LIGNIN BIODEGRADATION OF NITROGEN SUPPLEMENTED RED OAK (*QUERCUS RUBRA*) WOOD CHIPS WITH TWO STRAINS OF *PHANEROCHAETE CHRYSOSPORIUM*[1]

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(Received June 1990)

ABSTRACT

Red oak (*Quercus rubra* L.) wood chips were treated at two levels of nitrogen content (0.95% and 2.87% N level based on oven-dry wood chips) with either anhydrous liquid ammonia or ammonium nitrate in attempts to enhance the lignin degradation rate of a wild and mutant strain of *Phanerochaete chrysosporium.* As growth time increased, significant changes in weight loss, alkali solubility, alcohol/benzene extractive content, holocellulose content, and klason lignin content were observed.

Experimental results showed that lignin biodegradation rate of both a wild and a mutant strain of *P. chrysosporium* was increased by pretreating the wood chips with either liquid ammonia at low levels (0.95% N) or by treating wood chips with additions of ammonium nitrate at the 0.95% N and 2.87% N level. Treating red oak chips with liquid ammonia at the 2.87% N level not only caused a reduction in holocellulose content of red oak wood chips, but also inhibited the growth of both strains of *P. chrysosporium* during incubation. When ammonium nitrate was added to the red oak wood chips at the 2.97% N level, biodegradation capabilities of the wild strain were suppressed. However, the red oak wood chips treated to the 2.87% N level with ammonium nitrate did not affect the growth of the mutant strain of *P. chrysosporium.* An increase in lignolytic activity was found to occur using the mutant strain of fungus.

Significant differences in lignin and carbohydrate content of fungus-degraded wood were observed. The wild strain appeared to attack the lignin and carbohydrate constituents of wood simultaneously, resulting in a loss in both components as incubation time increased from 0 to 30 days. However, the mutant strain appeared to attack the lignin constituents while leaving the carbohydrate components largely intact. Comparison between the two strains (30 days of incubation) showed the red oak wood chips degraded by the mutant strain had a higher holocellulose content than did the wood chips degraded by the wild strain. These results suggest that the mutant strain may be the preferred fungus

[1] This study was supported by State McIntire-Stennis project 3062. We would like to express our appreciation to Dr. Ming Tien for providing us with the mutant strain of *Phanerochaete chrysosporium* used in this study.

Wood and Fiber Science, 23(4), 1991, pp. 533–542

to use in manufacturing biomechanical pulps and the biodegradation rate may be slightly increased with the addition of nitrogen to the substrate.

Keywords: Lignin, wood chips, *Phanerochaete chrysosporium,* red oak.

INTRODUCTION

During the past decade, a considerable interest has been shown in the application of biotechnology in the paper industry. The term "biopulping" or "biomechanical pulping" has been used to describe the application of using either white-rot basidiomycetes or their isolated enzymes to selectively remove lignin from wood followed by refiner mechanical treatment. The intent of these biological treatments was to reduce refining energy and improve pulp quality and paper strength.

Earlier studies showed that reductions in refining energy and potential gains in both pulp yield and pulp quality can be obtained from biopulping (Eriksson and Vallander 1980; Bar-Lev et al. 1982; Leatham and Meyers 1990, Leatham et al. 1990; Trotter 1990b). Most recently, Leatham and Meyers (1990) reported on the biomechanical pulping of aspen using different fungi prior to mechanical treatment. They reported that the fungi showing the best performance for increasing strength with aspen chips were rapid-growing white-rot fungi within the genera *Phlebia* and *Phanerochaete.* The four-week treatment with white-rot fungi generally increased strength and decreased optical properties. It was also reported that the optimum energy savings for pulping aspen chips to 100 ml CSF using a rotating-drum bioreactor was four weeks (Leatham et al. 1990).

