10 An overview of Biomechanical Pulping Research

MASOOD AKHTAR, ROBERT A. BLANCHETTE, GARY MYERS, and T. KENT KIRK

FUNGAL GROWTH AND DEGRADATION OF WOOD

Introduction

Although many microorganisms are involved in the decomposition of wood, fungi are the dominant decomposers in terrestrial systems. In nature, successions of different microbes, crucial to proper ecosystem functioning, recycle organic material. Because wood is composed of many different complex compounds, fungi use an array of processes to degrade woody tissues. The color and texture of the resulting wood is often used to categorize different forms of decay. The three main groups of decay caused by fungi are white-, brown-, and soft-rot.

White-rot fungi are of prime interest for use in various industrial processes utilizing lignocellulose because of their ability to degrade lignin. Most white-rot fungi degrade cellulose and hemicellulose in addition to lignin (Blanchette, 1991; Daniel, 1994; Eriksson et al., 1990). However, there is substantial variability, both among species and within strains of a species, in the amount and the sequence of each cell wall component that is degraded. Some white-rot fungi preferentially degrade lignin and some hemicellulose but do not remove very much cellulose (Blanchette, 1991). Fungi with the capacity to selectively degrade lignin may be utilized for a wide variety of applications: delignification of wood, straw, and other lignocellulosic materials, increasing their digestibility for use by ruminant animals: biopulping processes; and biobleaching and bioremediation of recalcitrant environmental pollutants like polychlorinated biphenyl compounds (PCBs) and dioxins (Blanchette, 1995; Eriksson et al., 1990; Kirk et al., 1993; Messner and Srebotnik, 1994). Technological advances in biological processing with white-rot fungi are already occurring and will undoubtedly become more common in the future.

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Fungal Growth and Cell Wall Changes

White-rot fungi rapidly colonize wood and utilize wood sugars and other easily accessible nutrients. Once the wood structure is exposed, the xylem is colonized in all planes, first via vessels (tracheids in conifers) and ray patenchyma cells. Nutrient reserves within ray parenchyma cells are captured during incipient stages of decay, and colonization of parenchyma helps to distribute the fungal mycelium throughout the sapwood. Some fungi, such as *Ceriporiopsis subvermispora,* produce abundant calcium oxalate crystals on fungal hyphae as well as manganese deposits in localized areas. The deposition of calcium and manganese occurs in wood after a relatively short incubation with *C. subvermispora.* Pine wood chips inoculated before biomechanical pulping will exhibit enormous amounts of these crystalline compounds after only a few weeks of incubation (Figures 10.1 and 10.2).

Considerable research has been conducted by the authors to evaluate the changes that occur in wood after pretreatment with white-rot fungi prior to mechanical pulping processes. During the early stages of colonization by white-rot fungi, little to no lignin is removed from the wood but substantial alterations occur within the cell walls. The first evidence of these changes was recognized when refined fibers were evaluated by Simons staining (Blanchette et al., 1992). This differential stain caused the cut ends of refined fibers to stain yellow-orange if they were fibrillated or had increased porosity. Wood chips incubated with *C. subvermispora* before being refined showed extensive yellowing of fibers, whereas fibers from untreated wood stained blue. As time of incubation with *C. subvermispora* increased, the extent and

