12 FungaI Treatment of Wood Chips for Chemical Pulping

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INTRODUCTION

Pulp producers have come under strong legislative pressure to reduce the emissions into water and air. Because a great put of the environmental problems were attributed to the use of chlorine in bleaching, the pulp companies had to undergo a dramatic change in their bleaching technologies. Elemental chlorine is no longer used in European Union (EU) countries and its use is being phased out in the United States. The number of companies using chlorine dioxide in ECF (elemental chlorine free) bleaching sequences in the EU is decreasing steadily. Most of the sulfite pulp is now produced totally chlorine free (TCF), and TCF bleached kraft pulp production is estimated as 15% of total pulp production. TCF pulp production is growing more slowly in the United States, and most industry observers predict that ECF will be the method of choice for the near future. However, the new bleaching chemicals—xygen, hydrogen peroxide, and ozone—have the disadvantage of being less selective than chlorine, leading to decreased strength properties at the same brightness level. Furthermore, the TCF bleached pulps contain a higher amount of lignin, resulting in increased yellowing (cf. Chapter 7 on ECF and TCF bleaching). Delignifying the pulp as much as possible during the cooking process is desired as a method of minimizing the disadvantages of the new oxygen bleaching chemicals. such as quality losses during bleaching. Extended cooking is the result of this strategy; however, this tool is limited because of heavy chromophore production at low kappa numbers.

Biotechnological processes are intrinsically friendly to the environment, and delignification is a biochemical process, basic to the earth's carbon cycle. White-rot fungi produce a very powerful delignifying enzyme system. It seems logical that the first attempts were already under way in the 1950s and 1960s to use these fungi to assist in pulp production (Lawson and Still, 1957; Reis and Libby, 1960; Kawase,

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1962). Since that time, the level of understanding of the biology of wood colonizing fungi and their enzyme systems has increased dramatically and new lignin-degrading enzymes have been found. This increased level of knowledge and the need for new pulping technologies led to a revival of the biopulping idea in the 1970s (Eriksson et al., 1976: Eriksson and Vallander, 1982; Henningsson et al., 1972). A great technological breakthrough in the use of white-rot fungi to reduce the energy consumption in mechanical pulping while improving paper strength was made by the Biopulping Consortium in Madison, Wisconsin (Akhtar et al., 1992; Otjen et al., 1987; Myers et al., 1988; cf. Chapter 10). The great potential of using these fungi to reduce the kappa number in sulfite pulping was demonstrated by Messner and co-workers (1992). The benefits of basidiomycetes for kraft pulping are still not clear and need further research. It was shown by Wall and co-workers (1994) that sap stain fungi also bring about kappa reduction in chemical pulping with very little change in yield and viscosity. Further fundamental and applied research is necessary to show how the potential of biopulping fungi to reduce the kappa number after cooking can best be converted into practical mill applications. Potentiral applications include (1) reduced bleach chemical use at the same or even improved pulp quality, (2) increased production without capital investment, and (3) removal of bottlenecks of an active alkali limited kraft mill.

This chapter provides information on the colonization of wood chips by fungi and the estimation of fungal biomass, discusses biopulping as a pretreatment of wood chips for chemical pulping not only by basidiomycetes, but also by sap-stain fungi, and outlines the achievements, the problems involved, the research needs, and the industrial potential of this biotechnology.

COLONIZATION OF WOOD CHIPS

Anatomy and Nutrition

The direct route of access into wood for all wood-colonizing fungi are the ray cells (Rayner and Boddy, 1988), which, because of their wideness, provide ample space for hyphal growth. Furthermore, the parenchyma cells of ray ducts function as storage cells, providing easily assimilated substances such as sugars, starch, or fats to the growing hyphae. Unlike the carbohydrates contained in wood cell walls, they are not lignified and their consumption does not need prior depolymerization of the lignin framework. Of utmost importance for the young growing hyphae is the high nitrogen content of the parenchyma cells. This nutritional setting also enables non-wood-decay fungi to colonize wood via the ray ducts.

In biopulping, wood chips are often supplemented with nutrients like corn steep liquor. Nevertheless, the storage nutrients still may play an important role in colonization, as even soluble compounds would not diffuse into a wood chip very deeply. It is well known from wood preservation that wood preservatives penetrate the wood for only a few millimeters in dip treatment technologies. Thus, the supplemented

nutrients will rather help to increase the fungal biomass at the surface of the wood chip, which in turn enables an even colonization of the deeper area of the chip.

Fungi able to metabolize triglycerides may densely colonize the resin ducts and almost completely remove their contents (Fischer et al., 1994). Fungi with this capability are especially interesting for their potential application to pitch problems in papermaking (cf. Chapter 18).

From the ray cells, the hyphae move into the longitudinal elements such as the tracheids. Usually not more than two hyphae are found within one tracheid. This also holds true for the center of supplemented chips in biopulping. The reason for that might be the low nitrogen content as well as the low oxygen tension. The main passage to the adjacent cells is provided by the pits. Even the bordered pits of spruce wood are penetrated easily by fungal hyphae. Many wood decay fungi are also able to penetrate the wood cell wall via bore holes and continue to grow on the other side of the wall.

As soon as the tracheids of the wood are colonized, the nutritional situation for the hyphae changes dramatically. The carbohydrates are complex polymers and are bound in a lignin matrix, whose depolymerization is a prerequisite to obtaining access to cellulose and hemicellulose. Lignin depolymerization is induced in most white-rot fungi during the idiophase, under nitrogen or carbohydrate starvation. when active fungal growth has decreased (Kirk et al., 1978). Some fungi are reported to become ligninolytic even in nitrogen-sufficient media (Moreira et al., in press). *Ceriporiopsis subvermispora* and *Dichomitus squalens,* two selective lignin-degrading fungi that are known as good biopulping organisms,. were found to belong to this group (Messner and Keller, unpublished work).

Micromorphology

The colonization of wood chips by selective white-rot fungi and the resulting micromorphological changes of the wood cell walls were studied in detail and reviewed elsewhere (Blanchette et al., in press; Messner and Srebotnik, 1994). These studies led to a better understanding of the biochemical process that causes the biopulping effect and generated the hypothesis that the agents that modify cell wall components during the first two weeks must be low molecular weight compounds (Messner and Srebotnik, 1994; Blanchette et al., in press). The nature of these compounds remains speculative and is currently the subject of intensive studies. Although valuable information was obtained from these studies, microscopic investigations can only provide insight into local events of colonization. Quantitative assessments cannot be drawn from such studies.

Quantification and Estimation of Fungal Biomass

One of the most important analytical parameters in fermentation processes is the estimation of fungal biomass. Because fungal biomass is a measure of the catalytic activity of the microbial cell at a given time, its measurement enables the control

and optimization of the process. The quantification of fungal biomass in plant biomass is a great problem because separation is impossible. Many indirect chemical methods, such as those involving the content of nucleic acid, protein, the cell carbon/phosphate ratio, polysaccharides, ATP, enzyme activities, immunological methods, and lipids and their derivatives, have been tested. All of them suffer from certain drawbacks or lead to more or less erroneous values (Gao et al., 1993; Singh et al., 1989).

The method best suited for the estimation of fungal biomass in wood chips seems to be the determination of the ergosterol content of the fungal mycelium. Ergosterol is the dominant sterol of fungal cell walls. It is produced only by fungi; it is not contained in plant membranes and, consequently, is not found in wood tissues. This method was first applied for decayed wood by Nilsson and Bjurman (1990) and was optimized by Gao and co-workers (1993). The principle steps are (1) extraction and saponification, (2) homogenization, (3) separation in petroleum ether, (4) redissolving in methanol, and (5) HPLC analyses at 282 nm (UV-detector). Wood chips were shock frozen in liquid nitrogen and ground in a hammer mill before this procedure. Cholesterol (2.0 mg/ml) was added as an internal standard after extraction to monitor losses during sample preparation (Amezeder et al., 1989).

In this work (Messner et al., unpublished results), biomass development of *C. subvermispora* CBS 347.63 was investigated during the first two weeks of the biopulping solid substrate fermentation process. Inoculation and fermentation of wood chips were done according to Akhtar and co-workers (1992).

Calibration of the Ergosterol Content A prerequisite for biomass determination in wood is the correlation of the fungal biomass, with the ergosterol content of a fungal biomass measured from a standing liquid culture. As shown in Figure 12.1, the correlation is very good in a medium containing 2% corn steep liquor and 2% glucose. After 14 days, 0.45 mg ergosterol/ml medium correlated with 4.5 mg dry weight of fungal biomass/ml medium.

Inasmuch as evaluation of the time course of biomass development in wood chips during biopulping was the goal of this study, it was first necessary to determine whether the ergosterol content calculated per mg of fungal dry weight remained constant with time. Figure 12.2 shows the increase in ergosterol content of the fungal mycelium up to approximately day 6. Between day 6 and day 14 the ergosterol content of the hyphae of *C. subvermispora* remained constant at a level of 1% of the dry weight. Conclusions regarding biomass development in wood under various conditions could not be made earlier than day 6.

Because *C. subvermispora* starts to grow on corn steep liquor (a medium rich in nutrients) and will have to adapt to grow on a very poor medium as it begins growth on unsupplemented material in the center of the wood chip, the influence of rich media as compared with poor media on the ergosterol concentration of the hyphae was investigated. In Figure 12.3 the ergosterol contents of mycelium grown in three different media (2% csl + 2% glucose; 2% csl; and wood extract) are compared. This figure shows that in rich media like 2% corn steep liquor plus 2% glucose (compare also Figure 12.2) the mycelium contains approximately 1% (dry weight)

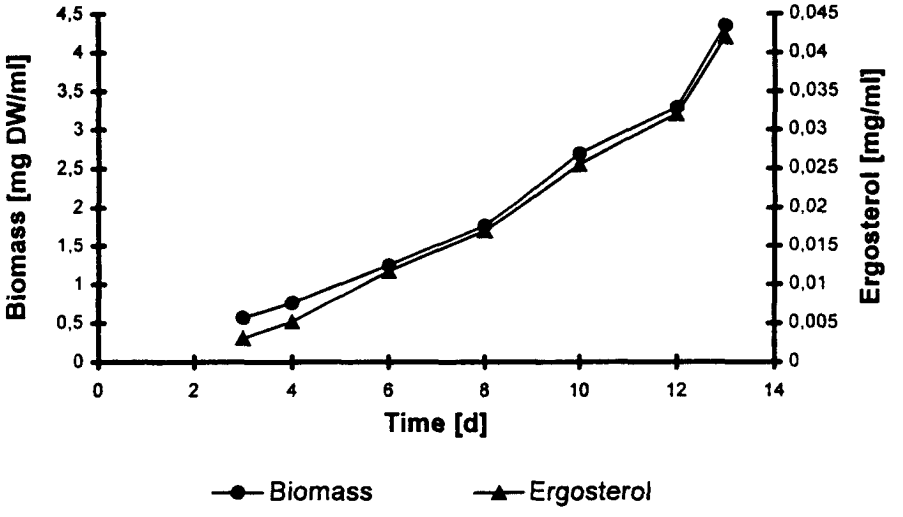

Figure 12.1. Biomass/ergosterol development of *Ceriporiopsis subvermispora* in static liquid culture.

ergosterol. The concentration decreases slightly to 0.7 in 2% csl without glucose and drops to 0.3% in the nutrient-poor wood extract medium. To calculate the fungal biomass from the ergosterol content of the wood for hyphae growing in different areas of the wood chip, we used 1% for the hyphae at the surface, 0.7% for the transient zone and 0.3% for hyphae at the chip center.