In a study completed in this laboratory, Oriaran (1989) showed that potential gains in both pulp yield and papermaking properties can be obtained if wood chips are fungally pretreated with *P. chrysosporium* prior to kraft pulping. Approximately a 5% gain in pulp yield was observed for the cooking conditions used after pretreating the wood chips with *P. chrysosporium* for a 30-day period. Not only were gains in pulp yields observed for fungus-degraded wood chips, but a reduction in refining energy was found to occur as fungal incubation time increased. Sheet strength evaluation studies of kraft pulps obtained from fungus-degraded wood showed that tensile and burst properties were increased, while tear properties decreased as fungal incubation time increased.

Based on earlier studies it can be concluded that not only were gains in pulp yield observed for pulping fungus-pretreated wood chips, but reductions in refining energy and improvements in sheet tensile and burst properties occurred. One critical factor that could affect implementation of this process for commercial applications is that the length of fungal incubation time was a lengthy 4 weeks. For most commercial papermaking operations this may be impractical (Trotter 1990a).

A potential way to enhance fungal degradation and reduce fungal incubation time is to pretreat wood chips with a strong swelling agent such as anhydrous ammonia. Leatham and Kirk (1983) found that the white rot fungi *P. chrysosporium, L. edodes,* and *P. ostreatus* grew on the three media and showed increased weight loss with increased nitrogen concentration. These fungi were reported to show no indication of inhibition by increased nitrogen, but rather the reverse was observed. Highly (1987) found *P. chrysosporium* grown in low nitrogen medium to exhibit high levels of H_2O_2 production, whereas at high nitrogen levels the

opposite was observed. Ammonia pretreatment not only increases the nitrogen content of wood (Chou 1987), it also changes the chemical structure of cellulose and the physical environment of the lignin in the lignocellulose material. Therefore, it is possible that substantial gains in lignolytic activity with a white-rot fungus can be achieved resulting in a shorter biotreatment process. Here we investigated the biodegradation rate of ammonia-pretreated red oak.

MATERIALS AND METHODS

Test materials

Red oak (*Quercus rubra* L.) wood chips were obtained from a local sawmill, then screened and dried to 12% moisture content in a controlled temperature/humidity chamber. Both oversized wood chips and fines were discarded. The remaining chips were stored in the chamber until needed.

Phanerochaete chrysosporium BKM-F-1767 and a ligninase overproducing mutant (Tien and Myer 1990) were maintained on potato-dextrose yeast-extract agar as outlined by Jodon and Royse (1979). Spawn was prepared and maintained according to methods described by Oriaran (1989).

Liquid ammonia treatment of red oak wood chips.

Anhydrous ammonia liquified in a 150-pound cylinder under 786 kPa pressure at 21 C was purchased from the Liquid Carbonic Company, State College, PA. A total of 370 g red oak wood chips (12% moisture content) was placed into a 2-l pressure resistant reactor (maximum pressure 13,790 kPa), sealed, and placed into a cold bath cooled with dry ice (carbon dioxide). The ammonia used to pretreat wood chips was determined according to methods described by Chang (1987).

For the low-pressure ammonia treatment, the reactor was heated to 25 C with an automatically controlled heater for 1 h. After treatment, the ammonia gas was released at a pressure of 1379 kPa to the atmosphere. For the high pressure ammonia treatment, the reactor was heated to 100 C in order to maintain the pressure below 12,411 kPa. The wood chips were treated for 1 h and the reactor was cooled in a cold water bath prior to releasing the excess ammonia to the atmosphere. Cooling the reactor prior to pressure release prevented an excess loss in moisture in the wood chips. The wood chips in the reactor were weighed before and after pretreatment so that the wood chip weight loss that occurred during ammonia pretreatment could be calculated.

Wood chip preparation and fungal incubation

One hundred gram samples were used in the fungal incubation experiments. Wood chips were supplemented with 2% glucose and 0.1 mM lysine and adjusted to a pH of 5.5 using buffer solution prepared from 1% NaOH and 1% acetic acid. The moisture content was adjusted to 55% (based on oven-dry wood chips) by directly adding tap water to the wood chips. Wood chips were placed into an autoclavable polypropylene bag (44.4 × 21 × 12.1 cm Fungi Perfecti, Olympia, WA 98507) containing an air microporous filter patch allowing air exchange, but precluding the entrance of contaminants. The bags with wood chips were autoclaved at 121 C for 45 min, cooled, and inoculated with the spawn from either

a mutant or wild strain of *P. chrysosporium.* The bags were heat-sealed, mixed by shaking, and placed in a 37 C constant temperature chamber for a period of 10, 20, or 30 days. During the fungal growth period, the bags were hand-shaken every other day in order to prevent clumping of the substrate by mycelial knitting.

