ENVIRONMENTAL ASPECTS OF BIOSULFITE PULPING

Gary M. Scott
Research Chemical Engineer
Marguerite Sykes
Res. Forest Products Technologist
Said Abubakr
Supervisory Chemical Engineer
USDA Forest Service
Forest Products Laboratory[1]
Madison, WI 53705

Masood Akhtar
Microbiologist
Michael Lentz
Research Specialist
Biotechnology Center
University of Wisconsin
Madison, WI 53706

ABSTRACT

In this study, we examined the effect of fungal pretreatment of wood chips prior to sodium- and calcium-based sulfite pulping. The pretreatment involved a 2-week incubation of loblolly pine chips with two strains (CZ-3 and L-14807 SS-3) of the white-rot fungus *Ceriporiopsis subvermispora*. Focus was on the kappa number and yield, effluent quality, and pulp bleachability after pulping. During sodium bisulfite pulping, the fungal pretreatment reduced the kappa number by 27%, with slightly lower pulp yield compared to the control. The two strains produced about the same results. However, during calcium-based sulfite pulping, strains CZ-3 and SS-3 reduced the kappa number by 48% and 21%, respectively, compared to the control, but had the same pulp yield as that of the control. Also, the CZ-3 pretreatment during calcium-based pulping reduced the effluent toxicity substantially compared to the control; BOD and COD remained unaffected. During calcium-based sulfite pulping, the brightness of the control pulp increased from 54% to 80%, whereas the brightness of the pretreated pulp (CZ-3) increased from 49% to 80%, with a comparable 4% hydrogen peroxide charge. These results clearly demonstrate that this fungal pretreatment is environmentally benign and offers much potential for sulfite pulping.

[1]The Forest Products Laboratory is maintained in coperation with the University of Wisconsin. This article was written and prepared by U.S. Government employees on official time, and it is therfore in the public domain and not subject to copyright.

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

INTRODUCTION

Increased public concern about the environment is having a rather large impact on the pulp and paper industry. The recently issued Environmental Protection Agency (EPA) regulations propose a dramatic reduction in allowable air and water discharges from chemical pulp and paper mills [1, 2]. In addition to kraft pulp mills, about 14 sulfite pulp mills are located in the United States (Table I), which also need to reduce the adverse environmental impact of their effluents. Paper-grade sulfite producers will also be required to produce totally chlorine-free bleached pulp [3, 4]. Thus, the sulfite industry must look for alternative technologies to meet EPA regulations.

Biopulping, defined as the fungal treatment of wood chips prior to pulping, may solve some of the problems associated with the traditional sulfite pulping processes. To date in our laboratory, we have focused only on the effect of fungal pretreatment of chips prior to refiner mechanical pulping. The 2-week process saved at least 30% electrical energy during refining, improved paper strength properties, and reduced the environmental impact of pulping [5, 6, 7, 8, 9]. However, a study [10] done in collaboration with the Technical University, Vienna, Austria, and a recent review [11] indicate that fungal pretreatment also offers a great advantage for magnesium-based sulfite pulping. We have further explored this approach and extended the use of fungal pretreatment for sodium- and calcium-based sulfite pulping with emphasis on reducing the environmental impact of the traditional sulfite pulping processes.

Table I. Sulfite mills in the United States [23]

Mill	Location	Production[a] (ton/day)
Ketchikan Pulp Co.	Ketchikan, AK	600
ITT Rayonier	Fernandina Beach, FL	430
Great Northern Paper Co.	Millinocket, ME	540
Finch, Pryn, and Co.	Glens Falls, NY	350
Proctor & Gamble	Mehoopany, PA	NA
James River Corp.	Camas, WA	450
Weyerhaeuser Paper Co.	Cosmopolis, WA	450
Scott Paper Co.	Everett, WA	500
ITT Rayonier	Port Angeles, WA	440
Wausau Paper Mills	Brokaw, WI	230
Cross Pointe Paper	Park Falls, WI	170
Badger Paper Mills	Peshtigo, WI	155
Georgia–Pacific Corp.	Port Edwards, WI	235
Weyerhaeuser Paper Co.	Rothschild, WI	200

[a]NA is not available.