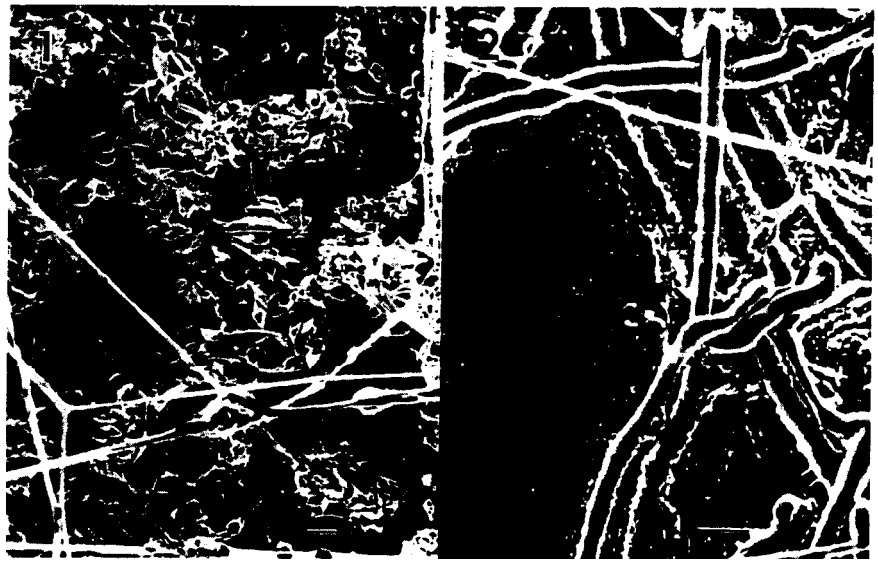


Figure 10.1 Colonization of pine wood by *Ceriporiopsis subvermispora.* Scanning electron micrograph of hyphae with calcium oxalate. Bar = 5 mm.
Figure 10.2 Colonization of pine wood by *Ceriporiopsis subvermispora.* Scanning electron micrograph of hyphae with manganese dioxide crystals. Bar = 5 mm.

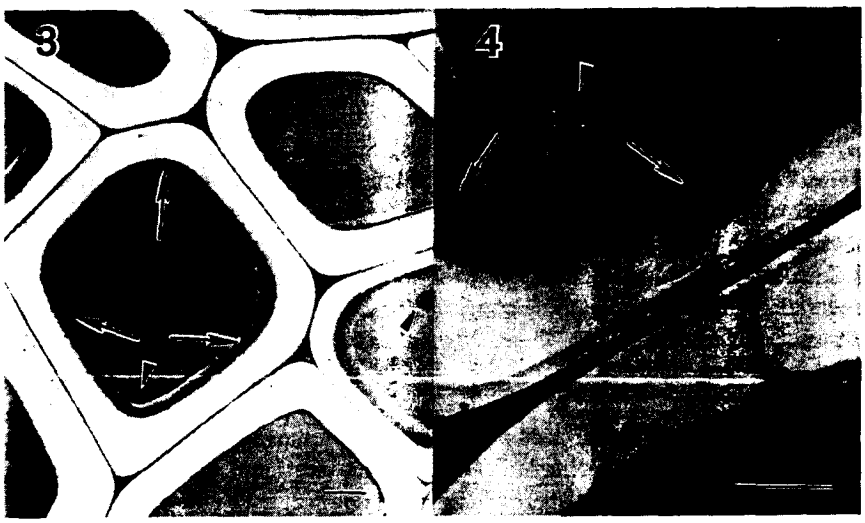


Figure 10.3 Transmission electron micrograph of wood colonized by *Ceriporiopsis subvermispora* stained with uranyl acetate. Hyphae in cell lumina *(arrowheads)* caused diffuse area of cell wall alteration within secondary walls *(arrows).* Dark staining by uranyl acetate shows extent of alteration. Bar = 2 mm.
Source: Blanchette. 1995.

Figure 10.4 Transmission electron micrograph of wood colonized by *Ceriporiopsis subvermispora* stained with uranyl acetate. Hyphae in cell lumina (arrowheads) caused diffuse area of cell wall alteration within secondary walls (arrows). Dark staining by uranyl acetate shows extent of alteration. Bar = 2 mm.
Source: Blanchette, 1995.

intensity of the yellow-orange stain in the fibers increased. Changes in cell wall characteristics and porosity appear responsible for the observed staining patterns (Yu et al., 1995).

Uranyl acetate staining and ultrastructural observations of the cell wall have provided additional evidence that changes in cell wall porosity occur early in the colonization process by white-rot fungi (Blanchette, 1995; Blanchette et al., in press). After two weeks of incubation, a zone of electron-dense staining occurred within cell walls directly beneath hyphae and along the outermost region of the secondary wall (Figure 10.3). In many cells, the staining affected the entire circumference of the secondary wall of colonized cell lumina. The depth of staining increased into the cell wall as incubation time was extended (Figure 10.4). Noninoculated wood observed after similar staining and sample preparation did not exhibit staining within the cell wall. Apparently, the cell wall architecture of sound wood does not allow penetration of uranyl acetate or binding to reactive sites on ceil wall components. In contrast, a zone of highly reactive secondary cell wall was found in cells colonized by the fungus, which demonstrates that appreciable changes were initiated in the wall in this early stage of colonization.

Immunocytochemical studies were also used to determine whether various-sized

enzymes could move into the cell wall after incubation of wood with *C. subvermispona*.. Treated and untreated wood was infiltrated with proteins of different molecular weights, followed by immunogold labeling. In tracheids from wood incubated for two weeks, gold-labeled insulin, with a molecular weight (MW) of 5,700D, was evident within the secondary wall in a narrow band around the circumference of the cell (Blanchette et al., in press). The penetration of the protein increased within the secondary wall after four and six weeks of incubation. Larger proteins, such as myoglobin (MW 17,600D), were observed within the wall four weeks after inoculation with *C. subvermispora*. No infiltration of the proteins was observed in untreated wood, indicating that the cell wall porosity was not sufficient to allow penetration.