Chemical analysis

Holocellulose, klason lignin, and extractive content of untreated, ammonium-treated, and fungus-degraded wood were determined using Technical Association of the Pulp and Paper Industry (TAPPI) Standards T-222-59-70, T-222os-71, and T-1200-75, respectively. The 1% alkali solubility of decayed wood chips was determined using the American Society for Testing and Materials (1980) method D1109-56. The percentage weight loss of fungus-degraded wood chips was based on the oven-dry weight of wood and comparisons were made between treated and untreated samples (control and ammonia treated samples). Kjeldahl nitrogen content of red oak was determined following the standard Association of Official Analytical Chemist.

Experimental design and analysis

Experiment I.—The objective of this experiment was to determine if ammonia treatment of red oak chips affected the lignin-degrading properties of a mutant and a wild strain of *P. chrysosporium.* The experiment was designed as a (3 × 2 × 4) factorial with four replications per treatment. The factors considered were three levels of ammonia treatment (control, low, and high nitrogen content based on oven-dry weight of wood chips), fungal type (wild and mutant), and four incubation times (0, 10, 20, and 30 days). Chip weight loss, 1% alkali solubility, extractive content, klason lignin, hemicellulose, and cellulose content were determined for each treatment.

Experiment II.—The objective of this experiment was to determine if any differences occurred in the fungal degradation rate of lignin between ammonia treatment and ammonia nitrate additions to wood chips degraded with a wild and mutant strain of *P. chrysosporium.* Similar procedures were followed in that ammonium nitrate was added directly to the polypropylene bags at a low level (0.95% nitrogen based on oven-dry weight of wood chips) and a high level (2.97%) to simulate conditions used in Experiment I. Glucose, lysine, pH, and moisture content adjustments were made on wood chips prior to incubation studies. A (3 × 2 × 2 × 4) factorial design was used (3 levels of nitrogen additives × 2 nitrogen sources × 2 fungal strains × 4 incubation periods). Data were analyzed using the Minitab (Ryan et al. 1986) and Statistical Analysis System (SAS 1983). Mean separation was determined by the Student-Newman-Keuls (SNK) procedure.

RESULTS

Liquid ammonia treatments

Physical changes were observed to occur in the red oak wood chips during liquid ammonia treatment trials. Red oak wood chips treated at the 2.97% N level became red in color and soft, and were easy to defiberize. However, the red color soon vanished and the chips became dry, brittle, and brown in color shortly after the bomb was opened and the liquid ammonia vaporized into the air. This color

TABLE 1. *Chemical composition of untreated, low pressure and high pressure liquid ammonia pretreated red oak wood chips.*

Treatment	Nitrogen[1,2] content (%)	Klason lignin (%)	Holocellulose content (%)	Alcohol/benzene extractive (%)	1% NaOH extractive
Untreated	0.27 a[3]	21.17 a	81.67 a	8.79 a	26.57 a
Low pressure	0.95 b	22.56 a	80.84 a	9.03 a	27.32 a
High pressure	2.87 c	28.96 b	70.78 b	20.22 b	38.34 b

[1] Means are the average of four replicates.
[2] Nitrogen, alcohol/benzene and 1% NaOH extractives content are based on the oven-dry weight of red oak wood chips. Klason lignin and holocellulose content are based on alcohol/benzene extractive free oven dry red oak.
[3] Means with the same letter within the same column are not significantly different ($P \leq 0.05$).

change could be attributed to the chromophoric amide groups introduced into the wood components.

Chemical analysis of ammonia-treated wood chips

No statistical differences ($P \leq 0.05$) in chemical composition occurred between untreated wood chips and those treated to the 0.95% N level using liquid ammonia (Table 1). However, significant differences in chemical composition occurred between nontreated chips and those chips treated with liquid ammonia at the 2.97% N level. About a 10% loss in holocellulose and an 8% increase in klason lignin content were measured. Undoubtedly the ammonia treatment rendered some of the holocellulose to behave "lignin-like" or the acid-soluble lignin condensed after treatment in the reactor.

