Environmental compatibility of effluents of aspen biomechanical pulps

Marguerite Sykes

ABSTRACT: *Biopulping, the fungal treatment of wood chips prior to mechanical refining, is an experimental pulping method that conserves energy and produces paper with enhanced physical properties. This study examines the toxicity and biochemical oxygen demand (BOD) of effluents from pulps incubated with either* Phanerochaete chrysosporium *or* Ceriporiopsis subvermispora, *two of the most effective fungi screened for use in biopulping. Effluents from fungus-treated pulps were substantially less toxic to aquatic life and had slightly higher BOD than effluents from untreated pulp. These findings suggest that biopulping is environmentally compatible.*

KEYWORDS: Biological tests, BOD, chemical oxygen demand, ecology, effluent treatment, effluents, mechanical pulps, pollution, Populus, toxicity.

Biopulping is an experimental pulping method that conserves energy and produces paper with enhanced physical properties *(1, 2)*. The environmental impact of this experimental process has not yet been determined, although there is a clear need for research in this area. Fungal metabolites or other biopulping by-products could substantially alter the waste load for mills switching from refiner mechanical pulping (RMP) to biopulping. This study examines the levels of biochemical oxygen demand (BOD), chemical oxygen demand (COD), and toxicity to aquatic life in effluents produced from refiner pulping of aspen chips with and without fungal pretreatment. The results were compared in an effort to assess the environmental compatibility of biopulping processes.

Screening studies have identified *Phanerochaete chrysosporium* and *Ceriporiopsis subvermispora* as two of the most effective fungi for use in biopulping. Both fungi are natural wood-decay fungi with no known toxic or pathogenic properties. Aspen chips were treated with either of these fungi prior to refining, and untreated effluents from these pulps were compared with effluent from conventionally refined aspen chips.

Sykes is a forest products technologist with the USDA Forest Service, Forest Products Laboratory, One Gifford Pinchot Drive, Madison, WI 53705-2398.

Background

High-yield mechanical pulping processes release organic materials (sugars, low-molecular-weight lignins, extractives) from wood, and these materials appear in the effluent stream *(3)*. BOD is a measure of the oxygen required to oxidize organic materials through the action of microorganisms. Untreated effluent streams from pulping contain varying levels of BOD, depending on wood species, pulping method, and pulp yield *(4, 5)*. A high level of BOD is detrimental to aquatic life forms because it forces them to compete for dissolved oxygen in lakes and streams.

Environmental guidelines restrict the composition of effluents from all stages of pulping and papermaking. To meet these guidelines for discharge into fresh-water sources, mills are equipped with treatment facilities to assure compliance with regulations set by the Environmental Protection Agency (EPA) for levels of BOD, total suspended solids (TSS), color, and pH. These regulations are based on an evaluation of the raw waste loads produced by various types of pulp production and on the best available treatment that is economically achievable *(6)*. State environmental agencies can impose additional regulations on maximum levels of individual compounds frequently occurring in effluents. In addition, toxicity of the effluent to aquatic life is monitored to assure continuous water-quality compliance. This is done by testing the effluent or downstream water for both acute and chronic toxicity *(7, 8)*.

I. Description of pulps*

Raw	Raw chips
Control	Control pulp
$Control_n$	Nutrient-enriched control pulp
C	*C. subvermispora* biopulp
C_n	Nutrient-enriched *C. subvermispora* biopulp
P_n	Nutrient-enriched *P. chrysosporium* biopulp

*All pulps were sterilized in an autoclave and incubated for 4 weeks, with the exception of the raw chips.

II. Effect of nutrient addition on effluent oxygen demand (raw aspen chips)

	Oxygen demand, g/kg o.d. pulp	
Raw aspen	BOD_5	COD
Without nutrients	18	40
With nutrients	38	68

Mill effluents could be monitored for as many as 100 potentially toxic constituents. However, even low levels of several benign compounds could interact synergistically to produce a toxic total effect *(9)*. Because the composition of biopulping effluents is not known, a general toxicity test was selected as the most efficient and appropriate way to evaluate these effluents.

The Microtox®* method of analysis is used as a rapid screening method for evaluating acute toxicity of untreated pulp mill effluents *(8)*. As effluents become more complex with the introduction of new pulping and bleaching technologies, a toxicity-based regulatory approach has been suggested as an alternative to the chemical-specific approach now in place for effluents *(9)*.