It is clear from these varied studies that white-rot fungi such as *C. subvermispora* can have a substantial effect on wood cell walls within a relatively short time after inoculation. Although lignin does not appear to be removed in significant amounts during the first few weeks of colonization by white-rot fungi, substantial modification of the cell wall occurs. These changes are now beginning to be identified and understood through the use of various recently reported histological and ultrastructural techniques (Akhtar et al., 1995; Blanchette, 1995; Blanchette et al., in press).

BIOPULPING

Introduction

The pulp and paper industry utilizes chemical or mechanical pulping methods, or a combination of these, to produce pulps of desired characteristics. Chemical pulping involves the use of chemicals to degrade and dissolve lignin from the wood cell walls, releasing cellulose fibers. Chemical pulping processes yield pulps with high strength; however, these processes are polluting, low-yield (about 40-50%), and expensive. In the United States, almost all chemical pulps produced from wood are made with kraft processes. Mechanical pulping involves the use of mechanical force to separate the wood fibers. Mechanical processes are high-yield (up to 95%) and produce paper with high bulk, good opacity, and excellent printability. However, these processes are energy-intensive and produce paper with lower strength and high color reversion (tendency to turn yellow with time), as compared with chemical processes. Kraft pulps are often blended with mechanical pulps to increase paper strength.

Biopulping, defined as the treatment of wood chips with lignin-degrading fungi prior to pulping, appears to have the potential to overcome some problems associated with conventional chemical and mechanical pulping methods. Biopulping is an environmentally friendly technology that substantially increases mill throughput or reduces electrical energy consumption at the same throughput in conjunction with mechanical pulping. Electrical energy is the major cost of conventional mechanical pulping. By producing stronger pulp with longer fibers and increased fibrillation,

biomechanical pulping may reduce the amount of kraft pulp required to increase pulp strength.

Fungal pretreatment of nonwoody plant materials, including kenaf and jute, has been found to be effective for mechanical pulping (Sabharwal et al., 1994a,b, 1995, 1996; Young et al., 1994; cf. Chapter 14). Recent studies suggest that fungal pretreatment is also effective for depitching pine wood chips (Fischer et al., 1994, 1996; cf. Chapter 18), for improving dissolving pulp (Christov et al., 1996a,b; cf. Chapter 13), for benefiting thermomechanical and chemithermomechanical pulping (Myers et al., 1996), and for reducing cooking time in sulfite pulping (Fischer et al., 1994; Scott et al., 1995a,b, 1996; cf. Chapter 12).

The following sections summarize biomechanical pulping research and describe key findings.

Review of Literature

The use of white-rot fungi for the biological delignification of wood was perhaps first seriously considered by Lawson and Still (1957) at the West Virginia Pulp and Paper Company research laboratory (now Westvaco Corporation). These researchers published a survey of the literature (72 lignin-degrading fungi), which pointed to the dearth of knowledge about the fungal degradation of lignin. Subsequent work showed that paper strength properties increased with the extent of natural degradation of pine by white-rot fungi (Kawase, 1962; Reis and Libby, 1960). In 1972, Kirk and Kringstad showed that pretreatment of aspen wood chips with *Rigidoporus ulmarius* reduced the electrical energy requirement during refiner mechanical pulping and produced stronger paper than did control chips (Forest Products Laboratory Internal Report).

Related work was done at the Swedish Products Laboratory (STFI) in Stockholm, and the first published repeat on biopulping per se demonstrated that fungal treatment could result in significant energy savings for mechanical pulping (Ander and Eriksson, 1975). That research resulted in a U.S. patent that described a "method for producing cellulose pulp" (Eriksson et al., 1976). Considerable efforts by the Swedish group were directed toward developing cellulase-less mutants of selected white-rot fungi to improve the selectivity of lignin degradation and, thus, the specificity of biopulping (Johnsrud and Eriksson. 1985). Although this research met with limited success, it provided valuable insights (Eriksson et al., 1983; Eriksson. 1990).