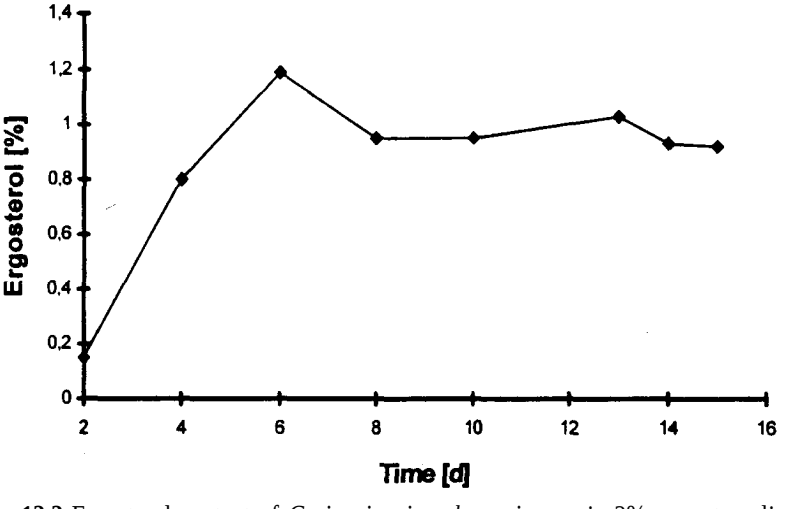

Figure 12.2 Ergosterol content of *Ceriporiopsis subvermispora* in 2% com steep liquor/glucose medium.

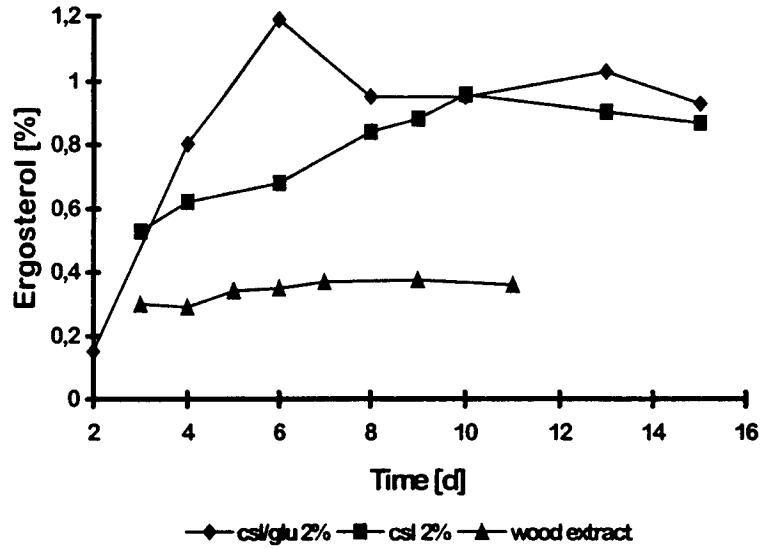

Figure 12.3 Ergosterol content of *Ceriporiopsis subvermispora* in different liquid media (cs1: corn steep liquor, glu: glucose).

Figure 12.4 shows the time course of the ergosterol content of wood chips over a period of 35 days. It increases until day 6 and remains constant until day 14, reaching a plateau of 0.045 mg ergosterol/g wood. After day 14, the ergosterol content of wood increases again, more or less constantly, and reaches 0.08 mg/g wood. Figure 12.4 shows that there is not very much difference between the chips supple-

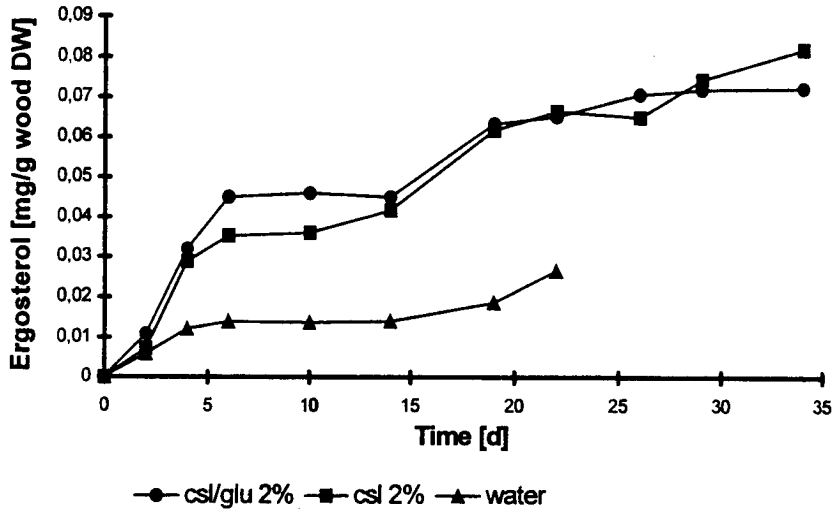

Figure 12.4 Ergosterol content of *Ceriporiopsis subvermispora* in wood chips (csl: corn steep liquor, glu: glucose).

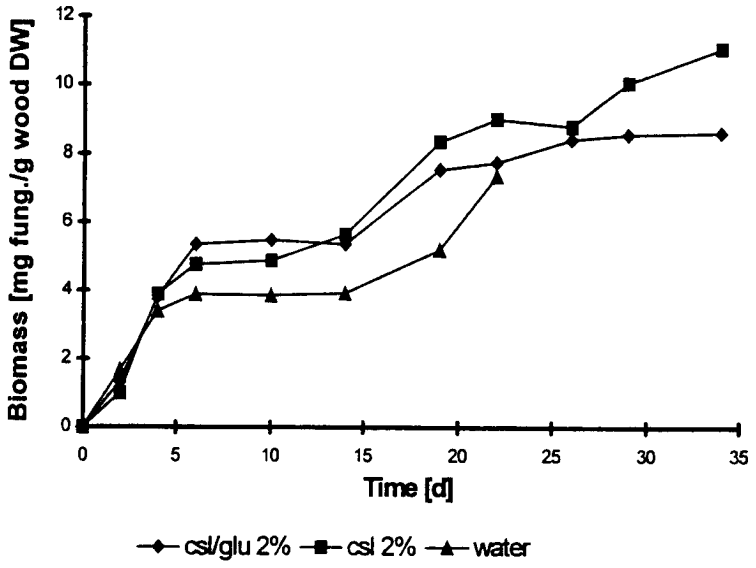

Figure 12.5 Fungal biomass in wood chips (csl: corn steep liquor, glu: glucose).

mented with the two rich media, but the ergosterol content of the chips supplemented only with water reaches a maximum of only 0.02 mg/g wood.

If the fungal biomass is calculated on an average basis of 0.7% ergosterol for the supplemented chips and on the basis of 0.3% for the unsupplemented chips, the biomass content of the unsupplemented chips reaches 4 mg/g wood in the plateau phase, which is relatively close to that of the supplemented chips at 5 mg/g wood (Figure 12.5).

A striking feature of the curve in Figure 12.5 is the plateau phase. The fungal growth stops between days 6 and 14. One possible explanation could be the temperature development in the 30-1 bioreactor (Figure 12.6). At all aeration rates a maximum temperature of approximately 32°C was reached at day 4, and between days 4 and 6 a slight decrease was observed, indicating a decrease in biological activity. Until day 14, the temperature remained constant between 32 and 33°C. The optimum temperature of this strain was determined to be 32°C. with a growth rate of 0.7 mm/day on malt agar media. At 35°C, fungal growth of 0.4 mm/day was measured. These data suggest that temperature was not the limiting factor for the growth plateau observed. Nevertheless, the temperature plateau correlates with the growth plateau.

One would assume that at the optimum growth temperature of 32°C. the temperature should continue to increase because of high physiological activity. A more likely explanation is that the CO_2 tension in the wood chips has reached a value that tiggers idiophasic conditions. The constantly decreasing temperature of the nonaerated fermenter even within the range of its optimum temperature is a support for this hypothesis. Under these conditions, aeration probably has little infiuence on the gas exchange between the center of a wood chip and its surface. More detailed

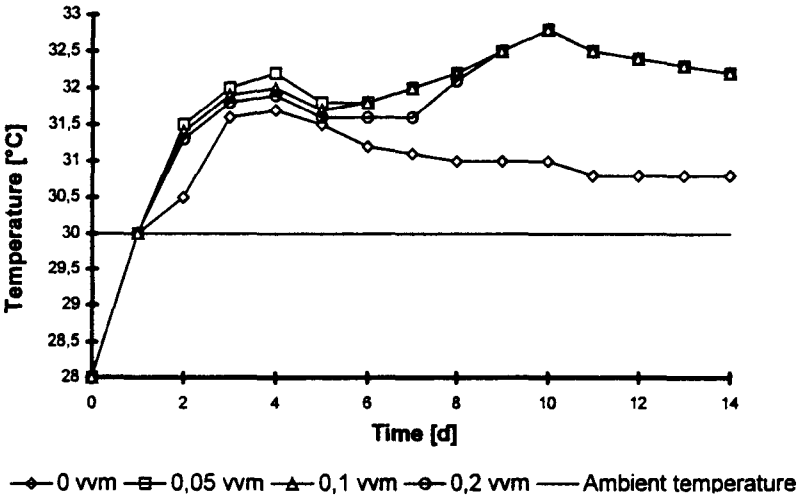

Figure 12.6 Temperature development at various aeration rates (vvm: volume/volume/min).

studies of the internal temperature in wood chips and O_2/CO_2 analyses are under way.

The ergosterol contents of the chips reflect the biological activity of the mycelium as shown by the temperature development. The ergosterol content was lowest in the nonaerated chips (Figure 12.7). Another proof for the assumption that idiophasic conditions are already reached in early stages of colonization is the weight loss curve (Figure 12.8). Weight loss starts after day 5 in supplemented chips and after day 10 in nonsupplemented ones; after 14 days, weight losses between 1 and 2% are reached. Weight loss continues until day 35, when it reaches 8%. If we consider that some depolymerization of the lignin framework must take place prior to carbohydrate degradation and that the ligninolytic enzyme system is produced only in the idiophase, the plateau of fungal biomass development, the temperature development and the weight loss curve are in good correlation to each other.