Conventional treatment for mill effluents includes a combination of a primary sedimentation treatment for removal of suspended solids and a secondary biological treatment *(10, 11)*. A legitimate concern about new pulping techniques is whether the treatment systems already in place will accommodate the waste loads and substances produced by the new process. Ideally, a new process would be more environmentally compatible than existing processes.

*The use of trade or firm names is for reader information and does not imply endorsement of any product or service by the U.S. Department of Agriculture.

Results and discussion

Effluent analysis

Effluents from aspen pulps with and without fungal pretreatment were evaluated for levels of BOD_5 (the amount of dissolved oxygen consumed by biological degradation of organic constituents during five days), COD, and toxicity. Because organic constituents in effluents oxidize slowly by biological means at 20°C, BOD usually is measured for a 5-day interval, when oxidation is approximately 65% complete. COD is almost always higher than BOD because more organic matter can be oxidized chemically than biologically.

Whereas chemical analyses measure the concentration of specific chemical compounds, toxicity tests provide an index of the bio-availability of toxic compounds *(12)* and measure the response to the combined effect of all component chemicals. Toxicity tests more accurately reflect the environmental impact of effluent components on receiving waters than do chemical-specific assessments.

Table I describes the pulps used in this study. The raw aspen chips (with no autoclaving, incubation, or added nutrients) were refined to provide a baseline for comparing biopulp runs and their respective sterile, incubated controls. The composition of the raw-chip effluent depended solely on the soluble organic material from the chip furnish.

Toxicity

Resin and fatty acid constituents of wood are extracted during mechanical refining, and these are a major contributor to effluent toxicity *(13, 14)*. The effluent from the RMP of raw aspen chips was the most toxic, as measured by the Microtox test method. Biopulping with either *P. chrysosporium* or *C. subvermispora* for 4 weeks significantly decreased the toxicity of aspen effluents, as seen in **Fig. 1**. Results for effluents of *P. chrysosporium* biopulps in **Fig. 2** suggest that an incubation longer than 2 weeks is required to decrease toxicity under the conditions used here. (Toxicity units in Fig. 1 are lower than in Fig. 2 because the data for Fig. 1 were obtained from a dilute effluent of 70 L/kg o.d. chips, compared with a concentration of 57 L/kg o.d. chips in Fig. 2.)

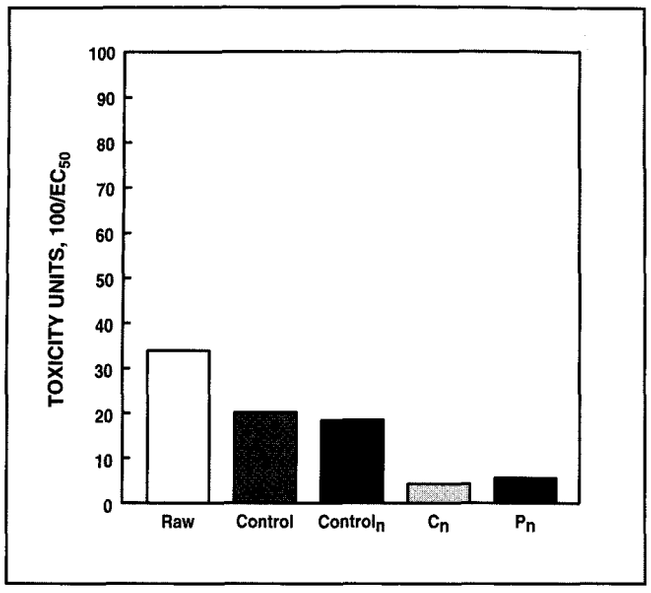
Because Microtox measures only one form of short-term acute toxicity, these results need to be confirmed with other bioassays that measure chronic, long-term toxicity. National Pollution Discharge Elimination System (NPDES) permits are based on acute and chronic tests using various organisms, including fish and daphnia, to test effluent toxicity.

Biological treatment is not a new concept in the paper industry. Mill treatment systems have been using bacterial action to decrease the BOD level in effluents for many years. In the MyCoR (mycelial color removal) system *(15–17)*, *P. chrysosporium* was initially used to remove color, but it was later discovered that this fungus also decreased effluent toxicity. The decrease of toxicity observed for the fungus-treated biopulps is consistent with biotreatment of mill effluents with MyCoR.