Weight loss, 1% alkali solubility, and alcohol/benzene extractives content

The weight loss and the 1% alkali solubility content of ammonia and ammonium nitrate supplemented red oak wood chips biodegraded by either a wild and mutant strain of *P. chrysosporium* for 10-day incubation intervals are summarized in Tables 2 and 3. Results showed a progressive increase in weight loss and alkali solubility as incubation time increased for both fungal strains. However, ammonia

TABLE 2. *Weight loss (%) of ammonia and ammonium nitrate supplemented red oak chips which were biodegraded by either a wild or mutant strain of* P. chrysosporium *for 10-day incubation periods.*

Incubation time (days)	Weight loss (%)				
	Control	Ammonia		Ammonium nitrate	
	0.27% N	0.95% N	2.87% N	0.95% N	2.87% N
	Wild-type strain of *P. chrysosporium*				
0	0.00 Aa[1,2]	0.00 Aa	0.00 Aa	0.00 Aa	0.00 Aa
10	2.89 Ba	2.32 Ba	0.32 Ab	2.15 Ba	0.35 Ab
20	5.36 Ca	6.78 Cb	0.34 Ac	6.43 Cb	0.79 Bc
30	7.45 Da	10.56 Db	0.89 Bc	9.89 Dd	0.82 Bc
	Mutant strain of *P. chrysosporium*				
0	0.00 Aa	0.00 Aa	0.00 Aa	0.00 Aa	0.00 Aa
10	2.56 Ba	2.54 Ba	0.12 Ab	3.56 Bc	2.57 Ba
20	4.59 Ca	5.89 Cb	0.25 Ac	5.78 Cb	3.62 Ca
30	6.32 Da	8.02 Db	0.48 Bc	7.12 Db	7.96 Dd

[1] Weight loss (%) was based on oven-dry weight of wood.
[2] Each measurement is an average of 4 replicates. Means with the same capitol letter in a column and means with the same small letter in a row indicates that no significant difference was observed at the 0.05% level of probability (Student-Newman-Keuls test).

TABLE 3. *1% NaOH extractives content (%) of ammonia and ammonium nitrate supplemented red oak chips which were biodegraded by either a wild-type or mutant strain of* P. chrysosporium *for 10-day incubation periods.*

Incubation time (days)	1% NaOH extractives content (%)				
	Control	Ammonia		Ammonium nitrate	
	0.27% N	0.95% N	2.87% N	0.95% N	2.87% N
	Wild strain of *P. chrysosporium*				
0	26.57 Aa[1,2]	27.32 Aa	38.34 Ab	26.57 Aa	26.57 Aa
10	29.57 Ba	37.32 Bb	37.39 Bb	39.00 Bb	27.56 Bc
20	35.23 Ca	40.52 Cb	38.00 Ac	39.58 Bb	28.02 Bd
30	39.43 Da	45.00 Db	38.56 Aa	43.00 Cb	28.32 Bc
	Mutant strain of *P. chrysosporium*				
0	26.57 Aa	27.32 Aa	38.34 Ab	26.57 Aa	26.57 Aa
10	27.57 Aa	30.23 Bb	38.02 Ac	27.57 Ba	30.26 Bb
20	30.42 Ba	31.53 Ba	38.92 Ac	33.65 Cb	33.75 Cb
30	31.48 Ba	33.23 Cb	37.92 Ac	35.26 Db	38.74 Dc

[1] One percent NaOH extractive content (%) was based on the oven-dry weight of wood.
[2] Each measurement is an average of 4 replicates. Means with the same capitol letter in a column and means with the same small letter in a row indicates that no significant difference was observed at the 0.05% level of probability (Student-Newman-Keuls test).

and ammonium nitrate treatment at the 2.87% N level resulted in a reduction in biodegradation activity by the wild-type *P. chrysosporium* strain. Similar observations were observed for the mutant strain; however, this occurred only for wood chips treated with ammonia at the 2.87% N level. Significantly higher weight losses were observed for those wood chips treated to the 0.95% N level and biodegraded with the wild and mutant strains of fungi compared to controls.