Eriksson and co-workers (1980) showed that chip colonization is not the rate-limiting step in biopulping. Samuelsson and co-workers (1980) applied the white-rot fungus *Phlebia radiata* and its cellulase-less mutant to chips and pulp. The wild type of fungus increased the amount of energy required to obtain a certain tensile index, whereas the mutant slightly decreased the energy requirement. These authors also reported that treatment of fiberized pulp had a greater effect than did treatment of chips, Eriksson and Vallander (1982) treated wood chips (with or without added glucose) with white-rot fungi, in most cases a cellulase-less mutant of *Sporotrichum pulverulentum (Phanerochaete chrysosporium). The* energy required for defibration and refining decreased somewhat with increasing treatment time. They reported an

increase in tensile strength for chips impregnated with glucose prior to fungal inoculation. However, fungal treatment of chips, whether or not amended with glucose, decreased the light-scattering coefficient and increased the light-absorption coefficient.

Subsequent work in collaboration with cuban scientists with bagasse using mutant strains of the white-rot fungus *P. chrysosporium* gave more promising results (Johnsrud et al., 1987). These authors reported that treatment of bagasse with a cellulase-less mutant was not sufficient by itself as a pretreatment for the production of high-yield pulp. However, fungai pretreatment combined with a modified soda process resulted in good quality pulp. The properties of the pulp were found to be comparable to those of pulps obtained with conventional chemimechanical processing.

In their early work, the Swedish scientists primarily used the white-rot fungus *Spootrichum pulverulentum* Novobranova in work on various aspects of biopuiping. At the same time, research by the U.S. group focused on the mechanism of lignin degradation by a white-rot fungus tentatively referred to as *Peniophora* "G." Both fungi were chosen because they grew and degraded lignin quite rapidly in comparison to other fungi, and they also produced copious conidia, which made them easy to manipulate. It was a surprise to both laboratories when the two fungi were found to be synonymous; the species is now classified as *Phanerochaete chrysoporium* (Burdsall and Eslyn, 1974).

Bar-Lev and co-workers (1982) showed that treatment of coarse thermomechanical pulp with the white-rot fungus *P. chrysosporium* prior to secondary refining reduced the energy requirement by 25 to 30% and increased paper strength properties. Similarly, Pilon and co-workers (1982) reported an increase in paper strength properties when refiner mechanical pulp was treated with several fungi; *Polyporus versicolor* gave the best results. Subsequently, Abuhasan et al. (1988) subjected chemithermomechanical pulp to the white-rot fungus *P. chrysosporium* for two weeks and reported a loss of about 30% lignin. Paper strength properties were improved and less energy was required when the pulp was refined in a PFI mill.

Akarnatsu and co-workers (1984) demonstrated that poplar chips treated with 10 species of fungi reduced refining energy during thermomechanical pulping: three fungi *(Trametes sanguinea, T. coccinea,* and *Coriolous hirsutus)* improved breaking length and tear strength properties. Nishibe et al. (1988) selected *P. chrysosporium* and a litter-degrading fungus, *Coprinus cinereus,* from among 86 strains of 65 species of fungi and studied their effects on first- and second-stage refining of thermomechanical pulp. They also reported an increase in some strength properties. Nishida and co-workers (1988) developed a screening method for lignin selectivity that was used to identify the strains used for subsequent studies. Nishida and co-workers (1992) obtained a U.S. patent on the use of lignin-degrading fungi for the treatment of primary mechanical pulp.

Setliff and co-workers (1990) investigated the use of *C. subvermispora* and *P. chrysosporium* on both hardwood and softwood chips. Aspen chips treated with *C. subvermispora* required 20% less energy for mechanical pulping, whereas similarly treated Norway spruce required 13% less energy. Strength improvements were also

noted with some wood species. Pretreatment of black spruce minichips with either fungus marginally improved strength properties. Treatment of refiner mechanical spruce pulp with *C. subvermispora* also improved strength properties.

Planes and co-workers (1990) treated wood chips from Argentinean willow *(Salix* sp.) with two strains of *P. chrysosporium* and one strain of *Trametes trogii,* and then subjected the chips to cold soda pulping. The fungal pretreatment resulted in considerable savings in refining energy and did not affect paper strength properties unless the treatment was carried out in the absence of nutrients. The effectiveness of the fungal pretreatment was attributed to the softening and swelling of wood fiber cell walls by fungal enzymes.

Kashino and co-workers (1991) demonstrated a successful biomechanical pulping process of softwood (spruce and red pine) using the hyperligninolytic fungus IZU- 154. After a 10- to 14-day fungal treatment of coarse mechanical pulp, a significant energy reduction (by one-third) and an increase in strength properties were achieved. The authors also extended their studies to coarse mechanical pulps from both hardwood (beech) and softwood (spruce) species (Kashino et al., 1993). A 7-day treatment of coarse hardwood mechanical pulp with the fungus reduced refining energy requirements by about one- to two-thirds and improved paper strength properties. The identity of IZU- 154 has not been revealed.