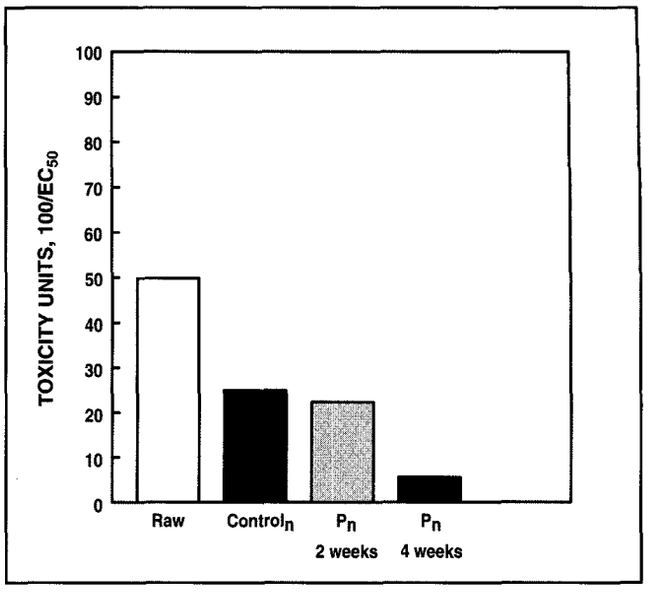
Nutrients and oxygen demand

Two issues affecting the optimization of biopulping are the addition of nutrient medium and sterilization of wood chips prior to fungal inoculation. The elimination of either requirement would be advantageous both economically and environmentally.

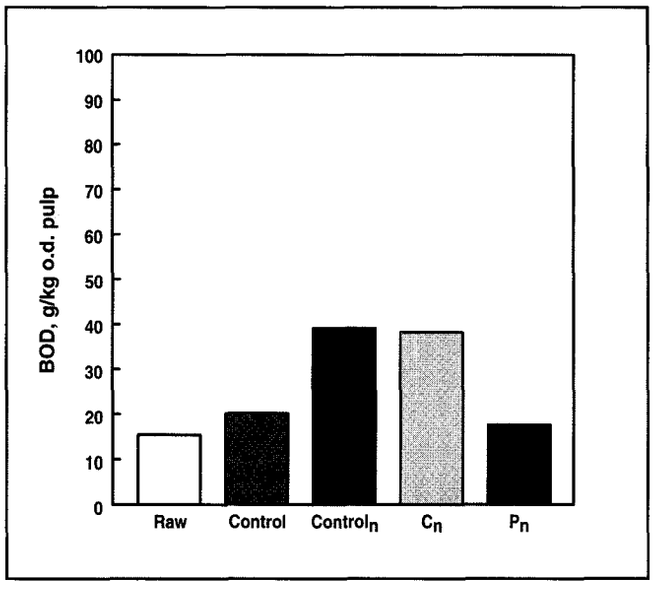
1. Toxicity of aspen pulp effluents with and without fungal pretreatment. All samples were sterilized and incubated for 4 weeks (except for raw chips). Effluent concentrations diluted to 70 L/kg o.d. chips.



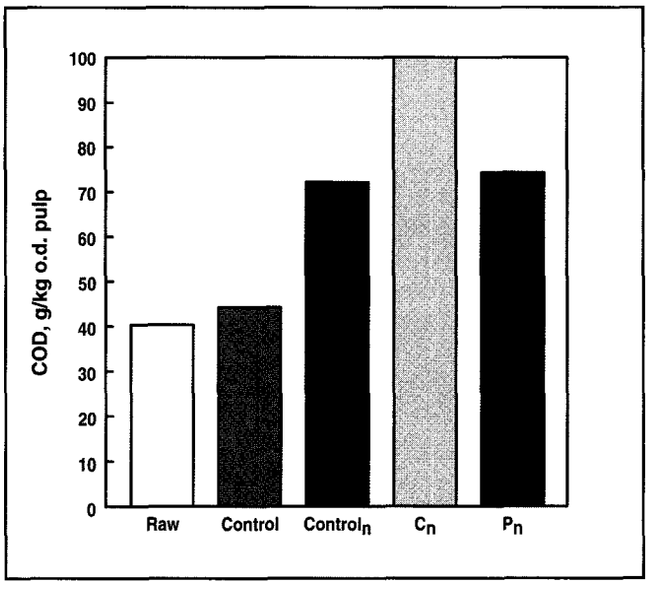
2. Toxicity of aspen pulp effluents with 2-week and 4-week *P. chrysosporium* pretreatment. The control sample was sterilized and incubated with nutrients for 4 weeks. Effluent concentrations diluted to 57 L/kg o.d. chips.