We hypothesized that the fungal pretreatment can be beneficial in several ways. The treatment can reduce the amount of cooking time, hence the energy that is required for pulping. The chemical demand can also be lessened due to prior degradation of the lignin by the fungus. It can also reduce the amount of bleaching that is required by selectively removing lignin from the wood by the fungus. Using these criteria, the effect of the best mechanical biopulping fungus *Ceriporiopsis subvermispora* [5] was studied on kappa number and pulp yield, effluent properties, and pulp bleaching characteristics after sulfite pulping.

BACKGROUND

Pulping processes have the potential to release organic materials (sugars, lignins, and extractives) as well as spent liquor into the effluent streams. BOD is a measure of the oxygen required to oxidize organic materials through biological action. The amount of BOD depends on the type of wood, pulping process, and yield [12]. A high level of BOD is detrimental to aquatic life because it depletes the available dissolved oxygen in lakes and streams. Pulp and paper mills must meet strict environmental guidelines when discharging effluent to waterways. Treatment facilities are used to treat the effluent to comply with discharge regulations set by EPA. Additional regulations may be imposed by state agencies. These regulations typically set levels for BOD, total suspended solids (TSS), color and pH. In addition, the toxicity of the effluent to aquatic life is measured to ensure compliance with regulations. Mill effluents contain many potentially toxic substances singly and in combination. A general toxicity test is selected as an efficient comparative evaluation of effluents. The Microtox method of analysis is used as a rapid screening method for evaluating acute toxicity of untreated pulp mill effluents [13, 14, 15].

The sulfite cooking process includes several pulping processes that differ in the base used for the pulping chemicals as well as the pH of the resulting liquor. Historically, sulfite pulping has been dependent on calcium-based liquor of high acidity (pH 1–2). Several U.S. mills still rely on this technology. However, in the last half century, the more soluble bases of sodium, magnesium, and ammonium have come into use. Furthermore, the use of these bases has extended the possible pH range to less acidic conditions so that we now have bisulfite pulping at a pH of 3-5 and neutral sulfite semichemical (NSSC) at a pH of 7-9, which use a sodium base. In the study reported here, results from a sodium-based bisulfite process and a calcium-based acid sulfite process are discussed. Previous work was done with a magnesium-based sulfite cook [11].

Conventional waste water treatment for mill effluents includes a combination of a primary sedimentation treatment for removal of suspended solids and a secondary biological treatment [16, 17]. A concern of using the fungal pretreatment is the change in the waste load that could result from the new process. Ideally, the new process would be more environmentally friendly than the existing process.

EXPERIMENTAL PROCEDURE

Wood Chips

Freshly cut loblolly pine (*Pinus taeda L.*) pulpwood-size logs were obtained from the Talladega National Forest in Talladega, Alabama. The logs were debarked and chipped to a size averaging 16 mm. The chips were bagged in plastic bags and frozen until used to present the growth of contaminating microorganisms.

Fungus

Two strains (CZ-3 and L-14807 SS-3) of the best biopulping fungus, *Ceriporiopsis subvermispora*, were used based on their superior mechanical biopulping performance [20, 7]. These strains were obtained from the Center for Forest Mycology Research at the USDA Forest Service, Forest Products Laboratory, Madison, Wisconsin. Cultures were continuously maintained in cereal culture and potato dextrose agar slants. Working cultures were prepared from the stock cultures as needed and refrigerated until used. Potato dextrose agar plate cultures were inoculated from a working culture and incubated at 27°C and 65% relative humidity for 10 days.

