lignin-degrading capability on glucose-supplemented hardwood and softwood pulp chips. As time (0, 10, 20, 30 days) of vegetative mycelial growth increased, significant changes in weight loss, alkali solubility, ethanol benzene extractives content, and klason lignin were observed for hardwood and softwood pulp chips. The highest amount of lignin (12% in red oak) was consumed by *P. chrysosporium* after a 30-day incubation period. Glucose-supplemented wood chips minimized holocellulose degradation while providing a significant increase in weight loss and delignification at the 20- and 30-day incubation periods.

Keywords: Biopulping, wood chips, hardwood, softwood, lignin, oyster mushroom, shiitake.

INTRODUCTION

Paper manufacturers continue to search for more efficient ways of producing high quality pulp at a lower cost. One approach for possibly lowering production costs that has received increased attention in recent years is biopulping (Erikson et al. 1980). Biopulping may allow selective enzymatic removal of nondesirable lignin contained in the cell walls. Selective enzymatic removal or utilization of lignin would lessen the dependence on chemicals in the pulping process and thereby reduce the environmental risks associated with chemical processing (Erickson et al. 1980).

For biopulping to become a commercial reality, a number of fundamental problems must be addressed: 1) promising lignin degrading fungi and their enzymes must be identified, 2) suitable wood species need to be selected, 3) the

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length of biological pretreatment must be determined, and 4) the uniformity of fungal degradation of wood chips must be considered.

Most investigators have used laboratory-scale techniques to study the biodegradation capacity of fungi. For example, Fenn et al. (1981), Kirk et al. (1978), and Leatham and Kirk (1983) have used different liquid culture media formulations to investigate the ligninolytic activity of various fungi on model compounds. The soil block and agar technique, as specified by the ASTM standards (D1414-76), utilizes 2.5- × 1.5- × 1.0- cm cross-sectional wafers. The modified agar method, as described by Setliff et al. (1983), was used to investigate the biological modification of wood chips and mechanical pulps. However, these methods are not readily applicable for use with commercial quantities of wood chips.

To help minimize the cost of biopulping, it may be feasible to couple the needs of the pulping industry with the methodology, and by-products of another industry such as the mushroom industry. Many exotic mushrooms are now produced commercially on supplemented wood chips (Royse 1985). Mushroom production is accomplished by allowing the fungus to colonize the wood chips for an optimum period of time (29 to 90 days), then inducing the fungus to produce sporocarps on the colonized substrate. During the vegetative growth stage, the fungus produces enzymes that degrade wood components into a usable food source. One possibility for efficient use of wood in biopulping would be to produce a high value product like shiitake (*L. edodes*) or oyster (*P. ostreatus*) mushrooms, followed by extraction of the remaining enzymes and/or use of the chips to make paper products.

Because of the relative ease of fungal preparation and handling, rye spawn was used as an effective means of uniformly establishing the fungal mycelium on wood chips. This methodology was used to examine the capacity of three white-rot fungi to selectively delignify nonsupplemented and glucose-supplemented wood chips.

MATERIALS AND METHODS

Test materials

Red Oak (*Quercus rubra*), white spruce (*Picea glauca*), and aspen (*Populus tremuloides*) were selected for degradation experiments because of their known differences in lignin composition and differences in lignin distribution within the cell wall. Pulp chips were screened, placed into polyethylene bags, and stored in a cold room (4 C) until needed.

Pleurotus ostreatus (WC 537) and *Lentinula edodes* (WC 305) were selected for this study because they are commercially used lines that have relatively high mushroom production capacity. These isolates are maintained on potato-dextrose yeast-extract agar as outlined by Jodon and Royse (1979). *Phanerochaete chrysosporium* (isolate BKB 1767) was selected because of its known ligninase production and was maintained in the laboratory as described by Kirk et al. (1981).

Spawn was prepared in 500-ml flasks by the addition of ingredients as follows: rye grain, 80 g; sawdust, 6 g; $CaCO_3$, 1 g; and tap water, 105 ml. The material was autoclaved for 45 minutes at 121 C, cooled, and inoculated. The mycelium was allowed to grow for 2 weeks, during which time the spawn was shaken twice weekly to prevent clumping.