3. Biochemical oxygen demand of aspen pulp effluents with and without fungal pretreatment. All samples were sterilized and incubated for 4 weeks (except for raw chips).



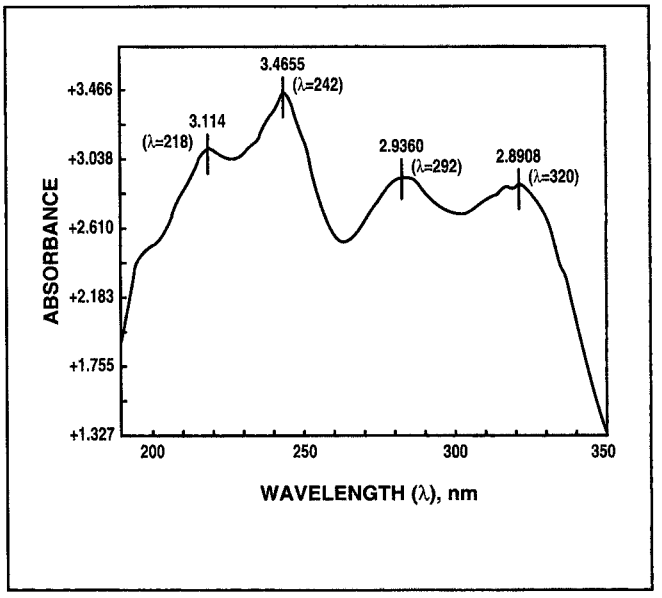
4. Chemical oxygen demand of aspen pulps with and without fungal pretreatment. All samples were sterilized and incubated for 4 weeks (except for raw chips).



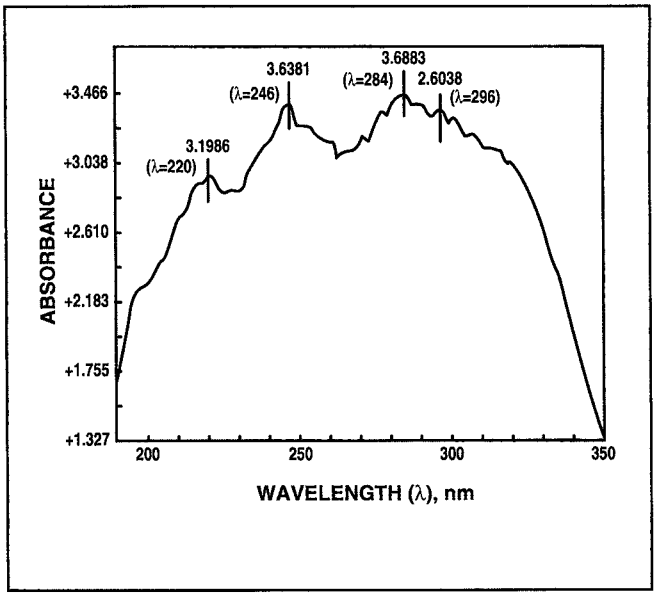
Addition of nutrients to the chips promotes vigorous fungal growth *(1, 18)*. As expected, we found that the nutrient medium contributed substantially to the BOD and COD load in effluents. **Table II** shows that when nutrients were added to raw wood chips, BOD_5 increased by approximately 20 g/kg o.d. chips and COD increased by approximately 28 g/kg o.d. chips.

P. chrysosporium. A 4-week fungal treatment with *P. chrysosporium* decreased the BOD_5 level to that of the control without added nutrients, as seen in **Fig. 3**. However, **Fig. 4** shows that COD following 4 weeks of fungal treatment with *P. chrysosporium* remained the same as that of the nutrient-enriched control. The decrease in BOD for *P. chrysosporium* implies that added nutrients were consumed by the fungus during the 4-week incubation period. The unaltered COD level might indicate a balance between consumption of nutrients and release of delignification products during fungal

5. UV spectrum of effluent from first refiner pass for RMP (sterilized control chips incubated with nutrients for 4 weeks)



6. UV spectrum of effluent from first refiner pass for *P. chrysosporium* biopulp (sterilized, fungus-treated chips incubated with nutrients for 4 weeks)



treatment. Regardless, COD levels in the effluents of both of the nutrient-enriched biopulps were higher than for the raw chips.

