Effect of fungal pretreatment on strength and optical properties of softwood and hardwood kraft pulps

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ABSTRACT *Douglas-fir and red-alder kraft pulps were treated with the fungus* Phanerochaete chrysosporium *Burds. under low and high nitrogen conditions for 14 days with periodic oxygen flushing. Significant improvement in the physicomechanical properties of the handsheets was observed for both Douglas-fir and red-alder kraft pulps. The magnitude of increase in the strength properties depended on pulp species and nitrogen level. For the Douglas-fir, the greatest increase in strength properties was for the pulp exposed to fungus under low-nitrogen conditions. Red alder showed the opposite trend, with the greatest increases observed for pulp exposed to fungus under high-nitrogen conditions. No significant change in pulp brightness or opacity was observed for either wood species.*

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Recent concerns about potential dioxin production during chemical bleaching of pulp have stimulated extensive research into alternative bleaching strategies. Although there are other chemical bleaching methods (*1*), the potential of biobleaching also has been explored (*2*). A variety of microbes appear to be capable of decomposing lignin degradation products, but only a few of the Basidiomycotina, specifically those capable of causing white rot, have been explored to any extent. White-rot fungi have varying capabilities to degrade lignin, and several methods of incorporating these organisms into the pulping process are being explored (*2*).

Exposure of hardwood kraft pulp to *Trametes versicolor* produced modest improvements in brightness while reducing pulp kappa number (*3*). The white-rot fungus showed no such effect in softwood pulps, however, confirming results in previous studies. On the other hand, a later study suggests that significant brightness improvements can be achieved when the fungus-treated pulp is subjected to an additional alkali-extraction procedure (*4*). Furthermore, fungal treatment has been shown to improve the mechanical properties of paper made from the colonized pulp (*5*).

Softwood pulps represent an important resource in North America. Proposed dioxin limitations in mill effluent would dramatically alter bleaching strategies for many mills, suggesting the need to reevaluate biological bleaching of softwood pulps.

Results and discussion

Two unbleached kraft pulps—one produced commercially using Douglas-fir and the other produced in a laboratory-scale reactor using red alder—were inoculated with the fungus *P. chrysosporium* and incubated for 14 days. The inoculum contained either a high-nitrogen growth medium or a low-nitrogen growth medium. Handsheets prepared from the incubated pulps were evaluated, and the results are shown in **Tables I and II** for the Douglas-fir and red-alder pulps, respectively.

The pH of the filtrate from the fungus-treated pulps was lower than that of filtrate from the nontreated control pulps. The treated pulps incubated under high-nitrogen conditions had a slightly lower final pH than the pulps incubated under low-nitrogen conditions. Glucose concentration for the fungus-treated pulps decreased substantially, particularly for the pulps incubated under high-nitrogen conditions. However, glucose in the media was never depleted. The presence of excess glucose, particularly in a non-nitrogen-limiting medium, should substantially increase mycelial growth, which might subsequently improve pulp strength.

Measured yields in fungus-treated pulps were higher than 100%, possibly reflecting the inability to separate fungal mycelia from the pulp fibers. Lignin content, as measured by kappa number, was relatively unchanged for both wood pulp species and nitrogen conditions, except for Douglas-fir

I. Strength and optical properties of Douglas-fir kraft pulp exposed to *P. chrysosporium* for 14 days under two nitrogen regimes*

		Fungus treated pulps			
	Untreated control	Low nitrogen medium		High nitrogen medium	
Glucose, g/L	2	1.09		0.34	
Final pH	5	4.5		4.4	
Freeness, mL CSF	748	718		682	
Yield, %	100.1	103.5		110.3	
Kappa number	32.2	29.7	(-7.8%)	33.1	(2.8%)
Density, g/cm³	0.35	0.38		0.45	
Burst index, kPa·m²/g	0.34	0.68	(100%)	0.43	(26.5%)
Tear index, mN·m²/g	11.70	18.60	(58.9%)	7.5	(-35.9%)
Tensile strength, kg/15 mm	0.83	1.36	(63.9%)	1.06	(27.7%)
Breaking length, km	0.90	1.57	(74.4%)	1.47	(63.3%)
Stretch, %	0.50	0.40	(-20%)	0.40	(-20%)
Fold	0.5	3.2	(540%)	0.8	(60%)
Brightness, %	26.52	25.87	(-2.5%)	25.78	(-2.8%)
Opacity, %	99.50	99.61	(0.1%)	99.48	(-0.02%)

*Handsheets were prepared from unbeaten pulp after fungal treatment. Data represent the average of six measurements. Values in parentheses represent percent change in property from nontreated control pulp.