Wood preparation and fungal growth

Anhydrous glucose (2% based on oven-dry weight of wood) was added to each bag containing 100 g of wood chips. The moisture content of the wood chips was adjusted to 50% by adding distilled water directly to the bags of wood chips. The mixture was autoclaved in special autoclavable polypropylene bags (30 × 60 cm; Thomas Scientific) at a temperature of 121 C for a period of 45 minutes. The sterilized wood chips were inoculated with spawn of each of the three white-rot fungi. The bags were heat sealed and the spawn was thoroughly mixed with the chips. The spawned wood chips then were placed into a controlled humidity growth chamber maintained at 25 ± 1 C and 95–98% relative humidity. Holes were punched in the bags with sterile needles after 5 days in the growth chamber in order to provide air exchange for the mycelium. Bags of chips were withdrawn for testing after 0, 10, 20, and 30 days in the growth chamber. For latter experiments, sterile air-flow polypropylene bags (44 × 22 × 12 cm) were used (Fungi Perfecti, Olympia, WA 98507).

Chemical analysis

Holocellulose, klason lignin, and extractive content of fungally degraded wood were determined using TAPPI standards T-222-su-70, T-222-os-71, and T-12-os-75, respectively. Hemicellulose content was estimated as a difference between holocellulose and alpha cellulose content values. The 1% alkali solubility of decayed wood chips was determined using the ASTM method D 1110-56. The percentage weight loss of fungally degraded wood chips was based on the oven-dry weight of wood and comparisons were made between treated and untreated samples (control).

Experimental design and analysis

Experiment I. This trial was designed to examine the capacity of three white-rot fungi to selectively delignify glucose-supplemented wood chips. The experiment was designed as a (4 × 3 × 2) factorial with three replications/treatment (Steel and Torrie 1980). The factors considered were incubation time (0, 10, 20, 30 days), fungal species (*L. edodes, P. ostreatus,* and *P. chrysosporium*) and wood species (red oak and white spruce). Chip weight loss, 1% alkali solubility, extractive content, klason lignin, hemicellulose, and cellulose content were estimated for each treatment.

Experiment II. This experiment was designed to determine the effect of glucose-supplementation on the degradation of red oak and aspen wood chips by *P. chrysosporium.* Aspen was selected instead of spruce because of its higher susceptibility to degradation by *P. chrysosporium.* Fungally degraded wood chips were evaluated in triplicate for chip weight loss, 1% alkali solubility, extractive content, klason lignin, hemicellulose and cellulose content. Data were analyzed using the Minitab (Ryan et al. 1986) and the Statistical Analysis System (SAS 1983). Mean separation was determined by the Student-Newman-Keuls (SNK) procedure.

RESULTS AND DISCUSSION

Weight loss and 1% alkali solubility

Changes in weight loss and 1% alkali solubility were statistically significant ($P \leq 0.05$) with respect to incubation time, wood species, and fungus used (Table

TABLE 1. *Mean weight loss (%) and 1% alkali solubility of sound and decayed red oak and white spruce wood chips through decay intervals of ten days each by* Pleurotus ostreatus, Lentinula edodes, *and* Phanerochaete chrysosporium.

Wood type	Incubation time (days)	Fungi					
		Pleurotus ostreatus		*Lentinula edodes*		*Phanaerochaete chrysosporium*	
		Weight[1] loss (%)	1% Alkali[1] solubility (%)	Weight loss (%)	1% Alkali solubility (%)	Weight loss (%)	1% Alkali solubility (%)
Red oak	0	0.00 aA[2]	35.04 aA	0.00 aA	35.04 aA	0.00 aA	35.04 aA
(*Quercus rubra*)	10	1.76 bA	36.04 bA	1.30 bA	35.98 bA	2.45 bB	39.76 bB
	20	3.06 cA	41.18 cA	2.41 cB	38.87 cB	4.45 cC	45.87 cC
	30	5.82 dA	46.28 dA	4.45 dB	43.25 dB	7.51 dC	49.94 dC
White spruce	0	0.00 aA	31.86 aA	0.00 aA	31.86 aA	0.00 aA	31.86 aA
(*Picea glauca*)	10	0.64 bA	31.92 aA	0.12 bB	31.88 aA	0.95 bC	31.98 aA
	20	1.34 cA	32.00 aA	1.02 cB	31.97 aB	1.80 cC	33.96 bC
	30	2.98 dA	36.88 bA	1.88 dB	33.58 bB	3.84 dC	38.93 cC

[1] Weight loss (%) and alkali solubility based on oven dried weight of sound wood chips.
[2] Each measurement is an average of 3 replicates. Means with the same small letter within a column and means with the same capital letter within a row indicate that no significant difference was observed at the 0.05% level of probability (Student-Newman-Keuls test).