Figures 5 and 6 are ultraviolet (UV) spectra of effluents from the control pulp and the *P. chrysosporium* biopulp, respectively. The biopulp effluent displayed an absorption peak not present in the control effluent, indicating the release of lignin-related compounds by selective fungal action on the chips *(19, 20)*.

C. subvermispora. When aspen chips were pretreated with *C. subvermispora*, the BOD_5 content of the effluent remained at the same level as that of the nutrient-enriched control aspen, as seen in Fig. 3. COD increased, as seen in Fig. 4, probably because of the release of lignin-related compounds by the fungal pretreatment.

BOD and COD levels in the effluents from chips treated with *C. subvermispora* were substantially higher than for the raw chips. These results suggest that an important factor in process optimization, from the viewpoint of effluent quality, is minimizing the addition of nutrients to the amount required to assure vigorous fungal growth. Excess nutrients were expressed in the effluent and increased the constituent level of BOD and COD. Apparently, vigorous fungal growth also produces delignification products that can be reflected in BOD or COD content of the effluent.

The high oxygen demand of components contained within effluents from aspen chips pretreated with *C. subvermispora* suggests that this fungus was not consuming the nutrients added to stimulate growth. Control chips and fungally inoculated chips were compared with and without the addition of nutrient-enriched media to confirm whether the presence of nutrients affected performance of *C. subvermispora*. We found that *C. subvermispora* grew vigorously even without added nutrients. **Figures 7 and 8** summarize the oxygen demand of effluents from these runs. Elimination of the nutrient medium was reflected in the lowered levels of BOD_5 and COD in the effluent. While the release of delignification products contributed to the COD level, the exclusion of added nutrients reduced both BOD_5 and COD levels to a more acceptable range.

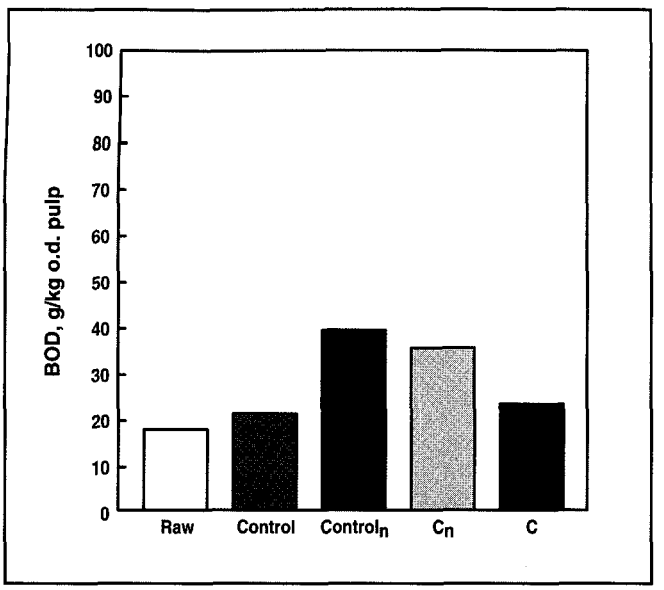
Aging of nonsterile chips

When nonsterile control and *C. subvermispora* inoculated chips were incubated for 4 weeks at 27°C with or without added nutrients, the toxicity and oxygen demand of the effluents decreased substantially compared with the effluent constituents of the raw aspen chips. (Data not shown.)

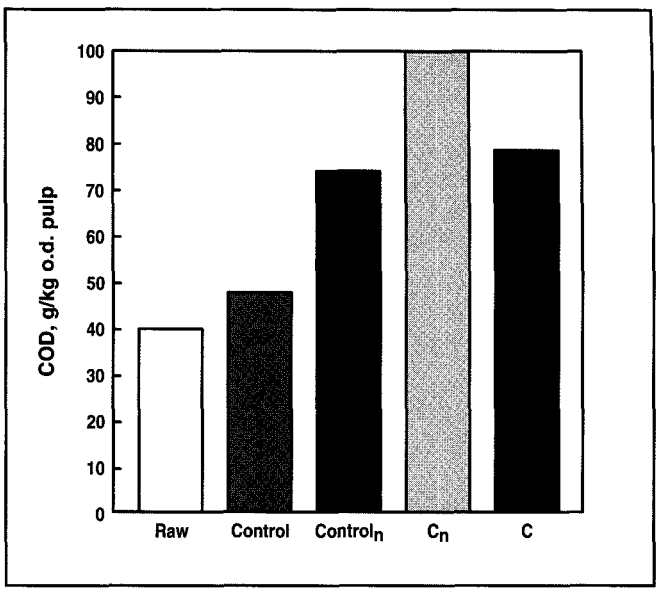
Table III illustrates that sterile chips are required for *C. subvermispora* to grow. This fungus was unable to compete with the other microorganisms that contaminated all of the nonsterile runs, essentially turning the fungus-treated runs into control replicates. The decrease both in toxicity and oxygen-demanding components compared with the raw aspen chips was unexpected. Because of the widespread contamination in the nonsterile runs, the resulting effluent data were initially discounted.