II. Strength and optical properties of red-alder kraft pulp exposed to *P. chrysosporium* for 14 days under two nitrogen regimes*

		Fungus treated pulps			
	Untreated control	Low nitrogen medium		High nitrogen medium	
Glucose, g/L	2	0.976		0.082	
Final pH	5	4.4		4.2	
Freeness, mL CSF	633	607		461	
Yield, %	99.9	104.9		111.9	
Kappa number	22.1	22.1	(0%)	23.3	(5.4%)
Density, g/cm³	0.43	0.44		0.50	
Burst index, kPa·m²/g	0.23	0.42	(82.6%)	0.63	(174.0%)
Tear index, mN·m²/g	6.20	7.20	(16.1%)	8.40	(35.5%)
Tensile strength, kg/15 mm	1.30	1.71	(31.5%)	1.95	(50%)
Breaking length, km	1.45	1.93	(33%)	2.30	(58.6%)
Stretch, %	1.10	1.40	(27.3%)	1.10	(0%)
Fold	0.5	2.0	(300%)	1.0	(100%)
Brightness, %	37.72	37.38	(-0.9%)	37.72	(0%)
Opacity, %	99.87	99.73	(-0.1%)	99.69	(-0.2%)

*Handsheets were prepared from unbeaten pulp after fungal treatment. Data represent the average of six measurements. Values in parentheses represent percent change in property from nontreated control pulp.

pulp under low-nitrogen conditions, where a decrease of almost 8% was observed. Low nitrogen levels have been reported to stimulate secondary metabolite production by *P. chrysosporium* (*6*).

Fungal treatment of Douglas-fir kraft pulp with *P. chrysosporium* under low-nitrogen conditions improved burst factor, tear index, tensile strength, breaking length, fold, and opacity, while brightness and stretch decreased. Similarly, fungus-treated Douglas-fir pulp under high-nitrogen conditions showed an increase in burst index, tensile strength, breaking length, and fold, while brightness and opacity decreased by an amount similar to that found under low-nitrogen conditions. However, tear index for the softwood pulp decreased significantly under high-nitrogen conditions.

Red-alder kraft pulp treated with the fungus under low-nitrogen conditions also showed an increase in burst index, tear index, tensile strength, breaking length, stretch, and fold. Brightness and opacity were either unaffected or showed a marginal decrease under both nitrogen regimes. Mechanical properties for the hardwood pulp showed greater improvement under high-nitrogen conditions, with the exception of fold and stretch.

One of the objectives of this study was to evaluate the enhancement of pulp brightness by fungal treatment. However, the hardwood pulps showed no change in brightness, while the softwood pulps showed a marginal reduction in brightness. This loss in brightness occurred even in the Douglas-fir pulp that showed a reduction in kappa number following treatment under low-nitrogen conditions.

The fungus-treated pulps in this study were not subjected to any alkali-extraction treatments. Recent studies (*4*) suggest that alkali extraction of softwood kraft pulp treated with *T. versicolor* enhanced lignin removal and subsequently increased pulp brightness. The treatment also increased pulp responsiveness to bleachability by a DED sequence (chlorine dioxide-alkali extraction-chlorine dioxide). It may be the case that lignin degradation is not the only factor involved in biobleaching. Xylanases are known to enhance delignification, presumably by liberating lignin from its complex with hemicelluloses (*7*).

Physicomechanical and optical properties of handsheets made from fungus-treated pulp depend on several factors, including fungal species, fungal strain, and treatment duration (*8*). Fungal treatment of both hardwood and softwood kraft pulps in our study improved the strength properties of the handsheets. However, the magnitude of the effect depended on pulp species and nitrogen levels.

The greatest increases in strength properties for Douglas-fir pulp were observed under low-nitrogen conditions, whereas red alder showed an opposite trend, with strength increasing under high-nitrogen conditions. These differences may reflect variations in the amount of fungal biomass, which may in turn affect strength properties. Alternatively, nutrient regimes may alter enzyme production, resulting in differential fiber attack and modification that could possibly affect subsequent fiber interactions in the pulp.

