PARTIAL DELIGNIFICATION OF UNBLEACHED KRAFT PULP WITH LIGNINOLYTIC FUNGI

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SUMMARY

Unbleached kraft pulp is partially delignified on incubation under specified conditions with ligninolytic fungi, thereby decreasing requirements for bleaching chemicals. Studies with Phanerochaete chrysosporium demonstrated important effects of nutrient nitrogen and molecular oxygen concentrations. Possible approaches to rate enhancement are suggested.

INTRODUCTION

Wood pulp produced in the kraft ("sulfate") process generally contains 5 to 8% by weight of residual, modified lignin, which gives the pulp a characteristic brown color. This residual "kraft" lignin is removed commercially by bleaching with chlorine and chlorine oxides. Chlorinated products derived from the kraft lignin during these bleaching procedures have been shown recently to be mutagenic (Ander et al., 1977), and they obviously represent a waste treatment problem because of both their toxicity and their dark color, which resists classical biological treatments. Alternative methods are needed for bleaching the pulp.

Metabolism of kraft lignins to CO_2 by a ligninolytic fungus was demonstrated with kraft-^{14}C-lignins prepared from labeled synthetic materials (Lundquist et al., 1977). The kraft lignin was metabolized more rapidly, in fact, than the original lignin. This finding suggested that the fungus examined, Phanerochaete chrysosporium, or other white-rot (lignin-degrading, wood-rotting) fungi might be used to bleach kraft pulps. Other work in our laboratory has identified parameters important in lignin metabolism by fungi (Kirk et al., 1978; Yang et al., 1979).

This note reports the results of preliminary studies of biobleaching with P. chrysosporium, and offers some suggestions for improving rate and selectivity.

METHODS

Experimental kraft pulps were prepared from southern pines, primarily Pinus taeda. Residual lignin in pulps was estimated from permanganate consumption by use of a standard method (TAPPI, 1976a), and is reported as "kappa number," equal to about 6x the weight percent of kraft lignin in the pulp. The pulps used here had kappa numbers of 24 and 28, indicating kraft lignin contents of approximately 4 and 5% respectively. Pulp viscosity was determined by a standard method (TAPPI, 1976b).

Experiments were done in 125-ml Erlenmeyer flasks. Each flask contained basal medium made of minerals, trace metals and vitamins (Kirk et al., 1978), pulp (~150 mg dry weight), buffer (except in one experiment noted, 0.01M o-phthalate) and a nitrogen source (unless otherwise stated, equimolar L-asparagine and NH_4NO_3, 0.2% N on a dry pulp basis). Glucose (33% of dry pulp) was added to some cultures.

Basal medium (+ glucose) was filter-sterilized in 10x concentration and added to the other components, which had been autoclaved (15 min., 121° C). Inoculation was with a spore suspension (Kirk et al., 1978) of P. chrysosporium 6251 (ATCC No. 34540). Final culture volume was 10 ml/flask. Incubation was without agitation at 39° C and 70 to 80% relative humidity.

Following incubation, the pulp with included mycelium was harvested by filtration, stirred for 1 h in 50 ml of 1 N NaOH, filtered and washed to neutrality with water, dried at 105° C, and then weighed and analyzed. All data are values obtained with at least 5 pooled replicate cultures. Controls were incubated without fungus and the pulps treated in the same way.

RESULTS

Various experiments demonstrated that the kappa number could be reduced by 50 to 75% in 6 to 8 days. Longer treatments resulted in greater reductions, but were not used for gathering the data reported here. During incubation, the pulp became substantially lighter in

color. Up to 50% of the cellulose was also depleted in 7 days, but this could be retarded by addition of glucose or any of several sugar sources such as malt extract, cane molasses, corn syrup, or starch.

Delignification was suppressed in cultures containing more nutrient nitrogen; in 7 days, reductions in kappa numbers were from 28 to 24 in 2.0% N, and 28 to 14 in 0.2% N. Several crude nitrogen sources, including malt extract, soybean grits, peptone, and casamino acids, gave delignification results similar to those with the defined source.

Delignification was greatly enhanced by incubating cultures under 100% O_2 instead of air; kappa numbers were reduced from 24 to 7 under O_2, and from 24 to 13 under air, in 7 days.

A preliminary examination was made of the delignifying ability of three white-rot fungi in addition to P. chrysosporium. Kappa numbers (originally 28) after treatment for 7 days with the fungi were: P. chrysosporium 11, Coriolus versicolor 16, Gloeoporus dichrous 21, and Lentinus edodes 24. The additional fungi were cultured at 24° C, and all were incubated in air.

Figure 1 gives the time course of decrease in kappa number in P. chrysosporium cultures containing 0.2% N and 33% glucose initially, and incubated under 100% O_2. No decrease in kappa number was observed prior to day 2. The subsequent change did not follow first order kinetics, but was biphasic: an initial rapid decrease followed after 4 days by a much slower decrease. Pulp viscosity also decreased in a biphasic pattern: from 15 to 9 kPa·s between 1.5 and 3 days after inoculation, then much more slowly to 6 kPa·s by day 8. At the time viscosity loss started, the added glucose had been decreased from 33% of the pulp (27mM) to approximately 17% (14mM); by day 5, the added glucose was gone. Loss in weight of the pulp occurred after the third day, and reached 12% by day 8.

Evaluation of chlorine consumption in a standard "CEDED" bleaching procedure to a brightness of 85 (See TAPPI, 1963) showed a decrease of 27% for a fungus-treated pulp sample in which the kappa number had been reduced from 24 to 17% (29% decrease).