It has been suggested, however, that the decrease in toxicity observed in effluents from runs treated with a fungal inoculum and incubated for 4 weeks might also occur if the chips were just aged for 4 weeks. This suggestion is especially relevant when considering how the chip stock used in

7. Biochemical oxygen demand of aspen pulps with and without nutrients. All samples were sterilized and incubated for 4 weeks (except for raw chips).



8. Chemical oxygen demand of aspen pulps with and without nutrients. All samples were sterilized and incubated for 4 weeks (except for raw chips).



these experiments was prepared. (Experimental conditions are described at the end of this report.)

It appears that the microorganisms associated with the nonsterile aspen chips reduce effluent toxicity to a similar extent as the white-rot fungi used to pretreat chips in biopulping. Toxicity is reduced in naturally aged chips through microbial consumption of fatty acids *(21)*. However, white-rot fungi—when permitted to colonize under sterile conditions—selectively affect the wood chips. This selectivity is demonstrated not only by the reduction of refining energy and enhanced paper strength properties, but also by the increase of delignification products in the effluents. Because all biopulping runs and their accompanying controls were sterilized prior to incubation, the toxicity reduction observed in treated runs can only be attributed to the fungal inoculum.

Waste load

Effluent data from optimized runs, where nutrients were eliminated, (Figs. 7 and 8), demonstrated that fungal pretreatment did not substantially alter the BOD load when compared with aspen RMP controls. Toxicity was unaltered by optimization, so these data are not shown. Although these observations were based on limited data, duplicate runs showed good agreement. Data presented in Figs. 1–4 are averages of four runs each, except for the two-week P_n value in Fig. 2, which is an average of two runs. Additional single runs are compared in Figs. 7 and 8, which accounts for the slight variation in control values compared with Figs. 3 and 4. The BOD_5 loads obtained for raw aspen (16–18 g/kg o.d. pulp) are within the range of published values [17.4 kg/ton of o.d. pulp (17.4 g/kg)] for the waste load of a generic-species groundwood pulp *(5)*. Thus we can assume that effluent-treatment systems currently in place should be able to accommodate effluents from biopulping.

It is well established that waste loads increase as pulp yield decreases, temperature increases, or pulping chemicals are added *(6)*. Consequently, the waste load produced by biopulping should be considerably lower and more benign than effluents currently produced by commercial chemithermomechanical pulp (CTMP) mills. Published values for BOD waste load—based on the EPA survey for estab-

III. Oxygen demand and toxicity of nonsterile chips after treatment with *C. subvermispora*

Pulp[a]	*BOD_5, g/kg o.d. pulp*	*COD, g/kg o.d. pulp*	*EPA toxicity units,*[b] *$100/EC_{50}$*
Raw chips	18	40	33
Control			
No nutrients	10	30	5
Nutrient enriched	12	33	9
Fungus treated			
No nutrients	10	30	6
Nutrient enriched	11	35	4

[a]All pulps incubated 4 weeks at 27°C, except for raw chips
[b]EC_{50} is a measure of toxicity (see the "Toxicity" subheading in the "Experimental" section).

lishing effluent guidelines—are 28 kg/ton of o.d. pulp (28 g/kg) for TMP and 95 kg/ton of o.d. pulp (95 g/kg) for CTMP *(5)*. One commercial CTMP mill reported a BOD_5 load of 45 kg/air-dry ton, or approximately 52 g/kg o.d. chips, for aspen CTMP *(22)*. *C. subvermispora* biopulp effluents contained approximately 24 g/kg, and *P. chrysosporium* effluents contained 20 g/kg at a comparable yield.