When the time course of the activity of ligninolytic enzymes in wood chips was measured. it was found that only manganese peroxidase was produced by *C. subvermispora* CBS 347.63, starting on day 4 and reaching a maximum of 7.2 units/g wood (dry weight) on day 10 (Figure 12.9). This, again, is in accordance with the stagnation phase starting on day 6.

Furthermore, it must be concluded from the strong biopulping effect in both mechanical and sulfite pulping after only 2 weeks of incubation, that a highly diffusible agent must be produced by the existing fungal mycelium, leading to chemical and physical changes within the wood cell walls. It appears that this agent is connected to the ligninolytic system and is produced under idiophasic conditions.

The increase in the ergosterol content after 14 days is another interesting phenomenon observed during the growth of *C. subvermispora* on wood chips. The

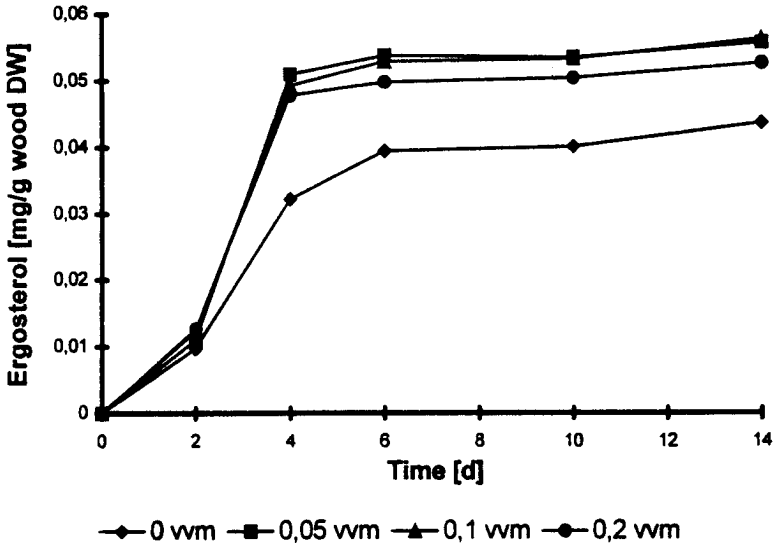

Figure 12.7 Ergosterol content of wood chips at various aeration rates (vvm: volume/volume/min).

increase coincides with a more intensive production of aerial mycelia, as the oxygen tension outside the wood chip is higher and allows intensive fungal gowth. Obviously, the degradation products of wood decay are converted into aerial mycelium. After only approximately 2 weeks, the mycelia of *C. subvermispora* can be seen clearly with the naked eye as a faint white velvet covering the wood chips.

BIOCHEMICAL PULPING

After it was shown by research carried out by the Biopulping Consortium in Madison. Wisconsin, that the pretreatment of wood chips with selective white-rot fungi is beneficial for mechanical pulping, yielding energy savings of nearly 50% (Akhtar et al., 1992, 1993; Leatham et al., 1990; Myers et al., 1988; cf. Chapter 10), the principle of biopulping was also tested for chemical pulp production.

Biosulfite Pulping

Biopulping, defined as the fungal treatment of wood chips with fungi prior to pulping, has been studied extensively at the USDA Forest Service, Forest Products Laboratory, Madison, Wisconsin, as a pretreatment for mechanical pulping. The fungal pretreatment of loblolly pine or aspen chips with *C. subvermispora* saves at least 30% electrical energy during mechanical refining, improves paper quality, and reduces the environmental impact of pulping (see Chapter 10 for details). Studies done in Vienna, Austria indicated that the fungal pretreatment also offers great advantage for magnesium-based sulfite pulping (Messner et al., 1992, 1993; Messner and Srebotnik,

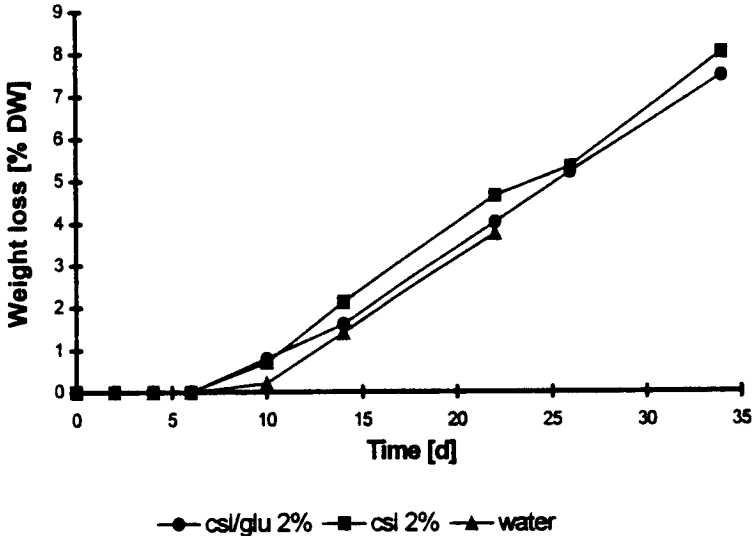

Figure 12.8 Weight loss (% dry weight) of wood chips as a function of time (csl: corn steep liquor, glu: glucose).

1994). This approach was extended to the use of fungal pretreatment for sodium- and calcium-based sulfite pulping by the USDA Forest Service, Forest Products Laboratory, with emphasis on reducing the kappa number, increasing pulp bleachability, and reducing the environmental impact of the traditional sulfite pulping processes. The other goal of the latter study was to develop a preliminary kinetic model, based on

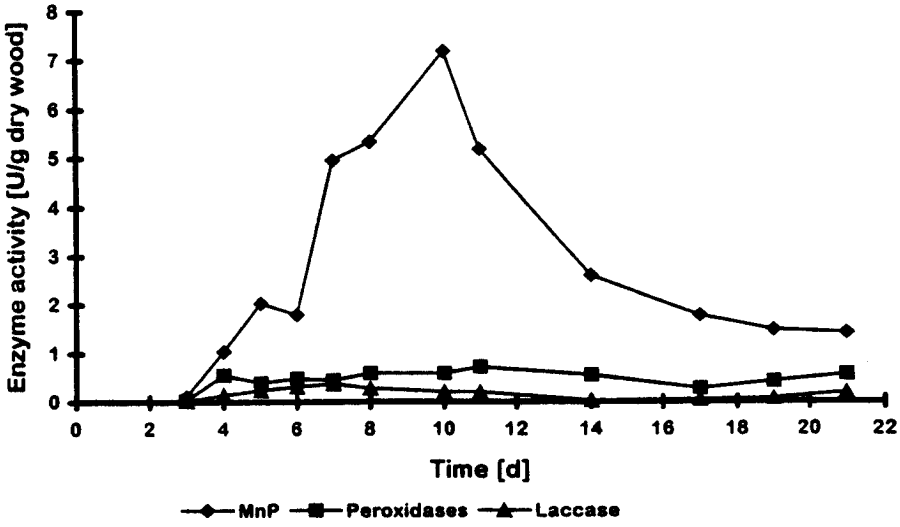

Figure 12.9 Enzyme development of Ceriporiopsis subvermispora in wood flakes (MnP: manganese peroxidase).

assumptions and data, to explain in part some of those results. This chapter describes the results of both groups.

Background. The sulfite pulping process includes several pulping processes that differ as to 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. 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 to 5 and neutral suifite semichemical (NSSC) pulping at a pH of 7 to 9, with a sodium base. In this chapter, we focus only on magnesium-, sodium-, and calcium-based sulfite pulping processes.

Biopulping in connection with sulfite pulping is of great interest for the European pulp and paper industry, especially in countries such as Germany and Austria. where most of the mills are sulfite based. The cooking process has generally changed in Europe from sodium bisulfite cooking to modem magnesium bisulfite or acid sulfite processes. The mills using the newer processes are reaching a very high environmental standard. Furthermore, totally chlorine-free bleaching sequences can be more easily introduced into a sulfite process than into a kraft process. In the United States, 14 sulfite mills are in operation (Scott et al., 1995 a, b). Recent EPA regulations propose dramatic reductions in air and water discharges, and new technologies are required to meet these regulations. Biopulping may be one method of complying with such standards.

Hypothesis In sulfite pulping the goal is to remove the lignin from wood, while leaving the cellulose and hemicelluloses unreacted. However, the process tends to somewhat degrade the carbohydrate portion of wood as the lignin degrading reactions occur. We speculated that the fungal pretreatment either consumes some of the lignin or modifies the lignin selectively in such a way that it would be easier to remove in the subsequent pulping process.

Furthermore, electron microscope studies indicated that the fungal treatment causes softening and swelling of wood cells. The possibility exists that these fungus-induced changes may result in improved chemical penetration during pulping operations and thus, in turn, could result in easily bleachable low-kappa pulps, reduced cooking times and temperatures, reduced pulping chemical loads, reduced effluent waste load, and so on, during sulfite pulping processes.

Magnesium-Bused Sulfite Pulping The method of pretreatment used in this study was similar to that used by Akhtar and co-workers (1992) for mechanical pulping. Prior to inoculation, the wood chips were steam sterilized to reduce the indigenous microflora, especially strains of *Trichoderma* spp. This fungus was found to control growth of biopulping basidiomycetes. The chips were inoculated with blended mycelia of selected strains suspended in a liquid nutrient medium and incubated in an aerated static-bed bioreactor at 28°C ambient temperature. The wood chips were cooked in a laboratory digestor according to the magnefite process.

After a first screening, five strains were selected to further study the biopulping effect on birch and spruce chips after 2 and 4 weeks incubation (Messner et al., 1992). The effect of fungal pretreatment was measured as a reduction of the kappa number after cooking, expressed in percentage of the untreated control, which had a kappa value of 24 after cooking. *C. subvermispora* turned out to be the most effective fungus, on birch as well as on spruce, reaching a kappa reduction of approximately 50% after 4 weeks on birch wood and approximately 30% on both wood types after 2 weeks of incubation. Even after 2 weeks of incubation, delignification during cooking can be greatly improved by fungal pretreatment. *Phlebia tremellosa, Phlebia brevispora,* and *D. squalens* also produced remarkable results after 4 weeks cultivation time, reducing the kappa number of birch wood pulp by 33 to 40%. *Phanerochaete chrysosporium* was the least effective fungus, yielding only 12 and 20% kappa reduction, respectively, after 2 and 4 weeks incubation time on birch wood. Surprisingly, no kappa reduction effect was observed on spruce wood chips. Further studies demonstrated that *P. chrysosporium* is a weak colonizer of spruce heartwood. The type of wood may have a great influence on the biopulping effect exerted by a particular organism. The time course of kappa reduction by *C. subvermispora* was almost identical on both wood types, indicating a lower wood species specificity for this fungal species.