No significant differences ($P \leq 0.05$) in alkali solubility were measured for wood chips pretreated with ammonia at the 2.87% N level and biodegraded with the mutant and wild-type strain of *P. chrysosporium.* However, statistical differences were observed for those wood chips treated with nitrogen at the 0.95% level. Higher alkali solubility contents were measured as fungal incubation time increased. Similar trends were observed in alcohol/benzene extractives content for fungus-degraded ammonia and ammonium nitrate supplemented red oak chips (Table 4).

Klason lignin content

The klason lignin content decreased from about 21% to 18% as fungal incubation time increased to 30 days for control samples using both a wild and mutant strain of *P. chrysosporium* (Table 5). Wood chips treated with liquid ammonia to the 0.95% N level and degraded exhibited a decrease in klason lignin (17%) using both fungal strains. Wood chips treated with liquid ammonia (2.97% N level), and degraded exhibited an increase in klason lignin content, and this was attributed to the ammonia-holocellulose reaction that rendered the holocellulose derivative insoluble in 72% H_2SO_4. However, this trend was not observed for those wood chips treated with ammonium nitrate and biodegraded with the wild-type and mutant strains of *P. chrysosporium.* In fact, a progressive loss in lignin content was measured for the mutant strain of *P. chrysosporium,* which was supplemented with ammonium nitrate at the 2.97% N level and fungally degraded for 30 days. These results showed that the liquid ammonia treatment of red oak chips at the

TABLE 4. *Alcohol/benzene extractives content (%) of ammonia and ammonium nitrate supplemented red oak chips which were biodegraded by either a wild or mutant strain of* P. chrysosporium *for 10-day incubation periods.*

Incubation time (days)	Alcohol/benzene extractives content (%)				
	Control	Ammonia		Ammonium nitrate	
	0.27% N	0.95% N	2.87% N	0.95% N	2.87% N
	Wild-type strain of *P. chrysosporium*				
0	8.79 Aa[1,2]	9.03 Aa	20.22 Ab	8.59 Aa	8.59 Aa
10	9.10 Aa	10.65 Ab	20.15 Ac	9.23 Aa	8.96 Aa
20	13.45 Ba	13.70 Ba	20.87 Ab	14.26 Ba	9.15 Ac
30	14.26 Ba	14.50 Ba	20.56 Ac	15.58 Cb	9.23 Ad
	Mutant strain of *P. chrysosporium*				
0	8.59 Aa	9.03 Aa	20.22 Ab	8.59 Aa	8.59 Aa
10	9.54 Aa	10.53 Bb	19.89 Ac	9.75 Ba	10.32 Bb
20	11.23 Ba	11.53 Ba	20.72 Ab	12.34 Ca	13.18 Ca
30	14.65 Ca	16.75 Cb	21.05 Ac	17.48 Db	15.27 Db

[1] Alcohol/benzene extractive content (%) was based on the oven-dry weight of wood.
[2] Each measurement is an average of 4 replicates. Means with the same capitol letter in a column and means with the same small letter in a row indicates that no significant difference was observed at the 0.05% level of probability (Student-Newman-Keuls test).

2.97% N level inhibited the growth of the wild-type and mutant strains of *P. chrysosporium.*

Holocellulose content

The holocellulose content of the liquid ammonia pretreated and ammonium nitrate supplemented red oak chips biodegraded by either a wild or mutant strain of *P. chrysosporium* for 10-day incubation periods is summarized in Table 6. A loss in holocellulose content was observed for untreated wood chips and those wood chips treated with both liquid ammonia and ammonium nitrate to the 0.95% N level and degraded with the wild-strain of *P. chrysosporium.* No reduc-

TABLE 5. *Lignin content (%) of ammonia and ammonium nitrate supplemented red oak chips which were biodegraded by either a wild-type or mutant strain of* P. chrysosporium *for 10-day incubation periods.*