1). A progressive increase in both weight loss and alkali solubility was associated with an increase in incubation time. Incubation time was more important than the fungus species used in reducing wood chip weight and increasing alkali solubility. The highest weight loss was observed for *P. chrysosporium,* while the lowest weight loss was observed for *L. edodes* after an incubation period of 30 days. Weight loss and alkali solubility was higher from red oak chips than from white spruce (Table 1). Approximately 20 additional days of incubation were required by each fungus to degrade the same amount of wood in spruce when compared to red oak (Table 1). This is not unusual since many fungi preferentially degrade hardwood compared to softwood and vice versa (Cowling 1961; Kirk and Moore 1972).

Extractive content

The ethanol benzene extractive content of wood chips increased as fungal degradation time increased (Table 2). This increase was statistically significant ($P < 0.05$) with treatment time, wood species, and fungus used. After an incubation period of 30 days, red oak extractive content increased from 3.48% to 6.45%, 5.23%, and 4.88% with *P. chrysosporium, P. ostreatus,* and *L. edodes,* respectively. In comparison, white spruce extractive content increased from 6.04% to 7.12%, 6.42%, and 6.19%, respectively, with the same fungi. Wood chips incubated with *P. chrysosporium* had the highest extractive content when compared to the other fungi.

These results support earlier findings by Setliff and Eudy (1981) who reported the tendency for extractive content to increase with an increase in the extent of wood decay. They reported that a 10 to 12% increase in extractive content occurred with different fungi on various wood species. Baldwin and Streisel (1985) observed significant changes in the ethanol benzene extractive content of hybrid poplar sapwood decayed to 5% weight loss with the fungus *Lentinula trabea.* Cowling (1961) found that brown-rot fungi markedly increase the solubility of wood in various solvents, whereas white-rot fungi do not substantially change the solubility

of wood. The difference in rate of change of solubility caused by white- and brown-rot fungi was most pronounced for 1% sodium hydroxide extractives.

The tendency for extractives content to increase with an increase in wood decay may be attributed to the increase in the phenol oxidizing activity and degradative products produced during decay. This may be especially true for red oak, a high density hardwood, known to contain a high proportion of phenolic materials such as d-catechin, ellagic acid, gallic acid, and proanthocyanin (Hillis 1962). These compounds are reported to be susceptible to gaseous and enzymatic oxidation (Hillis 1962). However, Kirk et al. (1981) suggested that the inability of certain fungi to oxidize phenols may not be associated with their inability to utilize lignin. In this study a gradual darkening of the wood chips was observed as decay progressed. These findings suggest that oxidation of polyphenolic material and the production of chromophoric groups are associated with decayed wood discoloration.

Klason lignin

The klason lignin content decreased as wood decay progressed in both types of wood (Table 2). These decreases in klason lignin content were statistically significant ($P \leq 0.05$) with respect to treatment time, wood species, and fungus.

These results are consistent with those reported by Kirk and Moore (1972), who observed a very slow rate of lignin loss in southern pine, Douglas-fir, and Sitka spruce with different white-rot fungi. The differences in the rate of decay between wood species may reflect the differences in lignin distribution in the cell-wall ultrastructure and the inability of the fungi to attack the cell walls. In red oak, more lignin can be found in the middle lamella (approximately 85%) than in other parts of the cell wall (Jackson et al. 1985). In spruce, however, the concentration of lignin in the middle lamella is approximately 73% while that in other parts of the cell wall is about 13% (Jackson et al. 1985). The total amount of lignin, however, is higher in the secondary wall. Although all three fungi decreased the lignin content with increased incubation time, wood chips decayed by *P. chrysosporium* resulted in the highest amount of lignin removal (Table 2). Wood chips incubated with *L. edodes* lost the least amount of lignin.