The nature of the observed differences in the mechanical properties of the fungus-treated pulps for the two wood species under the two nitrogen concentrations is unclear. Nevertheless, the results underscore the need to optimize fungus treatments for a specific application.

Conclusion

Treatment of red-alder and Douglas-fir kraft pulps with a white-rot fungus produced no significant improvements in brightness, suggesting that biobleaching with the chosen fungus does not reduce the need for chlorine bleaching. Fungal treatment did, however, produce substantial improvements in several paper strength properties. While the reasons for this effect are unclear, it is apparent that fungal treatments may represent one method for enhancing the physicomechanical properties of weak pulps. The effects noted here suggest that further fundamental studies are required to examine the physical and chemical basis for the observed enhancement of these mechanical properties.

Experimental

Fungus

The fungus used in this study was *Phanerochaete chrysosporium* Burds. (BKMF-1767) maintained on 1% malt/agar slants.

Pulp

Unbleached, commercially produced Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] kraft pulp was obtained from a local pulp mill and stored in plastic bags in a cold room at 4°C until needed. Red alder (*Alnus rubra* Bong.) kraft pulp was produced in a laboratory-scale reactor.

Inoculum preparation

Phanerochaete chrysosporium was aseptically transferred to 1% malt agar in petri plates and incubated at 28°C for 7-10 days. Two culture media, containing low and high nitrogen levels, were employed in this study. The media were a slight modification of that described in the literature (*9*). In addition to the basal medium III and thiamine, each medium contained three times the trace-element concentration and was buffered at pH 5.0 with 20m*M* 2,2-dimethylsuccinic acid. The nitrogen levels employed were 2.6m*M* and 26m*M*, supplied as equimolar amounts of l-asparagine and ammonium nitrate (*10*). Discs (1-cm diam.) were cut from the growing edge of the fungal mycelia and then transferred to a 250-mL Erlenmeyer flask containing 100 mL of sterile, high-nitrogen growth medium. The inoculated flasks were incubated for seven days, after which they were sterile filtered through a Buchner funnel containing Whatman No. 1 filter paper. The mycelia were washed three times with sterile deionized water and aseptically transferred to a sterile blender containing either low- or high-nitrogen medium. The contents were blended for 30 s.

Pulp inoculation and incubation

The unbleached kraft pulp was washed three times with deionized water, and about 5 g (o.d. basis) was added into sterile, 1-L Erlenmeyer flasks. The flasks were fitted with a rubber bung containing two glass-tube ports to enable flushing with oxygen (*11*). Each glass tube was plugged with cotton wool. The flasks and their contents were autoclaved at 121°C for 45 min. Then, 200 mL of low- or high-nitrogen growth media containing the blended mycelia was added to kraft pulp in the 1-L Erlenmeyer flasks, and the rubber bung ports were connected to a 10-cm-long latex rubber tubing that enabled the flasks to be closed with clamps.

The flasks were incubated in stationary culture at 28°C for three days. On the fourth day, each flask was flushed for 45 min with oxygen (*11*). Flasks were then incubated at 35°C and flushed with 100% oxygen on every third day. The higher temperature employed in the second incubation period more closely approximates the temperature optimum of the test fungus.

Fourteen days after inoculation, the contents of each flask were filtered through Whatman No. 1 filter paper. The filtrate was further purified through a 0.2-μm cellulose acetate membrane to remove fungal hyphae and spores. The filtrate was used to determine the final pH and glucose content of the residual growth media. The pulp was washed three times with deionized water and its yield was determined. After being dispersed in a blender for 30 s, the pulp was made into handsheets according to TAPPI Test Method T 205 om-81. Handsheets were compared with those made from pulp that was not exposed to fungus.

Analytical techniques

The initial and final pH of the growth media were determined. The final glucose content of the residual growth media was determined using a YSI 23A glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, Ohio).

Determination of physicomechanical and optical properties

The handsheets were conditioned at a controlled temperature (20°C) and humidity (50% RH) according to TAPPI Method T 402 om-88. Optical and mechanical properties were measured as specified in TAPPI Methods T 205 om-88, T 220 om-88, T 227 om-85, T 403 om-88, T 404 om-87, T 410 om-88, T 411 om-88, T 414 om-88, T 425 om-86, T 494 om-88, and T 511 om-88.□

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