Concluding remarks

Fungal pretreatment of aspen chips with either *Phanerochaete chrysosporium* or *Ceriporiopsis subvermispora* during biopulping decreased effluent toxicity. Effluents from RMP of raw aspen chips were significantly more toxic to aquatic organisms than biopulp effluents, as measured by Microtox.

The biochemical oxygen demand (BOD_5) for effluents from fungus-treated pulps were slightly higher than for RMP of raw chips. The chemical oxygen demand (COD) for effluents from fungus-treated pulps were considerably higher than for RMP of raw chips, probably because of the release of lignin-related products.

Addition of nutrients to the control chips increased BOD and COD in the effluent, as expected. BOD decreased after 4-week incubation with nutrient-enriched *P. chrysosporium*, while BOD remained unchanged following incubation with nutrient-enriched *C. subvermispora*. COD remained unchanged after 4-week incubation with nutrient-enriched *P. chrysosporium*, while COD increased in effluents from chips pretreated for 4 weeks with nutrient-enriched *C. subvermispora*.

Different white-rot fungi used for pulp pretreatment have differing nutrient requirements. Therefore, an important factor in process optimization is establishing the minimum amount of nutrient required to assure fungal growth. The BOD and COD data indicate that *C. subvermispora* does not require added nutrients.

Sterile chips are essential for colonization of the two white-rot fungi that we tested. Therefore, some method of chip sterilization is a process requirement for biopulping with these fungi.

Aspen biopulp effluents were less toxic, as measured by Microtox, and probably contained considerably lower BOD_5 and COD levels than aspen TMP or CTMP at comparable yields.

Experimental

Sample preparation

A uniform, controlled supply of chip stock was obtained from freshly cut aspen *(Populus tremuloides)* logs, which were chipped, mixed, and immediately frozen and stored in polyethylene bags. Chips were sterilized by autoclaving and then inoculated with either *P. chrysosporium* or *C. subvermispora* using pre-inoculated "seed" chips prepared for each fungus. Inoculated chip samples were then incubated for 4 weeks at a temperature appropriate for the optimum growth of each fungus (39°C for *P. chrysosporium* and 27.5°C for *C. subvermispora*). Control chips, run simultaneously with treated batches, were set up identically, excluding the fungal inoculum.

All samples (approximately 1.4 kg o.d. chips) were fiberized in a 305-mm single-rotating-disk atmospheric refiner at the time of harvest *(1)*. Additional "raw" chips—consisting of nonsterile, nonincubated aspen chips with and without the addition of nutrient medium—also were fiberized and effluent samples collected.

Approximately 50 L of 86°C water was used to refine 1 kg of chips. After we established that approximately 85% of the constituent BOD and COD was released during the first pass through the refiner, all effluents sampled were from first-pass slurries. This is consistent with the findings of Wong and others, who observed that most pollutants from thermomechanical pulp were released in the first stage of refining *(23)*. The resulting pulp slurries were mixed thoroughly before a 20-L representative sample from each slurry was removed. Samples were filtered on Buchner funnels to separate fibers from effluents. Filtrates were cooled in glass containers to 4°C and were submitted for analyses.

BOD_5 and COD analyses

Effluent samples were submitted to the Wisconsin State Laboratory of Hygiene (Madison, WI) for analysis. The BOD_5 and COD testing followed the accepted Standard Methods for Examination of Water and Wastewater procedures *(24)*. The BOD_5 analyses used the EPA-405.1 test method; COD level was analyzed according to EPA-410.1 titrimetric test method.

Toxicity

Effluent samples were submitted to the National Council for Air and Stream Improvement (NCASI) in Anacortes, WA, for Microtox testing. Microtox is a bioassay that uses luminescent bacteria for the test species. These organisms emit light as one endpoint of their metabolism. Light decreases when the bacteria are exposed to substances that interfere with their metabolism. The decrease in light emitted is proportional to the concentration of the toxic material. A linear relationship of sample dilutions and emitted light permits extrapolation of EC_{50}, a measure of toxicity. An EC_{50} is the effective concentration of a sample causing a 50% decrease in light output using the standard Microtox procedure (15°C, 15-min exposure) *(25)*. The EC_{50} measure of toxicity—which is comparable with the LC_{50} value obtained in conventional acute testing using salmon or Ceriodaphnia—was converted to EPA toxicity units for plotting data. EPA toxicity units were obtained by dividing 100 by the EC_{50} values.