Some investigators have used the term "specificity" to indicate the preference of certain fungi for lignin consumption. Setliff and Eudy (1981) and Setliff et al. (1983) have defined specificity as the ratio of lignin consumed to nonlignin components consumed. Using this ratio, both *P. chrysosporium* and *P. ostreatus* showed a high preference of selectivity. The amount of red oak lignin consumed by *P. ostreatus, L. edodes,* and *P. chrysosporium* was 8.3%, 1.3%, and 12.3%, respectively, after a 30-day incubation period. In comparison, the amount of white spruce lignin consumed by the same fungi was 4.9%, 2.9%, and 9.5%, respectively, after a 30-day period.

Holocellulose content

Statistical differences in hemicellulose and cellulose content ($P \leq 0.05$) were observed for spruce, but not for oak wood chips (Table 2). Kirk and Moore (1972) observed a decrease in sugar content of wood decayed by various fungi. However, no nutrient supplement (glucose) was added to the substrate. They reported a 15%

TABLE 2. *Extractive, klason lignin, hemicellulose, and cellulose contents of sound and decayed red oak and white spruce wood chips through decay intervals of ten days each by* Pleurotus ostreatus, Lentimula edodes, *and* Phanerochaete chrysosporium.

Wood type	Incubation time (days)	Fungi: *Pleurotus ostreatus* Extractive content	Klason lignin	Hemi-cellulose	Cellulose
Red oak	0	3.48 aA	21.25 aA	28.58 aA	46.60 aA
(*Quercus rubra*)	10	3.60 aA	21.18 aB	28.56 aA	46.56 aA
	20	4.97 bA	20.34 bA	28.52 aA	46.56 aA
	30	5.23 cA	19.48 cA	28.42 aA	46.52 aA
White spruce	0	6.04 aA	25.68 aA	24.38 aA	43.62 aA
(*Picea glauca*)	10	6.08 aA	25.62 aA	24.32 aA	43.58 aA
	20	6.24 aA	25.08aA	23.82 bA	43.52 aA
	30	6.42 bA	25.42 bA	23.40 bA	43.44 bA

[1] 1:2 ethanol-benzene extractive content determined using TAPPI standard T-12-os-75.
[2] Each measurement is an average of 3 replicates. Means with the same letter within a column and means with the same capital letter within a row indicate that no significant difference was observed at the 0.05% level of probability (Student-Newman-Keuls test).

weight loss with *P. ulmerius* while 3% of the glucan and 18% of the xylan were consumed. *Polyporus berkeleyi* resulted in 8% weight loss and a consumption of 3% of the xylan and 15% of the glucan. These observations indicate that sugars associated with hemicelluloses were readily consumed. Cowling (1961) also observed a decrease in the average DP (degree of polymerization) of cellulose in sweetgum sapwood decayed by both white- and brown-rot fungi. These data suggest that cellulolytic activity was suppressed by the addition of 2% glucose to the wood chip. Erikson and Vollander (1980) showed that the addition of glucose to a cellulose media repressed cellulose activity by *Sporotrichum pulverulentum* even at a glucose concentration of 50 mg per liter. Erikson and Hamp (1978) also reported that increasing the glucose concentration to approximately 50 mg l^{-1} in cultures containing 5.05% lignin resulted in an increase in the rate and extent of lignin decomposition. Lignin decomposition had essentially ceased after 20 days in cultures with an initial glucose concentration of 28 mM or less. Cowling (1961) pointed out that the catabolism of the carbohydrates may provide a source of energy for the fungi in the course of wood decay. This might be especially true in view of the role of glucose as the primary substrate in the glycolytic cycle.

Effect of glucose addition

Chemical components of biodegraded red oak and aspen as affected by glucose supplementation are summarized in Tables 3 and 4. At the initial stages of incubation there was a more profuse mycelial growth in the glucose-supplemented wood chips. Less time (6 to 10 days) was required for the mycelium to uniformly colonize glucose-supplemented substrates when compared to nonsupplemented substrates (12 to 15 days).

Higher mean weight losses, alkali solubilities, and extractive contents were detected for glucose-supplemented wood chips compared to nonsupplemented wood chips. The amount of holocellulose consumed by *P. chrysosporium* in aspen wood chips was 17% and 7% in the absence and presence of glucose, respectively. For red oak, the percent holocellulosic material lost to fungal decay was 15% for the nonsupplemented chips and 3.6% for glucose-supplemented chips.