In preparing the liquid inoculum, potato dextrose broth (4.8 g) and yeast extract (1.46 g) were added to 200 ml of distilled water and mixed well, and 100 ml of this medium was poured into two 1-L flasks. Each flask was autoclaved for 20 min at 121°C. After cooling to room temperature, each flask was inoculated with 10 plugs cut with a number 9 cork bore from a 10-day-old potato dextrose agar plate of the fungal culture. The flasks were then incubated at 27°C at 65% relative humidity for 10 days. Prior to use, the flasks containing the fungal biomass were decanted and washed with sterile distilled water to remove excess medium from the fungal biomass. The fungal biomass was then placed in distilled water and blended in a Waring blender twice for 15 s, each time at high speed; distilled water was then added to the suspension to make the total volume 100 ml (stock). About 0.86 ml of CZ-3 stock and 1.23 ml of L-14807 SS-3 stock producing 0.0005% (5 g/ton) inoculum of each strain (dry-weight basis) were diluted to 100 ml suspension, with 15 g of unsterilized corn steep liquor (0.5% on a dry weight basis) and an appropriate amount of sterilized water.

Chip Preparation and Bioreactor Inoculation

Frozen loblolly pine chips were thawed and thoroughly mixed to obtain uniform samples. Three static beds [20], each containing 1,500 g of chips (dry-weight basis), were decontaminated by using atmospheric steam for 10 min and then cooled

to room temperature. These bioreactors were inoculated with 100 ml of inoculum suspension of each fungal strain as mentioned. One noninoculated bioreactor served as control. About 55% moisture (wet-weight basis) in wood chips was maintained during fermentation. After receiving inocula, the bioreactors were shaken vigorously for uniform mixing. Each bioreactor was sealed and placed in a incubator at 27°C for 2 weeks and aerated with a specific aeration rate of 0.0227 L/L/min. At harvest, the fungus-treated and control chips were made into pin chips by processing the chips in a laboratory-scale atmospheric refiner equipped with "devil's teeth" plates. The chips were screened on a 3.2-mm mesh screen to remove fine material. The resulting chips averaged approximately 4 by 4 by 20 mm.

Sulfite Cooking

We pulped the chips in 90-ml bombs that were indirectly heated in an oil bath. The temperature was ramped from the initial 70°C to the final temperature over a period given in Table II, which also details the typical liquor conditions used. Additionally, the conditions given in Table II were varied to determine their effects on the process. For the calcium-based cooks, the liquor was obtained from a commercial source and titrated according to Tappi Test Method T 604. Results of this analysis are shown in Table III. Note that the liquor decreased in potency with time, requiring reanalysis for the later runs. The sodium-based liquor was prepared as needed from reagent grade sodium hydrogen sulfite.

Table II. Nominal cooking conditions used for sulfite pulping

	Sodium bisulfite	Calcium acid sulfite
Ovendry charge of wood (g)	10.0	10.0
Total sulfur dioxide on wood (%)	20.0	32.0
Combined sulfur dioxide on wood (%)	10.0	9.7–10.9
Liquor-to-wood ratio	4.0:1	5.9–6.8:1
Maximum temperature (°C)	161	140
Time to temperature (h)	1.5	5
Total cooking time (h)	5–6	9–10

Table III. Titration analysis of calcium-based cooking liquor

Cooks	Sulfur dioxide (%)		
	Total	Free	Combined
16–25	6.04	3.98	2.06
26–28	5.45	3.80	1.65

After charging the bombs with the wood chips and liquor, they were sealed and placed in the oil bath. The initial temperature of the oil bath was 70°C. The temperature was then ramped and held according to the specific cooking conditions. During the cook, the bombs rotated end-over-end to facilitate mixing and uniform heat transfer. No gas relief (as is commonly done in the industry) was performed during the cook.

At the conclusion of the cook, the bombs were removed from the oil bath and the reaction quenched by cooling the bomb to 90°C for 10 min in a water bath. The contents of the bombs were then disintegrated in a Waring blender for 5 min. The resulting pulp was washed over filter paper, dried, and the yield and kappa number were determined. The kappa number was determined using Tappi Test Method T 236. The spent liquor was analyzed using Tappi Test Method T 604 for residual pulping chemicals.