The effect of a 4-week fungal pretreatment of birch chips on fiber strength in handsheets was measured as tensile index and tear index after a beating time of 10 and 20 mins, respectively *(P. chrysosporium* not tested). After 10 min beating time, tensile index was reduced by 6 to 10% and tear index by 2 to 7%, whereas after 20 min a more pronounced decrease, 9 to 12% for tensile index and 6 to 31% for tear index, was measured. The decrease in strength of handsheets caused by *C. subvermispora* was the lowest.

A negative effect of the pretreatment of wood chips with *C. subvermispora* is a brown discoloration starting within the first few days of colonization. This discoloration is especially obvious when complex natural media are used to supplement the chips. As a result, an average brightness loss of 12% ISO was observed after the cooking of spruce chips (Messner et al., in unpublished results). The average brightness dropped from 62% ISO to 50% ISO. When these chips were bleached in a two-step laboratory bleaching sequence (EOP-P), a brightness level of 74% ISO was reached with the control. but only 70% ISO was obtained with the treated chips. The kappa number of the bleached, untreated control pulp was 6.3 as compared with 3.2 for the pulp produced from fugally treated chips. Although the kappa value after bleaching is reduced by almost 50% by fungal pretreatment, the chromophores remain somewhat resistant to the bleaching process.

When the chips were supplemented with a synthetic medium (Leatham et al., 1990), the discoloration was much less pronounced. At a similar kappa reduction after cooking and bleaching (38% and 51%, respectively), the brightness loss after cooking was only 1.5% ISO (brightness level 60.7) and only 0.8% ISO after bleaching (brightness level 50.8). It can be concluded that by optimizing the nutrient medium to support fungal colonization on the chip surface, the problem of brightness loss can be controlled. Unfortunately, no brightness gain was achieved after bleach-

ing, even with a kappa reduction of 50% for the bleached pulp, becuase of the chromophores produced.

The message to be drawn from these results is to avoid the production of chromophores. The aim of further development work should be to reduce the chromophore production not only by optimizing the media but also by optimizing the cooking conditions. The goal should be a gain in cooking time and/or a reduction in cook chemical use and an additional brightness gain after bleaching. A reduced cooking time would result in increased digestor capacity and increased production. In a laboratory experiment, the cooking time was reduced to 240 min (from 315 min for the untreated chips), reaching a kappa number of 25.4 for the treated chips supplemented with synthetic media. This is close to the kappa number 24.8 obtained with the control chips. The result was a brightness gain of 2.3% ISO. When the pulp was bleached, despite the shorter cooking time, the kappa number was decreased by 28% and a brightness gain of 3.5% ISO was observed. These positive results are due to less production of chromophores in the cooking process. Summarizing this work the final gain was 75 min reduction in cooking time, an increase in ISO brightness of 3.5%, and a decrease of the bleached kappa number by 28%. The results show that biosulfite pulping has the potential to increase productivity and to increase brightness. Further investigation is needed to optimize this process.

Sodium- and Calcium-Based Sulfite Pulping Scott and co-workers (1995a,b) investigated the effect of fungal pretreatment of loblolly pine chips *(Pinus taeda)* prior to sodium- and calcium-based sulfite pulping. The pretreatment involved a 2-week incubation of the chips with two strains (CZ-3 and L-14807 SS-3) of the white-rot fungus *C. subvermispora.* Focus was on the kappa number, yield, liquor consumption, pulp bleachability, and effluent analysis. Details about inoculum preparation, bioreactor inoculation, and incubation have been reported by Akhtar and co-workers (1996). At harvest the control and the fungus-treated 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 final material. The resulting chips averaged approximately 4 by 4 by 20 mm.

Pin chips were pulped in 90 ml bombs, which were indirectly heated in an oil bath. The temperature was ramped from the initial 70°C to the final temperature given in Table 12.1. The liquor was obtained from the Weyerhaeuser Paper Company (Rothschild, Wisconsin) and titrated according to Tappi test method T604. Results of this analysis are shown in Table 12.2. The sodium-based liquor was prepared as needed from reagent-grade sodium bisulfite ($NaHSO_3$). The kappa number was determined using Tappi test method T236. The spent liquor was analyzed using Tappi test method T604 for residual pulping chemicals.

Kappa Number and Pulp Held During sodium bisulfite pulping, the fungal pretreatment reduced both the kappa number and the pulp yield significantly, as compared with the control, regardless of the strains (Table 12.3). The yield decrease was perhaps due both to the dissolution of lignin and to the concurrent attack on the carbohydrates. The fungal pretreatment in this case seemed to accelerate the pulping

TABLE 12.1 Nominal Cooking Conditions Used for Sulfite Pulping.

Cooking Conditions	Sodium Bisulfite	Calcium-acid Sulfite
Wood (dry weight basis) (g)	10	10
Total SO_2 on wood (%)	20	32
Combined SO_2 on wood (%)	10	9.7–10.9
Liquor-to-wood ratio	4:1	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

Source: Scott et al., 1995a.

process but did not seem to change the selectivity between lignin and carbohydrates. However, during calcium-baaed sulfite pulping, strains CZ-3 and SS-3 reduced the kappa number by 48% and 21%, respectively, as compared with the control, but resulted in the same pulp yield as that of the control (Table 12.4). This indicates that calcium-based sulfite pulping is more selective toward lignin degradation.

Subsequent studies indicated that shorter cooking times can be used to teach the same kappa numbers as the control, thus increasing throughput and reducing energy consumption (Scott et al., 1995a, 1996). In addition to reducing the kappa number of the pulp, the possibility also existed that the fungal pretreatment could be used to reduce the amount of pulping chemicals that were consumed. Table 12.5 summarizes the amount of liquor charged and consumed for the control and the CZ-3-treated chips with the calcium-based cooking. The same amount of cooking chemicals was consumed in each case, approximately 0.24 g SO_2/g oven dried pulp. This shows that fungal pretreatment did not increase the amount of pulping chemicals needed for the process. Remember that the treatment resulted in a 48% reduction in the kappa number, which would make the pulp easier to bleach. This supports the theory that fungal pretreatment modifies the lignin in the pulp, making it easier to remove in the subsequent pulping step.

Bleaching Studies Sulfite pulps from these trials were disintegrated in a Warring blender. One obvious difference between the two pulps was the large shive content of the control pulp, indicating a most complete pulping with the fungal pretreatment. Large shives were removed to make a more homogeneous pulp. The resulting

TABLE 12.2 Titration Analysis of Calcium-based Cooking Liquor.

	Concentrations (%)
Total SO_2	5.745
Free SO_2	3.890
Combined SO_2	1.855

Source: Scott et al., 1995a.

TABLE 12.3 Yield and Kappa Number During Sodium Bisulfite Pulping (Cooking Time 5.12 to 5.25 h).

Treatment	Yield (%)	Kappa Number
Control	49.93	31.27
Treatment (CZ-3)	48.16	22.84
Treatment (SS-3)	48.20	22.90

pulp was subsequently bleached with conventional nonchlorine bleach chemicals. Chromophores were introduced in the fungus-treated chips during incubation and pulping, which decreased the initial brightness of this pulp as compared with the control.

Although the initial brightness of the control pulp was 5 points greater than that of the corresponding biopulps, the bleached brightnesses were comparable, indicating improved bleachability for the biopulps. Table 12.6 summarizes the bleach response to 4% hydrogen peroxide. The control pulp increased from 54% brightness to 80%, a gain of 26 points; the biopulps gained 31 points from an initial brightness of 49% to match the final 80% brightness of the control pulp with a comparable peroxide charge. 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 (Sharp and Lowe, 1993). Brightness did not benefit from a reductive bleach step with 1% FAS (formamidine sulfinic acid); the color, however, improved for the biopulps (Table 12.6). The yellow color component decreased from 9.90 for the biopulps to 2.80, and the control decreased from 9.70 to 3.90. Although the pulps had comparable bleached brightness, the biopulps appeared to be whiter because of less yellow content.

Effluent Analysis Effluent discharges from the pulp and paper industry have been drastically reduced during the past two decades, and the trend is toward further reductions. Pressures for lower discharge levels continue to come from both legislation and the marketplace. Regulations are being reviewed and modified in many countries. In the United States, the Environmental Protection Agency (EPA) is presumably very close to firming up new regulations that will undoubtedly cause sig-

TABLE 12.4 Yield and Kappa Number During Calcium-acid Sulfite Pulping (Cooking Time 9.50 h).

Treatment	Yield (%)	Kappa Number
Control	47.63	26.75
Treatment (CZ-3)	47.70	13.72
Treatment (SS-3)	47.80	21.05

TABLE 12.5 Comparison of the Consumption of Pulping Liquor During Calcium-acid Sulfite Pulping.

	Pulping Chemical ($g\ SO_2$/g oven dry pulp)	
Status of Pulping Liquor	Control	Treatment (CZ-3)
Charged	0.332	0.319
Consumed	0.240	0.234
Residual	0.082	0.086

Source: Scott et al.,

nificant modifications in processes and treatment systems. The EPA proposes a dramatic reduction in allowable air and water discharges from chemical pulp and paper mills. There are about 14 sulfite mills in the United States with varying production rates, from 155 to 600 tons per day. Like kraft pulp mills, all sulfite mills are required to reduce the adverse effect of their effluents. Thus, the sulfite mills must also look for alternative technologies to meet the various agencies' regulations.

Pulping processes release materials such as sugars, low molecular lignins, extractives, and so forth from wood, and these materials appear in the effluent stream (Eriksson, 1985). Environmental guidelines restict the composition of effluents from all stages of pulping, papermaking, and bleaching. To meet these guidelines for discharge into freshwater sources, mills are equipped with treatment facilities to assure compliance with regulations set by the 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 (Springer and Petterson,

TABLE 12.6 Bleaching Response of Pulps.

		Color[a] scale		
Treatments	Brightness (%)	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
Biopulps				
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*: black-white; a*: green-red; b*: blue-yellow

[b]Formamidine sulfinic acid

Source: Scott et al., 1995.

1980). State environmental agencies can impose additional regulations on maximum levels of individual compounds frequently occurring in effluents. Moreover, the toxicity of the effluents to aquatic life is monitored to assure continuous water-quality compliance. This is done through testing the effluent or downstream water for both acute and chronic toxicity (Firth and Backman, 1990; Johnson and Butler, 1991).