TABLE 2. *Extended.*

Fungi							
Lentinula edodes				*Phanerochaete chrysosporium*			
Extractive content	Klason lignin	Hemi-cellulose	Cellulose	Extractive content	Klason lignin	Hemi-cellulose	Cellulose
3.48 aA	21.25 aA	28.58 aA	46.60 aA	3.48 aA	21.25 aA	28.58 aA	46.60 aA
3.52 aA	21.20 aA	28.56 aA	46.60 aA	3.48 aB	21.14 aA	28.52 aA	46.57 aA
4.01 bB	21.08 aB	28.45 aA	46.57 aA	5.64 bC	19.84 bC	28.40 aA	46.54 aA
4.88 cB	20.97 bB	28.35 aA	46.56 aA	6.45 cC	18.65 cC	28.24 aA	46.48 aA
6.04 aA	25.68 aA	24.38 aA	43.62 aA	6.04 aA	25.68 aA	24.38 aA	43.62 aA
6.05 aA	25.66 aA	24.38 aA	43.60 aA	6.10 aA	25.56 aA	24.30 aA	43.56 aA
6.08 aB	25.40 aA	24.24 aA	43.55 aA	6.38 aC	24.66 bB	22.86 bB	43.20 aA
6.19 bB	24.91 bA	24.01 bB	43.48 bA	7.12 bC	23.24 cB	21.97 cC	42.60 bA

Simultaneous reductions in carbohydrates and lignin content occurred with the three fungi examined. Even where there was no significant change in the carbohydrate fraction, especially in the glucose-supplemented wood chips, a significant loss in the lignin fraction occurred. A more pronounced reduction in the lignin fraction was measured in the presence of glucose (27% and 22%) compared to the control samples (19% and 15%) for aspen and red oak, respectively, after the 30-day incubation period.

The justification for using glucose to protect the polysaccharide fraction in woody mass in the course of white-rot decay could be found in earlier studies by Erikson and Hamp (1978), Yang et al. (1980), Erikson et al. (1980), and Erikson and Kirk (1980). Erikson and Kirk (1980) noted that the addition of glucose provided an easily metabolizable carbon source to biodegrade lignin. They suggested that if wood chips were impregnated with glucose, the degrading organisms would preferentially metabolize the added glucose and naturally occurring, low-molecular-weight sugars rather than degrade the wood cell-wall hemicelluloses. Thus, the cellulosic and hemicellulosic fibers would be protected from degradation.

TABLE 3. *Mean weight loss (%), alkali solubility, extractive content, klason lignin, hemicellulose, and cellulose compositions of glucose and non-glucose treated sound and decayed red oak wood chips through decay intervals of ten days each by* Phanerochaete chrysosporium.

	Incubation time (days)	Weight[1] loss (%)	Alkali[1] solubility (%)	Extracive content (%)	Klason lignin (%)	Hemi-cellulose (%)	Cellulose content (%)
Red oak wood chips	0	0.00 aA	36.22 aA	5.41 aA	23.24 aA	29.20 aA	44.42 aA
(control)	10	3.35 bA	38.02 bA	6.48 bA	22.45 bA	27.45 bA	43.65 aA
	20	10.43 cA	43.62 cA	8.54 cA	20.12 cA	25.49 cA	41.28 bA
	30	14.14 dA	49.86 dA	9.17 dA	19.05 dA	22.43 dA	40.06 cA
Red oak wood chips	0	0.00 aA	36.22 aA	5.41 aA	23.24 aA	29.20 aA	44.42 aA
(+ glucose)	10	3.86 bA	38.91 bB	7.02 bA	21.86 aA	29.01 aB	44.39 aB
	20	14.87 cB	47.77 cB	9.80 cB	19.04 bB	28.14 bB	44.32 aB
	30	18.39 dB	53.92 dB	10.56 dB	18.07 cB	27.03 cB	43.78 bB

[1] Weight loss (%) and alkali solubility based on oven dried weight of sound wood chips.
[2] 1:2 ethanol benzene extractive content determined using TAPPI standard T-12-OS-75.
[3] Each measurement is an average of two replicates. Means with the same small letter (a, b, c) within each treatment column indicate that no significant difference occurred at the 0.05 level of probability (SNK). Similarly, means with the same capital letter (A, B) between both treatment columns indicate that no significant difference occurred at the 0.05 level of probability (SNK) compared at each decay period.

TABLE 4. *Mean weight loss (%), alkali solubility (%), extractive content, klason lignin, hemicellulose, and cellulose contents of glucose and non-glucose treated sound and decayed aspen wood chips through decay intervals of ten days each by* Phanerochaete chrysosporium.