Effluents

Filtrates of spent pulping liquor from bomb runs and wash water were made up to 3.75 L. These filtrates were considered the effluents compared for oxygen demand and toxicity in this study.

BOD and COD analyses.

Spent liquor samples were submitted to the Wisconsin State Laboratory of Hygiene (Madison, Wisconsin) for analysis. The BOD and COD (chemical oxygen demand) testing followed the accepted *Standard Methods for Examination of Water and Wastewater* procedures [21]. The BOD analysis used the EPA-405.1 test method; COD level was analyzed according to EPA-410.1 titrimetric test method.

Toxicity.

Spent liquor samples were submitted to the National Council for Air and Stream Improvement (NCASI) in Anacortes, Washington, 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) [22]. 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 analysis. Toxicity units were obtained by dividing 100 by the EC_{50} values.

Bleaching

Pulps were bleached with conventional oxidative and reductive chemicals after chelation with 0.5% DTPA (diethylenetriaminepentaacetic acid). Oxidative bleaching was done with 4% hydrogen peroxide, 2% sodium hydroxide, 3% sodium silicate, and 0.25% magnesium sulfate. All chemical charges were based on ovendried pulp. Bleaching was carried out at 12.5% consistency and 65°C for 90 min in plastic bags. Reductive bleaching was done with 1% FAS (formamidine sulfinic acid) and 0.1% sodium hydroxide at 5% consistency and 65°C for 1 h. The pulp was blanketed with nitrogen to minimize obviation of the FAS. After bleaching, pulps were neutralized with sodium hydrogen sulfite and washed to remove residual chemicals. Handsheets were prepared; brightness and color were read according to Tappi Test Method T 524 om 86.

RESULTS AND DISCUSSION

Results of a selected number of the cooks are detailed in Table IV. The remaining cooks used in this study were completed using variables such as cooking chemicals, maximum temperature, and ramp speed and are not presented here. Several cooks were tested extensively for liquor consumption, effluent change, and bleachability of the pulp and are presented here.

Kappa Number and Yield

The objective of sulfite pulping is to remove the lignin from the wood while leaving the cellulose and hemicelluloses unreacted. However, the sulfite process tends to somewhat degrade the carbohydrate portion of the wood as the lignin reactions occur. We speculated that the fungal pretreatment either consumes some of the lignin or modifies the lignin in such a way that it is easier to remove in the subsequent pulping process. The efficiency of the pulping is measured by the yield (dry weight of pulp per dry weight of wood charged) and kappa number, a relative measurement of the residual lignin in the pulp. The goal of these cooks was to reduce the lignin sufficiently to produce a more bleachable pulp.

Table II gives the nominal cooking conditions used for both sodium bisulfite and calcium sulfite pulping; Table IV summarizes the details of selected cooks. Table V summarizes the results for the sodium bisulfite cook at a time of 5.12 h to 5.25 h. The fungally treated chips resulted in a slightly lower yield and a 27% reduction in kappa number. Both are statistically significant ($p < 0.01$ and $p < 0.0001$ for the yield and kappa, respectively). During the cook, both the yield and the kappa decreased with time. The yield decrease was due both to the dissolution of lignin and the concurrent attack on the carbohydrates. The fungal pretreatment in this case seemed to accelerate the pulping process but did not seem to change the selectivity between lignin and carbohydrates. Figure 1 shows the kappa numbers for each pulping treatment at two times. An additional 30 min of cooking reduced the kappa number of the control to the same level as that of the fungally treated chips. As the reaction continued, the differences between the control and the fungal-treated chips decreased. This could be due to the difficulty in removing the residual lignin in this pulping process. Finally, no differences were seen between the two fungal strains: both CZ-3 and SS-3 gave comparable yields and reduced kappa numbers.