Incubation time (days)	Lignin content (%)				
	Control	Ammonia		Ammonium nitrate	
	0.27% N	0.95% N	2.87% N	0.95% N	2.87% N
	Wild-type strain of *P. chrysosporium*				
0	21.17 Aa[1,2]	22.56 Aa	28.96 Ab	21.17 Aa	21.17 Aa
10	20.75 Aa	21.36 Aa	28.40 Ab	19.43 Aa	20.95 Aa
20	18.56 Ba	17.89 Ba	28.54 Ac	18.67 Ba	19.71 Ab
30	18.43 Bb	16.43 Ca	27.96 Ac	17.01 Ca	20.45 Ad
	Mutant strain of *P. chrysosporium*				
0	21.17 Aa	22.56 Aa	28.96 Ab	21.17 Aa	21.17 Aa
10	20.98 Aa	20.78 Aa	28.56 Ab	19.46 Aa	19.93 Aa
20	19.43 Ba	18.63 Ba	28.98 Ac	17.61 Bb	17.65 Bb
30	18.96 Ca	17.96 Cb	28.75 Ac	16.32 Cb	16.32 Cb

[1] Lignin content (%) was based on weight of oven-dry alcohol/benzene extractive free wood.
[2] Each measurement is an average of 4 replicates. Means with the same capitol letter in a column and means with the same small letter in a row indicates that no significant difference was observed at the 0.05% level of probability (Student-Newman-Keuls test).

TABLE 6. *Holocellulose content (%) of ammonia and ammonium nitrate supplemented red oak chips which were biodegraded by either a wild or mutant strain of* P. chrysosporium *for 10-day incubation periods.*

Incubation time (days)	Holocellulose content (%)				
	Control	Ammonia		Ammonium nitrate	
	0.27% N	0.95% N	2.87% N	0.95% N	2.87% N
	Wild strain of *P. chrysosporium*				
0	81.67 Aa[1,2]	80.84 Aa	70.78 Ab	81.67 Aa	81.67 Aa
10	80.19 Ac	79.56 Aa	71.90 Ab	80.26 Aa	81.52 Aa
20	78.85 Ba	76.62 Ba	70.25 Ac	77.24 Ba	80.54 Ab
30	77.47 Ba	74.25 Cb	71.45 Ac	75.43 Cb	80.95 Ac
	Mutant strain of *P. chrysosporium*				
0	81.67 Aa	80.84 Aa	70.78 Ab	81.67 Aa	81.67 Aa
10	82.36 Aa	82.45 Ba	71.28 Ab	84.28 Ba	81.80 Ac
20	83.28 Ba	84.67 Cb	70.90 Ac	84.50 Bb	82.05 Ba
30	84.56 Ba	86.78 Db	71.32 Ac	86.90 Cb	84.25 Ca

[1] Holocellulose content (%) was based on oven-dry alcohol/benzene extractive free wood.
[2] Each measurement is an average of 4 replicates. Means with the same capitol letter in a column and means with the same small letter in a row indicates that no significant difference was observed at the 0.05% level of probability (Student-Newman-Keuls test).

tions in holocellulose content were observed for wood chips treated with liquid ammonia or ammonium nitrate at the 2.87% N level as fungal incubation time increased.

With the exception of those wood chips treated with liquid ammonia at the 2.87% N level, no reductions in holocellulose content occurred using the mutant strain as incubation time increased. In fact, a progressive increase in holocellulose content was measured as incubation time increased. Apparently holocellulose was only slightly degraded using the mutant strain as compared to the wild strain.

DISCUSSION

Ammonia treatment of red oak chips

A chemical analysis of the ammonia-treated chips showed clearly that significant changes in chemical composition occurred for those wood chips treated to the 2.87% N level. Earlier studies indicated that under certain conditions an ammonolysis reaction can occur between ammonia and the uronic acid components in wood causing bond cleavage in the hemicellulose (Wang et al. 1964). The decomposition of hemicellulose would account for the loss observed in this study. The degraded by-products were insoluble in 72% H_2SO_4 as shown by the marked increase in klason lignin content analysis. An increase in both alcohol/benzene content and 1% alkali solubility was observed for the pretreated wood chips.