The Microtox ™ method of analysis is used as a rapid screening method for evaluating acute toxicity of untreated pulp mill effluents (Firth and Backman, 1990). 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 (Johnson and Butler, 1991).

The BOD and COD contents of the pulps from the control and fungus-treated chips were almost identical. The BOD and COD values for both pulps were approximately 24 and 92 to 94 kg/kg wood chips, respectively. The principal toxic chemicals from sulfite pulping are resin acids, unsaturated fatty acids, and lignin degradation products (Springer, 1986). 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 response. Fungal pretreatment (CZ-3) during calcium-based pulping reduced effluent toxicity as measured by Microtox (Table 12.7). The control pulping effluent was 17.4 toxicity units as compared with 7.2 toxicity units for effluents from the fungus-treated samples. Some extractives are solubilized during pulping, which contributes to both oxygen demand and the toxicity of the resulting effluents. We assumed that the decreased toxicity was a result of fungal metabolism during incubation, which consumes some wood resins (Fischer et al., 1994), thereby improving effluent quality.

Kinetic Modeling Scott and co-workers (1995b) made an attempt to fit the data acquired to a simple kinetic model of the process. With this model, the changes that occur in the reaction can be discussed in a general way. For this model, wood is considered to be a system consisting of two fractions, lignin, L, and carbohydrates (cellulose and hemicellulose), C. The rate of dissolution of each of these components is assumed to be an nth order reaction. Because the data were collected at a constant temperature (that is, after the completion of the ramp to the final cooking time), temperature effects on the kinetics are not considered. In addition, the effect

TABLE 12.7 Toxicity Measurements of Liquor Effluent.

Treatments	EC50[a]	Confidence Interval	Toxicity Units
Control	5.74	(2.62–12.56)	17.4
Treatment (CZ-3)	13.82	(10.17–18.78)	7.2

[a] Measure of toxicity

Source: Scott et al., 1995a.

of liquor concentration is not considered in this simple model. The model is therefore given by the following:

$$dL/dt = k_L(L - L_0)^{n_L}$$

$$dC/dt = k_C(C - C_0)^{n_C}$$

where k_L and kc are the reaction rate constants; L_o and C_o are the amount of lignin and carbohydrates that are "unreactive," and n_L and n_C are the reaction orders; and t is the time of the reaction. Because of our simplifying assumptions, these two equations can be directly integrated to give

$$L = [k_L(1 - n_L)t + c_L]^{(n_L - 1)} + L_0$$

$$C = [k_C(1 - n_C)t + c_C]^{(n_C - 1)} + C_0$$

Where c_L and c_C are constants of integration. These values may then be used to calculate the resulting yield and kappa number as

$$\text{Yield} = L + C$$

$$\text{Kappa} = 650L/(L + C)$$

Using data from the cooks, the various parameters of the model were fit by minimizing the weighted sum of squared error. As a result of the range of the experimental data this produces a model that describes the behavior of the pulping process near the end of the cook. That is, the model is valid only in the region of 50% yield in both cases. Tables 12.8 and 12.9 summarize the fitted parameters to this model. The values given for L and C at the given time in each table are related to the constants of integration (c_L and c_C) and are essentially the initial conditions of the model. Figures 12.10 through 12.12 display the model fit for the sodium bisulfite

TABLE 12.8 Best Fit Parameters to the Kinetic Model for Sodium Bisulfite Cooks.

Parameters	Control	Fungal Pretreatment
L_o	0.0160	0.0122
k_L	0.0550	0.0440
n_L	3.70	3.23
L (3h)	0.155	0.089
C_o	0.468	0.458
k_C	0.0600	0.0485
n_L	2.81	3.00
C (3h)	0.592	0.554

Source: Scott et al., 1995b.

TABLE 12.9 Best Fit Parameters to the Kinetic Model for Calcium-acid Sulfite Cooks.

Parameters	Control	Fungal Pretreatment
L_o	0.00507	0.00709
k_L	0.0160	0.0366
n_L	1.94	2.46
L (7h)	0.0635	0.0724
C_o	0.486	0.480
k_C	0.0507	0.0493
n_L	2.89	3.00
C (3h)	0.496	0.497

Source: Scott et al., 1995b.

cooks, and Figures 12.13 through 12.15 display the results for the calcium-acid cooks.

We noted in the sodium bisulfite pulping that fungal pretreatment had little effect on the relative values of the kappa number when plotted against yield. Figure 12.10 shows that both the control and the treatment were similar in their relationship of kappa to yield. That is, for a given yield, both the control and the treatment had similar kappa numbers. However, the fungal pretreatment allowed the lower kappa and yield to be reaehed with less cooking time. Figures 12.11 and 12.12 show that for any given time. both the yield and the kappa number were lower for the treatment, indicating a greater extent of cooking.

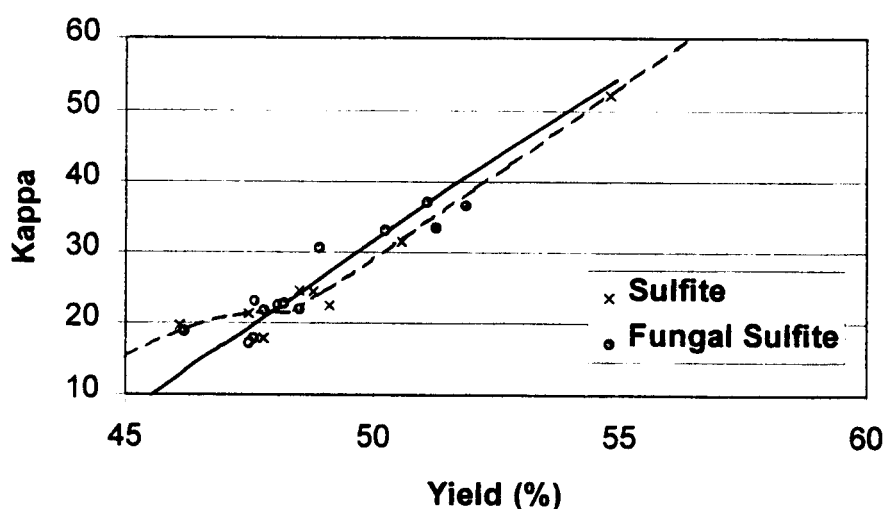

Figure 12.10 Kappa number compared with pulp yield for sodium bisulfite pulping. (Dotted and dashed lines show the fit of the respective models for the control and the fungal pretreatment.)

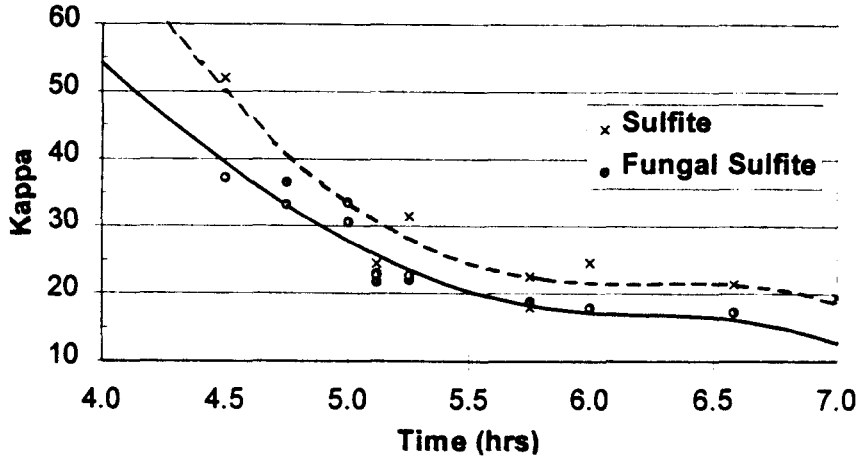

Figure 12.11 Kappa number compared with time for sodium bisulfite pulping. (Dotted and dashed lines show the fit of the respective models for the control and the fungal pretreatment.)

The parameters that are fit to the simple kinetic model also show some differences in the case of sodium bisulfite pulping (Table 12.8). For the treatment, the value of L (3h) (the amount of lignin remaining after 3 h of cooking) is significantly less than that of the control. Likewise, the amount of carbohydrate, C (3h), is slightly less for the treatment at this time.

This indicates that for the treatment, the extent of reaction is greater than that of the control at this time. Interestingly, in this case the rate constant for both lignin

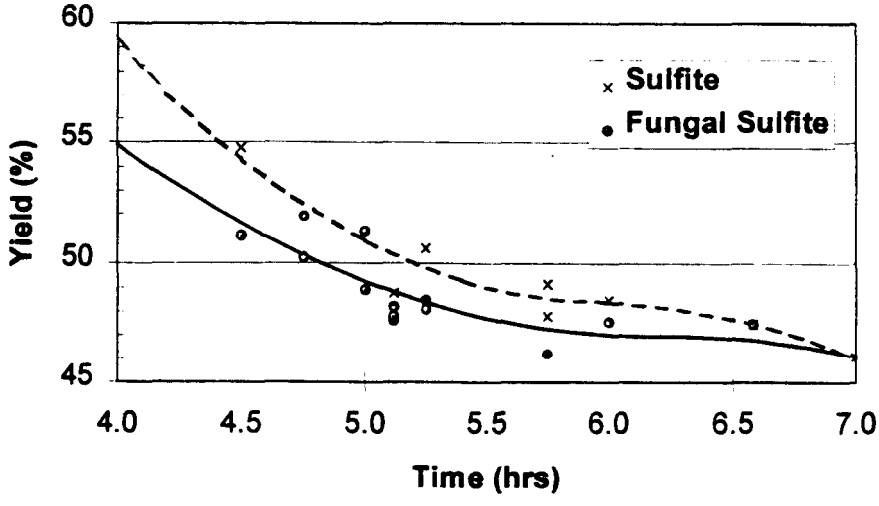

Figure 12.12 Pulp yield compared with time for sodium bisulfite pulping. (Dotted and dashed lines show the fit of the respective models for the control and the fungal pretreatment.)