Table IV. Summary of selected cooks

Treatment	Time (h)	Yield (%)	Kappa number	Cook number
Sodium-based cooks				
Control	5.25	50.6	31.5	9c
Control	5.25	49.7	30.7	10c
Control	5.25	49.5	31.6	11c
Pretreated (CZ-3)	5.25	48.5	22.1	9d
Pretreated (CZ-3)	5.12	47.6	23.1	12a
Pretreated (CZ-3)	5.25	48.1	22.7	12b
Pretreated (CZ-3)	5.12	47.8	21.8	13a
Pretreated (CZ-3)	5.12	48.8	24.5	24a
Pretreated (SS-3)	5.12	48.2	22.9	24c.
Control	5.75	49.1	22.6	13c
Pretreated (CZ-3)	5.75	47.8	17.9	24b
Pretreated (SS-3)	5.75	46.2	18.8	24d
Calcium-based cooks				
Control	9.50	47.2	26.4	17b
Control	9.50	47.1	26.2	21a
Control	9.50	47.6	28.1	25a
Control	9.50	47.7	25.7	25d
Control	9.50	48.0	26.4	26a
Control	9.50	48.2	27.7	26b
Pretreated (CZ-3)	9.50	48.0	15.6	23c
Tretreated (CZ-3)	9.50	47.8	12.9	25b
Pretreated (CZ-3)	9.50	47.8	13.8	26c
Pretreated (CZ-3)	9.50	47.2	12.6	26d
Pretreated (SS-3)	9.50	48.3	21.6	21c
Pretreated (SS-3)	9.50	47.3	20.5	25c
Control	10.00	44.9	13.5	21b
Control	10.00	44.7	14.5	23b
Pretreated (CZ-3)	10.00	45.9	11.2	23d
Pretreated (SS-3)	10.00	44.9	10.9	21d

Table V. Summary of sodium bisulfite cooks at cooking times of 5.12 h to 5.25 h

Treatment	Yield (%)	Kappa
Control	49.93±0.39	31.27±0.28
Pretreated (CZ-3)	48.16±0.21	22.84±0.98
Pretreated (SS-3)	48.20	22.90

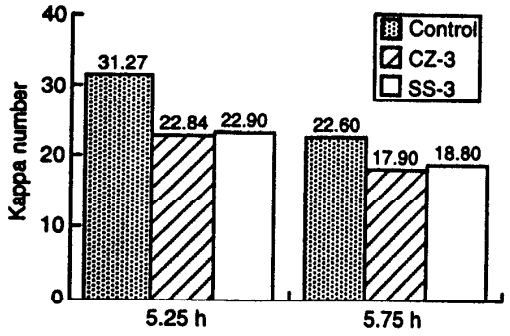

Fig. 1. Pulp kappa numbers for sodium bisulfite cooking.

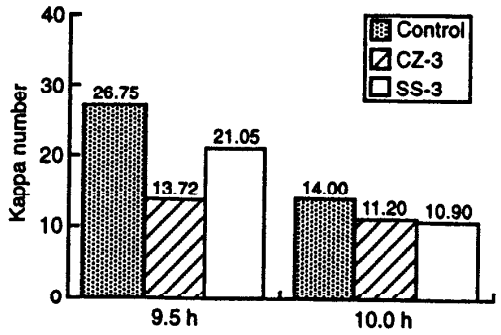

Fig. 2. Pulp kappa numbers for calcium acid sulfite cooking.

Table VI. Summary of calcium acid sulfite cooks at cooking times of 9.50 h

Treatment	Yield (%)	Kappa
Control	47.63±0.15	26.75±0.70
Pretreated (CZ-3)	47.70±0.12	13.72±1.78
Pretreated (SS-3)	47.80±0.69	21.05±0.84

Table VI summarizes the results for the acid sulfite cooks at a time of 9.50 h. Again, the fungal treatment resulted in a significant reduction in the kappa number. For strain CZ-3, the kappa number was reduced by 48% from approximately 27 to 14. The SS-3 strain reduced the kappa number by 21%. These differences are highly significant ($p < 0.0001$). In contrast to the sodium-based cooks, there were no differences in the yields between the control and the fungal-treated chips. This indicates that calcium-based sulfite pulping is more selective toward lignin degradation. Figure 2 shows the kappa number for each treatment at 9.50 h and 10.0 h. Again, an additional 30 min of cooking was required for the control to reach the same kappa number as the biotreated chips (SS-3). Also, as in the case of the sodium-based cooking, the differences between the control and the treatments were reduced to approximately 20% in kappa. Interestingly, a comparison of cooks 23d with 21b and 23b in Table IV shows the fungal treatment resulted in a yield increase. These results need to be confirmed with additional experimentation .