An explanation as to why liquid ammonia treatments at the 2.87% N level inhibited the growth of the wild-type and mutant strains of *P. chrysosporium* is not clear. It has been known for some time that anhydrous liquid ammonia could be used to pretreat lignocellulose material to enhance its *in vitro* digestibility. For example, Waiss and his co-workers (1972) treated rice straw with liquid ammonia to obtain a product containing about 1.3% nitrogen, and the *in vitro* digestibility of the treated straw was increased to about 62%. Chou (1987) in a similar study utilized ammonia under supercritical conditions (100–250 C) to pretreat ligno-

cellulose material and reportedly reached near theoretical conversion of cellulose to sugars.

Only a few researchers reported on the inhibiting effects ammonia had on wood fungal degradation. Chang (1987) reported liquid ammonia treatments to decrease the digestibility of alpha cellulose. He attributed the loss in digestibility to imide formation during ammonia treatment at high temperatures.

Biodegradation of red oak chips with a mutant and wild strain of P. chrysosporium

A comparison of the biodegradation rates of red oak using the mutant and wild-type strain of *P. chrysosporium* showed that significantly higher ($P \leq 0.05$) weight losses were obtained for those wood chips treated with ammonia and ammonium nitrate at the 0.95% N level compared to controls. Weight loss increased as incubation time increased; however, for the mutant strain, only slight reductions in weight loss were observed for those wood chips treated with either liquid ammonia or ammonium nitrate at the 2.87% N level. For the mutant-type strain, significantly higher weight losses were observed for ammonium nitrate treated wood chips at the 2.87% N level. Only slight reductions in weight loss were measured for wood chips treated with ammonia at the 2.87% N level.

Chemical analysis of the biodegraded wood chips showed no statistical differences ($P \leq 0.05$) in lignin content for all treatments after the 10-day incubation period. As the incubation period increased beyond 10 days, significantly lower lignin contents were measured. Statistically lower lignin contents were measured for wood chips treated with ammonia and ammonium nitrate at the 0.95 N level using both fungal strains. No clear trends could be established in lignin content reductions for both types of nitrogen treatments. The lowest lignin content levels were measured for those wood chips treated with ammonia nitrate at the 0.95% and 2.87% N levels and biodegraded with the mutant-type strain after 30 days of incubation.

Holocellulose content of wood chips treated with liquid ammonia or ammonium nitrate at the 2.87% N level decreased with an increase in incubation time using the wild-type strain of fungus. However, an increase in holocellulose content was measured for a number of test conditions with the mutant strain. Clearly, the mutant strain should produce a biodegraded wood chip rich in holocellulose. These biodegraded chips should produce a thermomechanical pulp with higher yields compared to untreated wood chips. Earlier studies showed that pulp yields were positively correlated to the holocellulose content of biodegraded wood (Oriaran 1989). On the basis of these observations, the mutant strain appears to have decided advantages over the wild strain.

CONCLUSIONS

The following conclusions can be drawn from this study:

1. Liquid ammonia treatments of red oak wood chips to the 0.95% N level appeared to enhance fungal degradation using both the wild and mutant strain of *P. chrysosporium,* whereas liquid ammonia treatments to the 2.87% N level inhibited the growth of both the wild and mutant strain of *P. chrysosporium.* Ammonia nitrate treatments to the 2.87% N level inhibited only the growth of the wild strain.

2. With the exception of wood chips treated with liquid ammonia at the 0.95% N level, the mutant strain appeared to biodegrade wood chips at a faster rate than did the wild-type strain. The holocellulose content for mutant degraded wood was higher than that of the wild-type strain of degraded wood as fungal incubation time increased; whereas, the klason lignin content for mutant degraded wood was lower than that of the wild-type strain of degraded wood as incubation time increased.
3. The results obtained from this study indicate that the mutant strain of *P. chrysosporium* would have decided pulping advantages to that of the wild strain. In addition, low nitrogen additions to wood chips should be considered in order to increase the biodegradation rate of wood for future biopulping studies.

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