	Incubation time (days)	Weight[1] loss (%)	Alkali[1] solubility (%)	Extractive content (%)	Klason lignin (%)	Hemicellulose (%)	Cellulose content (%)
Aspen wood chips (control)	0	0.00 aA	33.28 aA	3.26 aA	19.75 aA	28.11 aA	49.88 aA
	10	3.57 bA	36.02 bA	3.96 bA	19.20 bA	27.24 bA	47.80 bA
	20	9.58 cA	40.14 cA	4.76 cA	16.55 cA	24.62 cA	45.62 cA
	30	12.43 dA	42.65 dA	5.89 dA	16.02 cA	21.08 dA	43.62 dA
Aspen wood chips (+ glucose)	0	0.00 aA	33.28 aA	3.26 aA	19.75 aA	28.11 aA	49.88 aA
	10	4.02 bA	34.62 bB	4.12 bA	18.45 bB	28.02 aB	49.24 aB
	20	12.72 cB	42.86 cB	5.60 cB	15.48 cB	26.84 bB	48.85 bB
	30	16.93 dB	45.92 dB	6.24 dB	14.27 dB	25.96 cB	46.52 cB

[1] Weight loss (%) and alkali solubility based on oven dried weight of sound wood chips.
[2] 1:2 ethanol benzene extractive content determined using TAPPI standard T-12-OS-75.
[3] Each measurement is an average of two replicates. Means with the same small letter (a, b, c) within each treatment column indicate that no significant difference occurred at the 0.05 level of probability (SNK). Similarly, means with the same capital letter (A, B) between both treatment columns indicate that no significant difference occurred at the 0.05 level of probability (SNK) compared at each decay period.

In an earlier study by Erikson and Hamp (1978), and reported by Yang et al. (1980), wood polysaccharide degrading enzymes of white-rot fungi were reported to be generally induced during growth on their substrates and repressed during growth on the end products of polysaccharide hydrolysis. This characteristic, according to Yang et al. (1980), makes glucose a potent repressor of endo-1-4-β-glucanase in *Sporotrichum pulverulentum.* Erikson et al. (1980) found that a 1% glucose impregnation of pine wood blocks considerably increased the rate of lignin degradation. A weight loss of 15.7 $\pm$ 1.8% was reported for glucose impregnated wood compared to 9.3 $\pm$ 1.3% for the control samples after 3 weeks of decay. By using red alder thermomechanical pulp (TMP) as the source of lignocellulose, Yang et al. (1980) showed that the addition of 35% glucose to the basal culture medium completely suppressed the attack on the wood polysaccharides in 2 weeks without significantly affecting lignin degradation by *P. chrysosporium* in cultures with 0.12% added nitrogen.

Because the edible mushroom species degraded lignin at a slower rate than *P. chrysosporium,* they would be less likely candidates for use directly as biopulpers. However, although lignin biodegradation parameters such as aeration, moisture content, and temperature were not examined, even *P. chrysosporium* would appear too slow to be used on a commercial scale directly as a biopulping fungus. The most feasible approach appears to be to produce a high value mushroom product then, either extract the remaining enzymes or use the chips directly to make paper products. These studies are now underway.

SUMMARY AND CONCLUSIONS

The following conclusions can be drawn from this study:

1. Treatment time was a more important factor in affecting wood chip weight loss and alkali solubility than the specific fungus or wood species used.
2. The addition of 2% glucose to the wood chip substrate prior to inoculation suppressed holocellulose degradation.

3. The rate and extent of total wood degradation as shown by weight loss and lignin utilization with fungi were higher for red oak than for white spruce. At any given level of treatment, lignin consumption by all fungi was lower for white spruce than for red oak.
4. All three white-rot fungi showed a preference to selectively degrade and utilize lignin on glucose supplemented chips. *P. chrysosporium* exhibited a faster rate of mycelial growth and produced more metabolic breakdown products. It also utilized the highest amount of lignin in both wood species in comparison to *P. ostreatus* and *L. edodes*. *L. edodes* was the slowest growing and resulted in the least amount of lignin consumed.
5. Rye grain spawn as routinely used in the mushroom industry is a convenient means for inoculating pulp chips for laboratory studies and may have a practical application for commercial-scale pulp pretreatment facilities.

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