As a result of the timing of the experiments, the potency of the cooking liquor differed between several of the calcium-based cooks through degradation of the chemicals (Table IV). Statistical analysis of the data showed that the change in liquor did not produce statistically significant differences in the results.

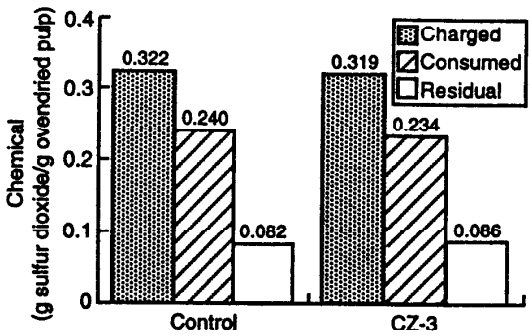

Fig. 3. Comparison of the consumption of pulping liquor by cooks.

Consumption of Cooking Liquor

In addition to reduction in the kappa number of the pulp, the possibility also exists that the fungal treatment would reduce the amount of pulping chemicals that are consumed. Figure 3 summarizes the amount of liquor charged and consumed for the control and the CZ-3 treated chips with the calcium-based cooking. Each data bar represents the average of two cooks (cooks 25a, 25b, 25c, and 25d). The same amount of cooking chemicals were consumed in each case: approximately 0.24 g sulfer dioxide/ g ovendried. pulp. Thus, the fungal pretreatment did not increase the amount of pulping chemicals needed for this process. Remember that the treatment resulted in a 48% reduction in the kappa number that will presumably make the pulp easier to bleach. This supports the theory that the fungal treatment modifies the lignin in the pulp, making lignin easier to remove in the subsequent pulping step.

Effluents

Toxicity.

The principal toxic chemicals from sulfite pulping are resin acids, unsaturated fatty acids, and lignin degradation products [18]. Loblolly pine contains extremely high levels of resins and lignin. Not only do these compounds contribute to effluent toxicity to aquatic organisms, but they inhibit pulping and subsequent bleach responses.

Fungal pretreatment substantially reduced effluent toxicity as measured by Microtox. The control pulping effluent was 17.4 toxicity units compared with 7.2 toxicity units for effluents from the biotreated samples. Table VII summarizes the test results. Previous pulping experiments of biotreated loblolly pine mechanical pulps also demonstrated reduced toxicity when compared with control effluents. Some extractives are solubilized during pulping, which contributes to both oxygen demand and toxicity of resulting effluents. We assumed that the decreased toxicity was a result of fungal metabolism during incubation that consumes some wood resins [10], thereby improving effluent quality.

Oxygen demand.

The BOD and COD contents of the biosulfite and control pulps were almost identical. The BOD value for both pulps was approximately 24 g/kg wood chips. The BOD values for both pulps were tempered by a substantial pH adjustment required to bring the pH 2.5 effluents up to the 6–8 pH range for biological testing.

The low pH of the effluents did not affect the COD analyses. However, little difference existed in the levels of COD for the two effluents: 92 g/kg chips for the control and 94 g/kg for the biotreated chips.

Bleaching

Sulfite-pulped chips from these trials were disintegrated in a Waring blender. One obvious difference between the two pulps was the large shive content of the control pulp, indicating a more complete pulping with the biotreatment. Large shives were removed to make a more homogeneous pulp. The resulting pulps were subsequently bleached with conventional nonchlorine bleach chemicals. Chromophores were introduced in the biotreated chips during incubation and pulping, which decreased the initial brightness of this pulp compared with the control. Although the initial brightness of the control pulp was 5 points greater than the corresponding biotreated pulp, the bleached brightnesses were comparable, indicating improved bleachability for the biotreated pulp. Table VIII summarizes the bleach response to 4% hydrogen peroxide. The control pulp increased from 54% brightness to 80%, a gain of 26 points; the biotreated pulp gained 31 points from an initial brightness of 49% to match the final 80% brightness of the control pulp with a comparable peroxide charge.