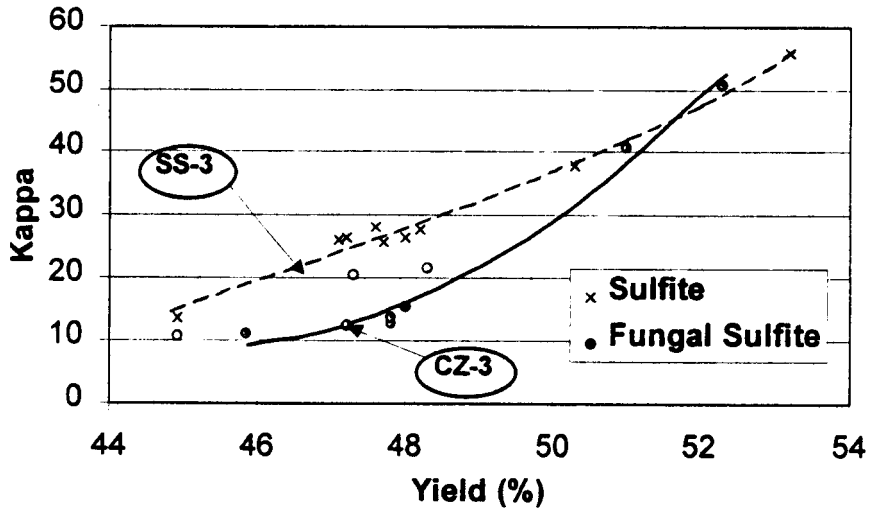

Figure 12.13 Kappa number compared with pulp yield for calcium-acid sulfite pulping. (Dotted and dashed lines show the tit of the respective models for the control and the fungal pretreatment.)

and carbohydrate degradation is less for the treatment. However, it is generally understood that as cooking proceeds, the lignin becomes more difficult to remove. Therefore, the results may indicate that the reaction has proceeded to the point where the more difficult lignin is being removed.

In contrast to the sodium bisulfite pulping, we noted that the calcium-acid cooks

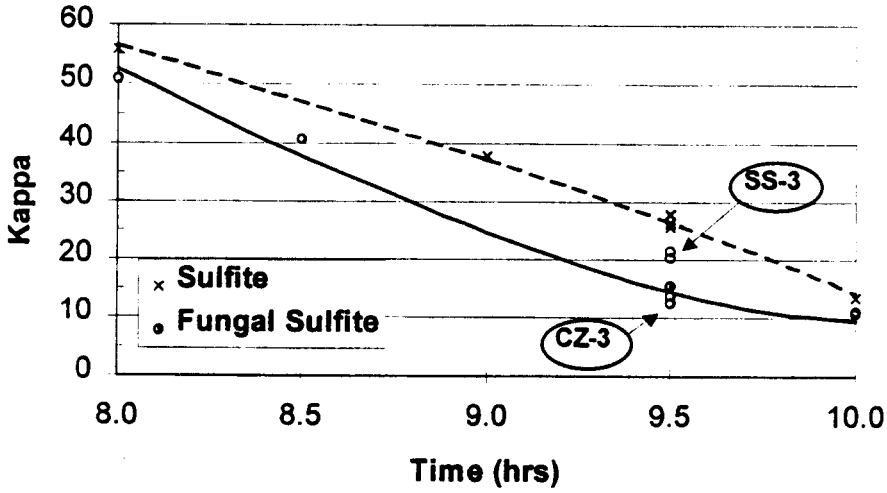

Figure 12.14 Kappa number compared with time for calcium-acid sulfite pulping. (Dotted and dashed lines show the fit of the respective models for the control and the fungal pretreatment.)

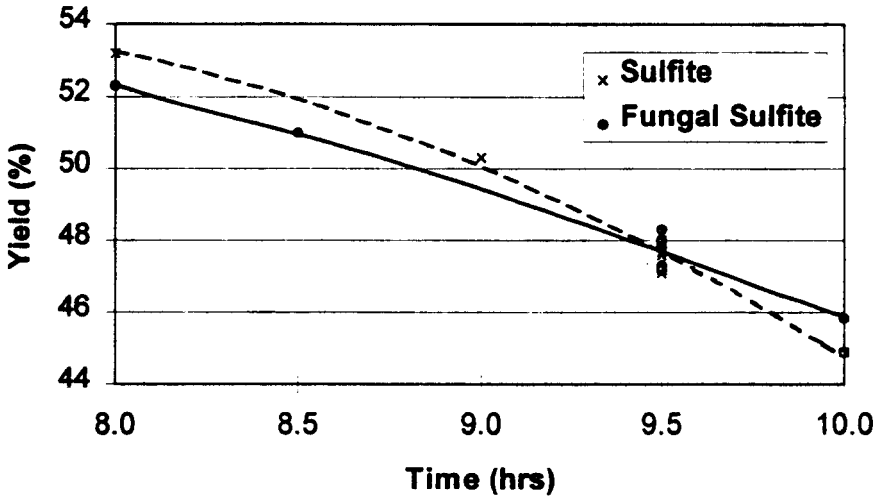

Figure 12.15 Pulp yield compared with time for calcium-acid sulfite pulping. (Dotted and dashed lines show the fit of the respective models for the control and the fungal pretreatment.)

had a significant effect on the relative values of kappa number and yield. As previously mentioned in regard to calcium-based cooking, the two strains of fungus showed different results. Only the CZ-3 data were used to fit the model. Figure 12.13 shows that for yields from 46 to 50%, the treatment had a kappa number approximately 10 points less than the control for the same yield. Figure 12.14 shows that at the same cooking time, the kappa number for the treatment was approximately 10 points less. Figure 12.15 shows the evolution of the yield with time. As can be seen, there is little difference between the yields for the control and the treatment. In fact, at 9.5 h, the lines representing the models cross and both predict the same yield. As can be seen in Figure 12.14, the kappa number is still significantly less than that of the control at this point.

Table 12.9 gives the fitted model parameters for the calcium-acid sulfite process. As can be seen, there is little change in the parameters representing the degradation of the carbohydrates. This indicates that the treatment has little effect on the carbohydrates and their subsequent reactions in sulfite pulping according to this model. However, the parameters for lignin degradation did change, and the treatment resulted in art increase in the reaction rate constant, k_L, and the order of the reaction, n_L. Both of these effects would lead to an increase in the rate of lignin degradation.

Future Work The fungus used during sulfite pulping, *C. subvermispora,* is the best fungus for mechanical pulping. However, it may not necessarily be the best fungus for biochemical pulping. Moreover, it is important to emphasize here that during biomechanical pulping of loblolly pine chips, strain SS-3 appeared to be superior to strain CZ-3 (Akhtar et al., 1997); however, these strains during sulfite pulping produced the opposite results. Thus, not only additional fungi, but also different strains

of the same fungus, must be evahtated. Parameters such as brightness, color stability, strength, and optical properties of the resulting pulps must be optimized. Sophisticated models, such as those that account for changes in the liquor composition, would aid in the interpretation and optimization of the biopulping process.

Biokraft Pulping

Biokraft pulping, the use of fungal pretreatment to obtain benefits during kraft pulping, has not received as much attention as biomechanical pulping. Kraft pulping involves the selective removal of lignin from wood, and white-rot fungi are capable of degrading and modifying lignin. Therefore, it has been commonly believed that pretreatment with white-rot fungi could aid the kraft pulping process.

An early study by Adamski and co-workers (1987) showed that pretreatment of wood chips with the white-rot fungi *Phellinus pini* and *Stereum hirsutum* resulted in a decrease in the refining energy needed for kraft pulping. A more extensive study was performed on kraft pulping of aspen and red oak pretreated with *P. chrysosporium* (Oriaran et al., 1989, 1990, 1991). Fresh, screened aspen and red oak chips were autoclave and then inoculated with a rye spawn of *P. chrysosporium* BKM-1767. The chips were supplemented with 2% glucose (dry weight basis) and adjusted to 50% moisture (dry weight basis). The chips were incubated at 25°C in polypropylene bags for 10 to 30 days. Holes were punched in the bags with a sterile needle to allow air exchange. For red oak chips, pulp yield as a function of kappa number increased by 3 to 5% afier 30 days treatment (Oriaran et al., 1991). For the treated chips. pulp with a similar kappa number and higher yield could be produced with one-third less cooking time. In addition, the pulp responded better to refining, was more hydrophilic, and showed increased tensile and burst indices. A decrease in tear strength was observed. For biomechanical pulping, large increases in tear strength are generally observed in combination with little change in burst and tensile strengths (Akhtar et al., 1996). Although the yield during pulping increased, high yield losses (4% after 10 days and 18% after 30 days) were observed during fungal pretreatment. Unbleached handsheets showed large reductions in brightness that increased with treatment length. Similar reductions have been observed for biomechanical pulping (Akhtar et al., 1996) and for sulfite pulping with white-rot fungi. Bleachability of the pulp was not reported. The results obtained for aspen chips were similar to those reported here for red oak chips (Oriaran et al., 1990).

A large number of white-rot fungi isolated in South Africa were screened for their ability to reduce kappa number without significantly affecting yield during micro-kraft pulping (Wolfaardt et al., 1996). The strains that performed best for micropulping— *S. hirsutum, Pycnoporus sanguineus,* and *Coriolus versicolor*— were used for larger-scale studies on sterile, nutrient-supplemented pine chips. A 9-week fungal treatment resulted in decreases in kappa number of up to 17%. However, yield decreased and active alkali consumption increased.

Although sap-stain fungi are not capable of degrading lignin, them have been reports of kraft pulping benefits obtained with the sap-stain fungus *Ophiostoma piliferum* (Wall et al., 1994, 1996). All results presented here were obtained with

the commercially available strain of *Ophiostoma piliferum* Cartapip 97. A two-week treatment of nonsterile, unsupplemented northern softwood chips with *O. piliferum* resulted in a 9% reduction in kappa number, with improved bleachability and with little change in yield and viscosity (Wall et al., 1994). The pulp was bleached to 91.9% brightness with a 9% reduction in chemical use during the first chlorination stage. In addition, strength properties were maintained and the treated pulp responded slightly better to refining. A similar study was performed on an aspen mixture with a 3-week treatment time (Wall et al., 1996). Under given cooking conditions, *O. piliferum* treatment reduced the kappa number by up to 29%. Alternatively, a given kappa number was achieved at approximately 20% less active alkali with treated chips than with untreated chips (Figure 12.16). Figures 12.17 and 12.18 show that these results were achieved with little effect on yield or viscosity. Pulping benefits have also been observed at the mill scale for sulfite pulping of aspen chips (Wall, unpublished) and for kraft pulping of aspen chips (Rocheleau et al., unpublished results).