Ultraviolet spectra

The UV spectra in the 190-nm to 350-nm range were measured using a 1-cm quartz cuvette in a Hewlett Packard scanning diode array spectrophotometer (Model 8452A). Samples were glass-fiber filtered prior to spectra measurement. The diode

configuration results in spectra increments of 2 nm.

Literature cited

1. Myers, G. C., Leatham, G. F., Wegner, T. H., *et al.*, *Tappi J.* 71(5): 105(1988).
2. Setliff, E. C., Marton, R., Granzow, S. G., *et al.*, *Tappi J.* 73(8): 141(1990).
3. Eriksson, K-E., *Tappi J.* 68(7): 46(1985).
4. Rogers, I. H., Davis, J. C., Kruzynski, G. M., *et al.*, *Tappi* 58(7): 136(1975).
5. Springer, A., Industrial Environmental Control for Pulp and Paper, John Wiley & Sons, New York, 1986, p. 22.
6. Springer, A. and Petterson, R. C., Environmental Control in Pulp and Paper Chemistry and Technology (J.P. Casey, Ed.), John Wiley & Sons, New York, 1980, Vol. 2, p. 1191.
7. Walden, C. and Howard, T. E., *Tappi* 60(1): 122(1977).
8. Firth, B. and Backman, C., *Tappi J.* 75(12): 169(1990).
9. Johnson, I. and Butler, R., *Paper Technol.* 32(6): 21(1991).
10. Lacey, W., in *Riegel's Handbook of Industrial Chemistry,* Van Nostrand Reinhold, New York, 1983, p. 14.
11. Norton, S., *Res. J. Water Poll. Control Fed.* 63(4): 462(1991).
12. Pulping effluents in the aquatic environment, Special Report No.89-08, National Council for Air and Stream Improvement, New York, Dec. 1989, p. 12.
13. Mutton, D. B., in *Wood Extractives and Their Significance to the Pulp and Paper Industry* (W.E. Hillis, Ed.), Academic Press, New York, 1962, p. 333.
14. Leach, J. M. and Thakore, A. N., *Tappi* 59(2): 129(1976).
15. Pelliman, J. and Joyce, T. W., *TAPPI 1990 Environmental Conference Proceedings,* TAPPI PRESS, Atlanta, p.1.
16. Campbell, A. G., Gerrard, T. W., Joyce, T. W., *et al.*, *TAPPI 1982 Research and Development Conference Proceedings,* TAPPI PRESS, Atlanta, p. 209.
17. Eaton, D. C., Chang, H-M., Joyce, T. W., *et al.*, *Tappi* 65(6): 89(1982).
18. Crueger, W. and Crueger, A., Biotechnology: A Textbook of Industrial Microbiology, Singuer Assoc., Inc., Sunderland, MA, 1984, p.49.
19. Kirk, T. K. and Chang, H-M., *Holzforschung* 28(6): 217(1974).
20. Reid, I., Abrams, G., and Pepper, J., *Can. J. Botany* 60: 2357(1982).
21. Wong, A., Breck, D., and Costantino, J., *Pulp Paper Can.* 81(3): T72(1980).
22. Multiclient report, Jaakko Poyry, Inc., Lidingo, Sweden, 1985, p.3.
23. Wong, A., Breck, D., and Costantino, J., *Pulp Paper Can.* 81(3): T72(1980).
24. *Standard Methods for the Examination of Water and Wastewater,* 17th edn., American Public Health Assoc., American Water Works, and Water Pollution Control Federation, American Public Health Assoc., Washington, D.C., 1989.
25. *Rapid Screening Method,* Microtox Information Sheet M-10, Microbics Corp., Carlsbad, CA.

This research was supported in part by the Biopulping I Consortium, which consisted of 21 pulp and paper and related companies, the University of Wisconsin Biotechnology Center (UWBC), and the Forest Products Laboratory (FPL). I thank Timothy Hall and the NCASI for Microtox testing; Masood Akhtar and Michael Attridge for their cooperation in providing required effluent samples; and T. Kent Kirk, Theodore H. Wegner, Edward Springer (all from FPL), John W. Koning, Jr. (UWBC), and Pat L. Hoekstra (Jaakko Poyry, Inc.) for valuable discussions.

Received for review Feb. 5, 1993.

Accepted May 10, 1993.

The Forest Products Laboratory is maintained in cooperation with the University of Wisconsin. This article was written and prepared by U.S. government employees on official time, and it is therefore in the public domain and not subject to copyright.