Table VII Toxicity measurements of liquor effluent

Cook	EC_{50}[a]	Confidence interval	Toxicity units
Control	5.74	(2.62–12.56)	17.4
Biosulfite	13.82	(10.17–18.78)	7.2

[a]Measure of toxicity.

Table VIII. Bleaching response of pulps

Treatment	Brightness (%)	Color[a] scale L*	a*	b*
Control				
Initial	54	84	2.40	9.70
4% hydrogen peroxide	80	94	-1.00	5.75
1% FAS[b]	80	94	-0.77	3.94
Biosulfite				
Initial	49	81	2.00	9.90
4% hydrogen peroxide	80	94	-0.68	5.53
1% FAS	80	94	0.33	2.75

[a]L* is black-white; a* is gren-red; b* is blue-yellow.
[b]Formamidine sulfinic acid.

In addition to brightness, pulp color is also important to the papermaker. Pulp color is evaluated by the L*, a*, b* scale that measures black-white, green-red, and blue-yellow components, respectively [19]. Brightness did not benefit from a reductive bleach step with FAS; the color, however, improved on the biotreated pulp (Table VIII). The yellow color component decreased from 9.90 for the biosulfite pulp to 2.80, and the control decreased from 9.70 to 3.90. Aklthough the pulps had a comparable bleached brightness, the biosulfite pulp appeared to be whiter because of less yellow content.

CONCLUSIONS

Results show that the fungal pretreatment of the wood had several beneficial effects on the pulping process. The fungal pretreatment significantly reduced the kappa number of the resulting pulp in both the bisulfite and acid sulfite cooks. In the case of the acid sulfite cooks, this was done without adversely affecting the yield. For the same cooking time, the pulp can be cooked to a lower kappa number. Alternatively, shorter cooking times can be used to reach the same kappa number as the control, thus increasing throughput and reducing energy consumption. We also noted that in the case of the acid sulfite process, the amount of shives in the pulp was qualitatively reduced compared to the control. This results in a higher screened yield for the biosulfite process. A slight improvement in the color properties of the biosulfite pulp was also noted.

The spent liquors from both the control and the biosulfite process were also investigated for environmental aspects. Little significant difference was found between the BOD and COD of the spent liquors. However, the biosulfite process resulted in a significantly reduced toxicity of the spent liquor.

Future work needs to be directed at accessing the effects of the biotreatment on the pulp and optimization of the process. Brightness and color stability of the resulting pulp needs to be investigated. It is expected that the significantly decreased lignin content will produce a pulp that is more resistant to color reversion. Strength and mechanical properties of the pulp also need to be explored. The fungus used in this study, *C. subvermispora,* is the best mechanical biopulping fungus discovered to date. However, it may not necessarily be the best fungus to use for biochemical processes. Thus, additional fungi need to be screened for their biochemical pulping efficacy. Finally, the process needs to be extended to additional species and different pulping chemistries, including neutral sulfite and kraft.

ACKNOWLEDGMENTS

This research was supported in part by the Biopulping II Consortium, which consisted of four pulp and paper and related companies, the University of Wisconsin Biotechnology Center, and the Forest Products Laboratory. We thank Weyerhaeuser Paper Company of Rothschild, Wisconsin, for supplying the calcium acid sulfite liquor. We also thank Timothy Hall and the NCASI for the Microtox testing and Eric Horn for the kappa number determination and liquor titrations.

LITERATURE CITED

1. Chlorine-based bleaching EPA rule to curb paper industry use. *C&EN.* November 8:5 6. 1993.

2. Federal Register, Part II Environmental Protection Agency. 40 *CFR Parts 63 and 430,* 58(241):66196. December 17, 1993.