A study by Rocheleau and co-workers (submitted for publication) compares the efficacy of the sap-stain fungus *O. piliferum* to that of the white-rot fungus *Phlebia tremellosa* NRRL 21253 for kraft pulping. Fresh aspen chips were treated with a commercial preparation of *O. piliferum* or a fresh suspension of *P. tremellosa.* The chips were not sterilized or supplemented with addtional nutrients. The chips were stored in loosely closed plastic bags at room temperature for up to 6 weeks. The control chips were incubated in the same manner but were not inoculated. At each time point (0, 1, 2, 3, and 6 weeks), duplicate kraft cooks were performed for each treatment and for the aged control. Average values for the 3-week treatment are shown in Table 12.10. As compared with the aged control, both treatments decreased kappa number with little effect on yield, pulp brightness, viscosity, or strength prop etties. The decrease in kappa number was greater for *P. tremellosa* (14.3%) than for

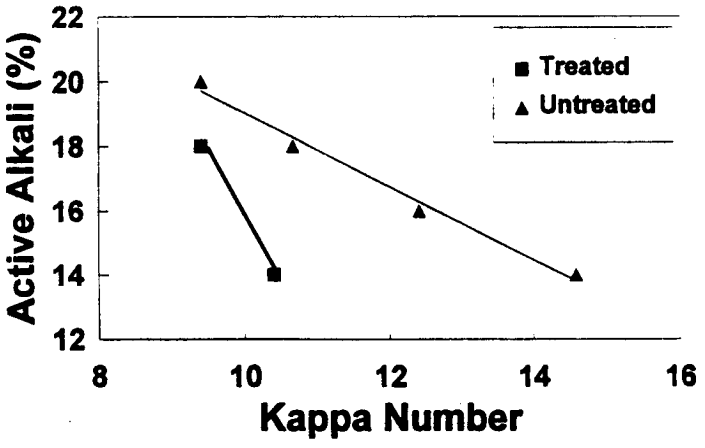

Figure 12.16 Effect of *Ophiostoma piliferum* treatment on active alkali requirement during kraft pulping.

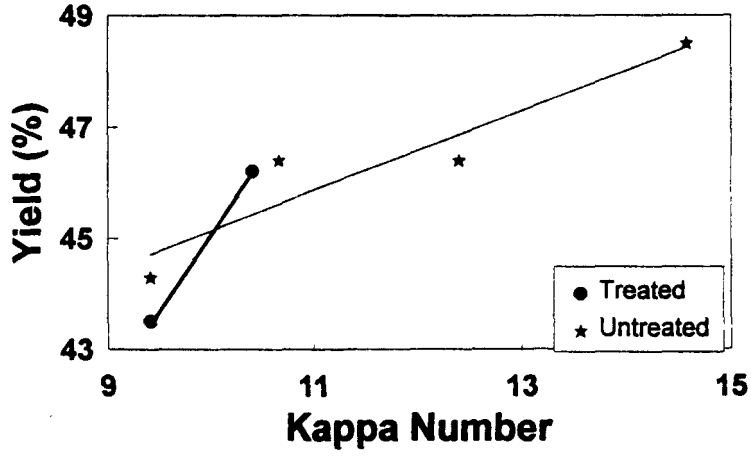

Figure 12.17 Effect of *Ophiostoma piliferum* treatment on yield during kraft pulping.

O. piliferum (5.8%). In tlis experiment, treatment with *P. tremellosa* did not cause the pulp brightness decrease observed with most white-rot fungi for biokraft, biosulfite, and biomechanical pulping. Biomechanical pulping with a different strain of *P. tremellosa* under different inoculation and incubation conditions resulted in a 24% reduction in pulp brightness (Akhtar et al., 1996). Maintenance of pulp brightness in this study may be the result of using nonsterile, unsupplemented chips.

Both white-rot fungi and sap-stain fungi have potential for use with kraft pulping. The challenge is to find applications that fit readily into existing mills and that offer

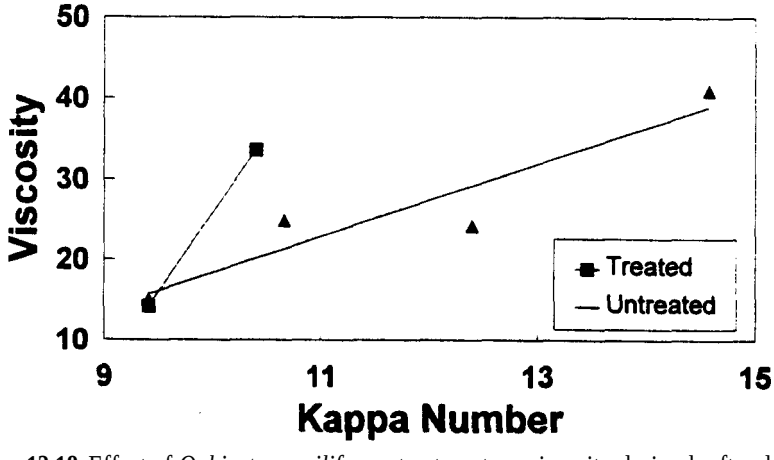

Figure 12.18 Effect of *Ophiostoma piliferum* treatment on viscosity during kraft pulping.

TABLE 12.10 Physical Properties of Kraft Pulps

Property	Fresh Chips	Aged Control	*Ophiostoma piliferum*	*Phlebia tremellosa*
Pulp Yield	55.7	54.8	54.9	54.7
Kappa Number	15.4	15.2	14.5	13.2
ISO Brightness	32.4	33.0	32.9	33.7
Viscosity	48.5	46.4	47.6	45.9
CSF[a]	617	611	639	623
Tear Index	6.78	7.44	7.65	7.90
Tensile Index	60.7	61.1	59.2	54.1

sufficient savings to motivate mill use. Fungal pretreatment appears to make wood chips more amenable to pulping. For white-rot fungi in particular, further screening is necessary to identify those strains that decrease kappa number and increase refinability and strength without detrimental effects on yield pulp brightness, and active alkali consumption. Identification of those strains most practical for large-scale production and wood yard use is also importain. In addition, chip incubation and pulping conditions must be optimized to better take advantage of the benefits of fungal treatment.

In most cases, pretreatment results in reductions in kappa number under equivalent pulping conditions. Mills under pressure to reduce chlorine use could benefit because a lower kappa number coming out of the digester results in less bleach chemical use in the bleach plant. If cooking conditions were changed to reach an equivalent kappa number, cook time or active alkali use could be decreased. Mills could increase throughput and get more productivity from the existing capital investment with a shorter cook time. Mills that have bottlenecks in their recovery operations would benefit from decreased active alkali concentration. These mills could increase pulp production if a lower active alkali concentration were used. Unlocking the potential of biokraft pulping will require improved screening programs specifically targeted toward kraft pulping and intensive efforts to better understand the mechanism of biopulping.

WHAT CAUSES THE BIOPULPING EFFECT?

As discussed earlier, both ascomycetes and basidiomycetes have been shown to be useful for biochemical pulping. For biosulfite pulping, kappa number reductions are much more dramatic with basidiomycetes than with ascomycetes. For basidiomycetes, kappa number reductions of 30 to 50% are common; whereas, with ascomycetes, kappa number reductions of 7 to 10% have been observed. For biokraft pulping, kappa number reductions were only slightly better for basidiomycetes than for the ascomycete, *O. piliferum.*

We hypothesize that two mechanisms are itvolved in biopulping: (1) modification of the wood cell wall, most likely by a diffusible agent, and (2) improved

penetration and diffusion of cook chemicals caused by the action of the fungus on the chip. For biosulfite pulping, the first mechanism appears to be dominant for basidiomycetes. However, the results obtained with *O. piliferum* suggest that the second mechanism is also involved. For biokraft pulping, both mechanisms appear to be involved.

Ascomycetes such as *O. pilferum* are not capable of degrading or modifying wood cell wall components such as cellulose and lignin. Some strains of ascomycetes produce hemicellulases; however, degradation or modification of hemicellulose by these fungi is not expected to be significant. Therefore, biopulping with ascomycetes must involve a mechanism other than cell wall modification.

The Role of Cell Wall Modification

Differential staining with safranin and astra-blue is a useful method of localizing the delignification process within wood cell walls using light microscopy (Srebotnik and Messner, 1994). Astra-blue is incorporated into cellulose fibers only in the absence of lignin (Maacz and Vagas, 1963; von Aufsess, 1973), whereas safranin stains lignin regardless of whether cellulose is present. In addition to the investigations made on birch (Srebotnik and Messner, 1994), the progress of delignification was studied on spruce chips after 2 weeks of incubation with *C. subvermispora* and also in the fibers during the sulfite cooking process (Heimel and Messner, unpublished results). The initiation and progression of delignification in spruce chips during fungal colonization is very similar to that in birch. One of the routes of delignification starts in the cell walls of the ray cells and progresses via the middle lamellae into the S_2 layers of the adjacent cell walls. The second route starts on the surface of the lumina of those cells colonized by hyphae of *C. subvermispora.* As long as no delignification takes place, the cell walls and middle lamellae are stained red. These regions turn blue as soon as an area of the cell wall becomes deilignified. After 2 weeks incubation, the walls of ray cells, the lumen surface of the tracheids, and, in some cases, the S_2 layers were stained blue, indicating delignification. In the much thicker walls of the latewood cells, the delignification process had progressed much further than in earlywood. An attack on the middle lamellae had not taken place in any of the sections.

After a cooking time of 60, 120, and 240 min, samples of pulp produced from untreated and treated chips were investigated for delignification by safranin/astra-blue staining. The course of delignification within a wood tissue during cooking of untreated chips is also described by Bosshard and Holzkunde (1984). No differences were observed between pretreated and untreated chips. Nevertheless, the course of delignification over time offers insights into the topographic effect of biopuiping. After 120 min, the middle lamellae started to be disintegrated. The cell walls were still lignified. After 240 min, all the fibers were separated from each other and the cell walls of the earlywood were delignified, whereas the thick latewood cell walls still contained lignin. It can be concluded that the effect of the fungal pretreatment is not to help dissolve the middle lamellae, as they seem to be easily degraded by the cooking chemicals anyway, nor to delignify the earlywood cells, which follow

next, but much more to facilitate the delignification of the thick latewood cell walls, which happens last. As described earlier, after 2 weeks, the latewood cell walls were preferentially attacked by the organism's diffusible ligninolytic system. In other words, the sections of the wood cell wall delignified last during conventional sulfite pulping were preferentially attacked by the fungus. The action of the fungus on the latewood cell walls is certainly an important factor in biosulfite pulping.

It has long been hypothesized that the strong lignin-degrading enzyme systems produced by white-rot fungi constitute the major cause of the biopulping effect. Modification or degradation of hemicelluloses may also be involved. The biopulping effect can be observed at very low weight losses (<5%). This low weight loss may be attributed to the degradation of extractives and other low molecular compounds found in the ray cells. Under these conditions, there are no obvious differences in the morphology of the wood fiber. In other words, good biopulping is observed when the major cell wall components-lignin, cellulose, and hemicellulose—are largely intact.