DISCUSSION

These results demonstrate that ligninolytic fungi can partially delignify kraft pulp. The observed concomitant reduction in the amount of chlorine required for subsequent chemical bleaching was expected, because chlorine requirements generally parallel kappa number. "Biobleaching" is impractically slow as described here, but there are possibilities for rate enhancement. Also, cell-free systems might result from further understanding of lignin metabolism and production of the ligninolytic system.

Results from experiments with synthetic ^{14}C-lignins (Kirk et al., 1978) and with a representative lignocellulosic (Yang et al., 1979) are paralleled by the observations here of the accelerating effect of oxygen and depressing effect of nutrient nitrogen, and in the lag period before degradation began. Investigations with the synthetic lignins have shown recently that lignin metabolism is faster in cultures maintained under an atmosphere of 40 to 60% O_2 than under air or 100% O_2 (unpublished), an effect not examined with the kraft pulps. Further study of oxygenation of the pulp during biobleaching is an obvious approach to rate enhancement. A second observation made with the ^{14}C-synthetic lignins is that lignin metabolism occurs only after nutrient nitrogen has been depleted and a subsequent lag phase has passed (Keyser et al., 1978). This is probably responsible for the 2-day lag seen here before decrease in kappa number began (Fig. 1). Further work has shown that addition of nutrient nitrogen delays appearance of the ligninolytic system, or suppresses it if it is already present (Keyser et al., 1978). This accounts for the deleterious effect of high nitrogen levels observed here, and suggests that mutants derepressed for the nitrogen control would not exhibit such a pronounced lag phase. Such mutants might also be used with higher nitrogen levels, favoring rapid growth and more rapid delignification. Presence of mycelium in the pulp might enhance certain properties. At the low nitrogen levels employed here, however, mycelium was a negligible component of the pulp.

The biphasic pattern of fungal delignification suggests that a portion (≅ 50%) of the kraft lignin in the pulp used here differed from the remaining lignin in some way; biodegradability, accessibility, or attachment to the carbohydrates are possibilities. Time required to remove the more susceptible portion of the lignin was less than 2 days after delignification began.

The pulp was partly depolymerized in the experiments reported here despite the addition of glucose, which did, however, retard pulp weight loss. The rapid initial viscosity loss between the second and third days (Fig. 1) might have reflected hemicellulose depolymerization. The slower subsequent decrease suggests that cellulose depolymerization also occurred, despite the presence (until day 5) of the repressor glucose (cf. Eriksson and Hamp, 1978). Ligninolytic mutants of this fungus lacking celluase activity have been reported (Ander and Eriksson, 1976).

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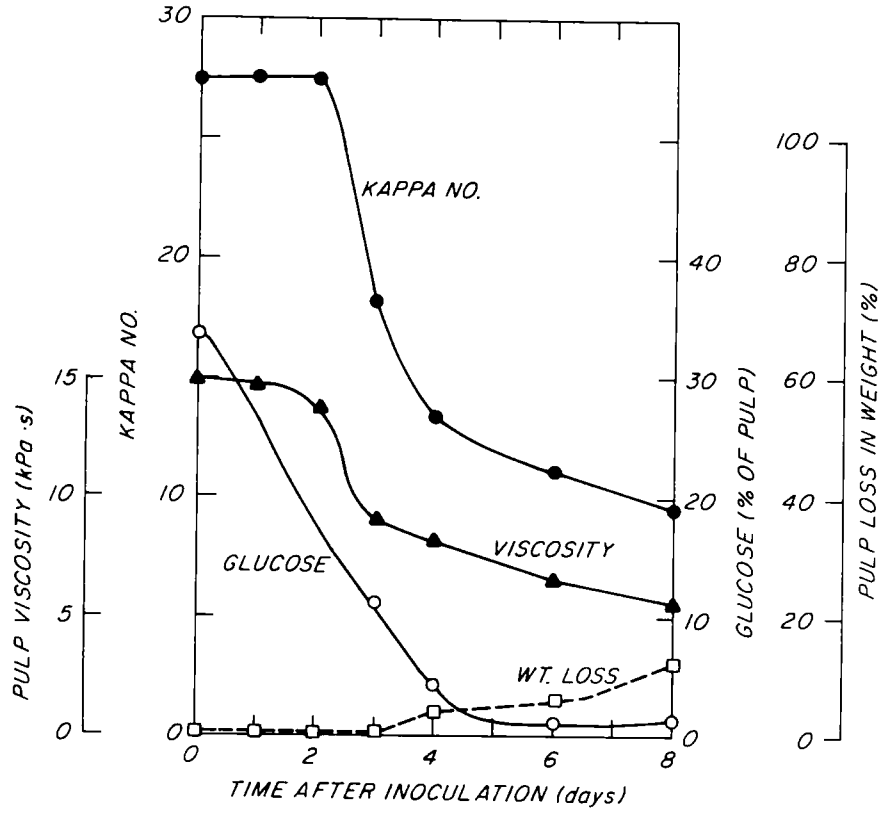

Figure 1. Changes in kappa number, viscosity and weight of kraft pulp, and concentration of added glucose, during incubation with Phanerochaete chrysosporium. Cultures initially contained 150 mg of pulp, basal medium with 0.2% nutrient nitrogen and 33% glucose (dry pulp bases), and 0.01 M 2,2-dimethylsuccinate as buffer (pH 4.5). Incubation was under 100% O_2. (M 148 305)

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