3. Federal Register, Part II Environmental Protection Agency. J0 *CFR Parts 63 and 430,* 58(241):66120, December 17, 1993.

4. Ricketts, J.D., Considerations for the closed-cycle mill, *Tappi Journal,* 77(11):43–49 (1994).

5. Akhtar, M., Biomechanical pulping of aspen wood chips with three strains of *Ceriporiopsis subvermispora, Holzforschung,* 48:199–202 (1994).

6. Akhtar, M., Attridge, M.C., Myers, G.C., et al., Biomechanical pulping of loblolly pine with strains of the white-rot fungus *Ceriporiopsis subvermispora. Tappi Journal,* 75(2):105–109, (1992).

7. Akhtar, M., Attridge, M.C., Blanchette, R.A., et al., The white-rot fungus *Ceriporiopsis subvermispora* saves electrical energy and improves strength properties during biomechanical pulping of wood, *In* : Kuwahara, M., and Shimada, M., ed., *5th International Conference on Biotechnology in the Pulp and Paper Industry,* May 1992, Tokyo, Japan, Uni Publishers Co., 3–8,

8. Kirk, T. K., Koning, J.W., Burgess, R.R., et al., Biopulping: A glimpse of the future? Research Paper FPL–RP–523, USDA Forest Products Laboratory, Madison, WI, 1993.

9. Sykes, M., Environmental compatibility of effluents of aspen biomechanical pulps, *Tappi Journal,* 77(1): 160–164 (1994).

10. Fischer, K., Akhtar, M.,Blanchette, R.A., Burnes, et al., Reduction of resin content in wood chips during experimental biological pulping processes, *Holzforschung,* 48:285–290 (1994).

11. Messner, K., and Srebotnik, E., Biopulping: An overview of developments in an environmentally safe paper-making technology, *FEMS Microbiology Reviews,* 13:351–364 (1994).

12. Rogers, I.H., Davis, J.C., Kruzynski, G.M., et al., Fish toxicants in kraft effluents, *Tappi Journal,* 58(7):136–140 (1975).

13. Walden, C. and Howard, T.F., Toxicity of pulp and paper mill effluents, *Tappi Journal* 60(1):122–125 (1977).

14. Firth. B., and Backman, C., Comparison of microtox(r) testing with rainbow trout (acute) and *Ceriodaphnia* (chronic) bioassays in mill wastewaters. *Tappi Journal,* 73(12):169–174 (1990).

15. Johnson, I., and Butler, R., Paper-mill effluents a move to toxicity-based-consents, *Paper Technology,* 32(6):21 (1991).

16. Lacey, W., *Riegel's Handbook of Industrial Chemistry,* Van Nostrand Reinhold, New York, 1983. p. 14.

17. Norton, S.C., Pulp and paper effluent management, *Research Journal of the Water Pollution Control Bederation,* 63(4):462 –471 (1991).

18. Springer, A., *Industrial Environmental Control for Pulp & Paper,* John Wiley & Sons, New York, 1986.

19. Sharpe, P.E., and Lowe, R.W., The bleaching of colored recycled fibers, *In:* 1993 Tappi Pulping Conference Proceedings, Tappi Press. Atlanta, 1993, p. 1205.

20. Akhtar, M., Attridge, M.C., Myers. G.C., and Blanchette, R.A., Biomechanical pulping of loblolly pine chips with selected white-rot fungi. *Holzforschung,* 47(1):36–40 (1993).

21. American Public Health Association, *Standard Methods for the Examination of Water and Wastewater.* American Public Health Association, American Water Works, and Water Pollution Control Federation, Washington, DC, 1989.

22. Microbic Corp., *Rapid Screening Method.* Carlsbad, CA., Microtox Information Sheet M–10.

23. Pulp & Paper, 1994 *Lockwood-Post's Directory of the Pulp, Paper and Allied Trades,* Miller Freeman, Inc., San Francisco, 1993.