A number of immunolabeling and pore size distribution studies have shown that lignin-degrading enzymes cannot penetrate intact wood cell walls (Messner and Srebotnik, 1994). Additional work by Messner and Srebotnik has shown that even after complete delignification and separation of wood fibers by *C. subvermispora* and *D. squalens,* damage to the fiber structure was often negligible. Lignin-degrading enzymes and size marker proteins were not capable of penetrating these fibers. Therefore, it appears unlikely that direct action of enzymes on the wood cell wall is the cause of the biopulping effect. Most likely, a low molecular weight diffusible agent formed by the action of one or more ligninolytic enzymes causes the biopulping effect. The identity of this low molecular agent is not known and is the subject of much speculation and concerted research (Blanchette et al., in press; Messner and Srebotnik, 1994). Many of the agents proposed are not capable of degrading the nonphenolic structures in lignin. However, biopulping may not require degradation of nonphenolic structures; modification and weakening of lignin rather than bulk removal of lignin appears to be sufficient to give the biopulping effect.

The Role of Penetration and Diffusion

Ascomycetes act by rapidly colonizing wood chips, degrading extractives, and growing throughout the interior of a wood chip. Scanning electron micrography has shown that the commercially available strain of *O. piliferum* rapidly colonizes resin canals, disrupts ray parenchymal cells, and ruptures pit membranes (Blanchette et al., 1992). Figure 12.19 shows *O. piliferum* hyphae colonizing tracheids and penetrating pit membranes. Pit membrane penetration is shown at higher magnification in Figure 12.20A; colonization of resin canals is shown in Figure 12.20B. Similarly, many species of white-rot fungi have been shown to degrade extractives, disrupt ray parenchymal cells, and rupture pit membranes (Fischer et al., 1994; Sachs et al., 1989). In addition, white-rot fungi alter the wood cell wall by degrading and modifying cellulose, lignin, and hemicellulose and by forming bore holes through the wood cell wall (Sachs et al., 1991).

Figure 12.19 *Ophiostoma piliferum* growing on wood chips with hyphae colonizing tracheids and penetrating pit membranes. (Bar in photo = 50 μm. Photo courtesy of Professor Blanchette of the University of Minnesota.)

We hypothesize that obtaining a more even distribution of reactants (cooking chemicals) throughout the reaction site (the wood cell wall) is an important part of the biopulping mechanism for both basidiomycetes and ascomycetes. Cooking chemicals come into contact with the wood cell wall via two mechanisms: penetration and diffusion. Penetration involves the bulk flow of cooking chemicals into the wood chip under the influence of a pressure gradient. Diffusion involves the transport of dissolved cooking chemicals as the result of a concentration gradient.

Poor penetration and poor diffusion of cooking chemicals can cause nonuniformities in pulp production and can result in the exposure of the interior of the wood

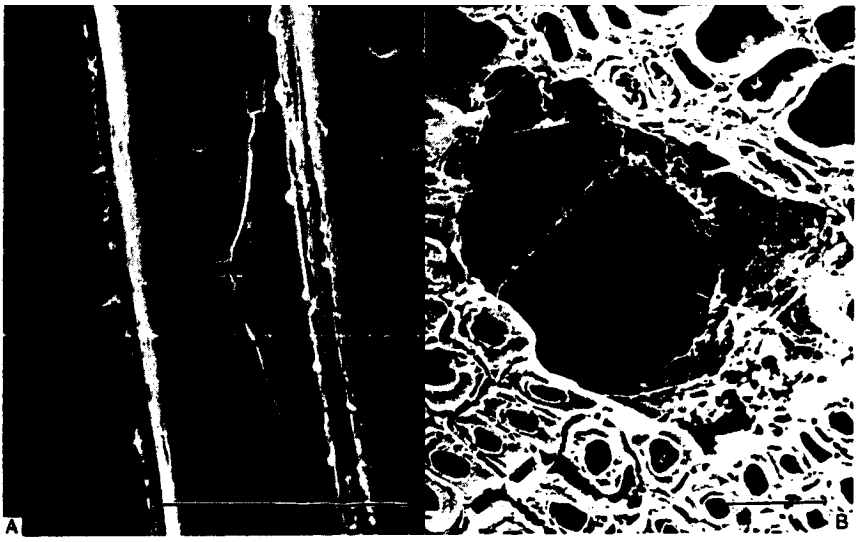

Figure 12.20 Radial section of wood chip showing hyphae of *Ophiostoma piliferum colo*nizing a tracheid and penetrating a bordered pit. (A) Transverse section of wood chips with hyphae colonizing a resin canal. (B) (Bar in photo = 50 μm. Photo courtesy of Professor Blanchette of the University of Minnesota.)

chip to low levels of cooking chemicals. In this case, the exterior of the wood chip is overcooked and the interior is undercooked. The pulp produced has high reject levels, and pulp parameters such as strength and kappa number have high levels of variability.

Poor penetration of cook chemicals can be caused by high levels of extractives (Chen, 1994), the presence of air in the chip (Gustafson et al., 1989), or intact pit membranes (Wardrop and Davies, 1961). Chips are commonly presteamed prior to pulping to remove air from the chips. Poor penetration can be caused by an inadequate presteaming step. Penetration can be improved by the use of digester additives such as surfactants (Chen, 1994). These additives act by wetting and emulsifying the extractives. Chen showed that the use of a digestor additive in an unbleached kraft mill resulted in a 10-point decrease in kappa number at the same level of rejects (1994).

Both basidiomycetes and ascomycetes are expected to improve penetration of cook chemicals by degrading extractives and rupturing pit membranes. This hypothesis is supported by the kratl pulping studies performed with *O. piliferum.* Measurement of the penetration rate in untreated chips and chips treated with a variety of fungi is required to more fully understand the role of penetration in biopulping.

Poor diffusion of cooking chemicals is commonly caused by oversized chips. Because of the high alkalinity of kraft cooking liquor, the diffusion coefficient is the same for the three chip dimensions-longitudinal, radial, and tangential. Therefore, the shortest path, chip thickness, is the key variable for diffusion. A chip thick-

ness of 3 mm or less is required to avoid diffusion limitations (Talton and Cornell, 1987). A uniform chip size distribution is also important in reducing the impact of diffusion limitations. Recently, mills have been investing in thickness screening equipment to gain good control of chip size and, thereby, reduce pulp nonuniformities caused by poor diffusion.

White-rot fungi may increase the effective diffusion rate of cooking chemicals through the wood cell wall by chemical modification of the wood cell wall. If the effective diffusion rate were increased, uniform pulp could be obtained from larger chips. Moving to larger chip sizes would save mills money by decreasing chipping costs and reducing fiber losses during the chipping process. Measurement of the effective diffusion rate of cooking chemicals in untreated and treated chips would be an important contribution to our understanding of the role of diffusion in biopulping.

INOCULUM PRODUCTION

The choice of inoculum has a major impact on all microbial processes. The inoculum should be a stable, pure, vigorous, high-quality culture of the desired microorganism. The culture should be adapted to grow on the desired substrate, in this case, wood chips. In the case of biopulping, a commercial inoculum would be preferred, one that is produced at a site other than the pulp mill. Therefore, the inoculum must be stable during transportation and storage. Large-scale inoculum production is discussed in Chapter 15.

Two methods have been proposed for the production of inocula of biopulping organisms: liquid fermentation (Gerin et al., 1993) and solid-state fermentation (Kerem et al., 1992). Liquid fermentation is a common, well-understood technology that generally offers high cell yields at low cost (Atkinson and Mavituna, 1991). Various drying procedures, such as freeze-drying, fluid bed drying, and spray drying, are available to stabilize the culture (Kadam, 1991). Solid-state fermentation is a less developed and commercialized technology. Solid-state fermentation methods are recognized as practical for the production of mushroom spawn and select types of food products (Wood, 1989). However, the exploitation of this technology for other applications has been limited. Solid-state fermentation may offer select advantages for biopulping, such as better adaptation to growth on wood. Nevertheless, the absence of a commercially available solid-state fermentation system and the challenges of applying a solid product uniformly to wood chips in a commercial wood yard make developing a practical solid-state inoculum a daunting task.

For some organisms, such as *O. piliferum,* fermentation and stabilization process scale-up is possible with direct application of existing fermentation and stabilization technology. The commercially available inoculum of *O. piliferum* Cartapip ™97, is stable at room temperature (4 to 25°C) for 90 days and at -20°C for 1 year. The inoculum is well adapted to growth on wood and is easily applied under mill conditions, as described in Chapter 18.

Other organisms, including many strains of white-rot fungi, are more difficult to produce and stabilize. Some strains are produced at very low yields in liquid

fermentation. Therefore, a reliable method of producing a shelf-stable inoculum of white-rot fungi using liquid fermentation is needed.

CONCLUSIONS

The results reviewed and presented in this chapter demonstrate the great potential of fungal pretreatment of wood chips prior to chemical pulp production. The most prominent benefit of fungal pretreatment is improved effects on cooking, leading to kappa numbers as much as 30% lower than those of the control pulp in sulfite cooking, after only 2 weeks of fungal treatment. The benefits for kraft pulping are less obvious, and more research is needed to better understand biokraft pulping. The choice at the time is to select the best fungus. White-rot fungi are clearly superior to sap-stain fungi for sulfite pulping, but their application may suffer from the drawback of increased chromophore production. The high kappa reduction obtained with fungal treatment coincides with the strategy of extended cooking; however, because bleached pulp of high brightness is the desired final product, methods of preventing chromophore production by basidiomycetes must be developed. One empirical approach will be the development of improved chip supplementation media or the elimination of the need for nutrinent supplementation. If the chromophore production induced by basidiomycetes were controlled, they would probably be more effective than sap-stain fungi for chemical biopulping.

The biochemical basis for the modifications taking place in the wood cell wall and leading to increased delignification is still far from being understood. We can only assume that highly diffusible low molecular weight molecules in connection with the lignin-degrading enzyme system are responsible for the changes. It will certainly take some time to understand fully the biochemical process involved in biopulping, and then more time to learn to control this process. More applied research is also needed to find out what kind of wood, what kind of chemical pulping processes (including the new methods of pulp production like the ASAM process; cf. Chapter 3). and which bleaching sequences are most amenable to fungal pretreatment and which will deliver the highest benefits.

The interpretation of the results obtained with sap-stain fungi is less complex because no cell wall alteration takes place and the effect is assumed to be the result of improved penetration of cook chemicals. Because the major wood cell wall components are not chemically modified, the kappa number reduction during sulphite pulping is lower but no brightness losses are involved. Improved penetration and diffusion of cooking chemicals may also be involved in chemical biopulping with white-rot fungi. More mechanistic studies on penetration and diffusion rates in chips treated with a variety of fungi are necessary to better understand the biopulping mechanism.

The application conditions and inoculum preparation for sap-stain fungi such as *O. piliferum* have already been developed. Method(s) to produce commercial inocula of basidiomycetes have yet to be investigated. The choice of organism will depend on the economic evaluation of the biokraft process. We must point out that

biopulping coincides perfectly with environmentally safe production strategies and can be implemented in existing production plants without major changes.

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