Patel and co-workers (1994) reported the use of the white-rot fungus *Antrodiella* sp. RK1 in the biomechanical pulping process. After a four-week fungal pretreatment of aspen chips, the electrical energy necessary for fiberization by mechanical pulping was reduced by about 26% and strength properties were improved.

Pearce and co-workers (1995) screened 204 isolates of natural wood decay fungi for biomechanical pulping of eucalyptus chips. Some of these fungi saved 40 to 50% electrical energy in refining and resulted in greater brightness of unbleached pulp, as compared with that of pulps from untreated control chips. Some of the strains were found to be effective on unsterilized wood chips. In another study, Schmidt and co-workers (1996) reported a novel method for growing selected white-rot fungi on unsterilized wood chips. Chips in compressed bales inoculated with *P. chrysosporium* showed reduced energy requirements for mechanical pulping. Other details on biopulping research have been described in review articles and the literature cited therein (Akhtar et al., 1997a; Eriksson and Kirk, 1985; Kirk et al., 1992,1993,1994; Messner and Srebotnik, 1994).

Biomechanical Pulping

As the preceding discussion indicates, fungal pretreatment of wood chips for mechanical pulping can have certain benefits. Accordingly, a comprehensive evaluation of biomechanical pulping was launched in 1987 at the USDA Forest Service, Forest Products Laboratory (FPL), under the auspices of a Biopulping Consortium. The Consortium involved the FPL, the Universities of Wisconsin and Minnesota, and pulp and paper and related companies. The overall goal was to evaluate the technical feasibility of fungal pretreatment with mechanical pulping to save energy and/or improve paper strength. In addition, it was assumed that fungal pretreatment would

have less environmental impact than chemical pretreatment (which the results verified). The consortium research was conducted by fungal, pulp and paper, enzyme, molecular genetics, economics, engineering scale-up, and information research teams (see Kirk et al., 1993). However, in this chapter, we will focus on only the work conducted by the fungal and pulp and paper research teams; key findings are described in the following sections.

Selection of Lignin-Degrading Fungi Wood delignified by fungi has been a curious phenomenon in forests because of the unusual white colonization of the residual' wood, which consists primarily of cellulose. Investigators reported the occurrence of naturally delignified wood and examined its unique characteristics more than a century ago (Hartig, 1878; Philippi, 1893). In most temperate forests, delignified wood occurs in localized regions; the wood has the appearance of white-pocket or white-mottle rot. However, in some forests, such as the temperate rain forests of southern Chile, dead *Nothofagus trees* may be extensively delignified by fungi (Agosin et al., 1990; Blanchette, 1991; Dill and Kraepelin, 1986). Entire fallen trees have been found with the wood almost completely delignified by fungi. The residual decayed wood is composed of cellulose and occurs in such abundance that farmers in Chile use it as feed for cattle (Gonzalez et al., 1986, Philippi, 1893).

Investigations to characterize fungi that cause selective delignification and to elucidate patterns of cell wall degradation were recently reviewed (Blanchette, 1991, 1995; Daniel, 1994; Eriksson et al., 1990). Many white-tot fungi can preferentially remove lignin from wood under laboratory conditions (Figure 10.5) (Table 10.1). However, the incubation time necessary to achieve extensive lignin removal from wood is very long. Commercial applications of these fungi to remove large concentrations of lignin from wood will apparently not be immediately realized because of the time needed for delignification. However, recent biopulping experiments have shown that after only relatively short incubation times, appreciable changes take place in the treated wood, resulting in substantial benefits to the mechanical pulping process. Dramatic decreases in the electrical energy used to refine wood and increases in paper quality have repeatedly been demonstrated during biomechanical pulping experiments (Akhtar, 1994; Akhtar et al., 1993; Blanchette et al., 1992; Kirk et al., 1993).

The screening of white-rot fungi for the most efficient strains for biomechanical pulping has followed procedures for selecting fungi that preferentially degrade lignin (Blanchette et al., 1988, 1992; Otjen et al., 1987). Fast-growing species that rapidly colonize wood and show a preference for degrading lignin have been successfully used for biomechanical pulping. Recently, new methods of screening fungi for their potential in biomechanical pulping have been developed using Simons staining (Akhtar et al., 1995; Blanchette et al., 1992). Wood treated with various species or strains of white-rot fungi can be refined and stained, using Simons staining methods, to predict the amount of energy savings that will occur during biomechanical pulping. This relatively fast and easy method of fiber evaluation can be used to obtain reliable information on which fungi are able to provide the greatest

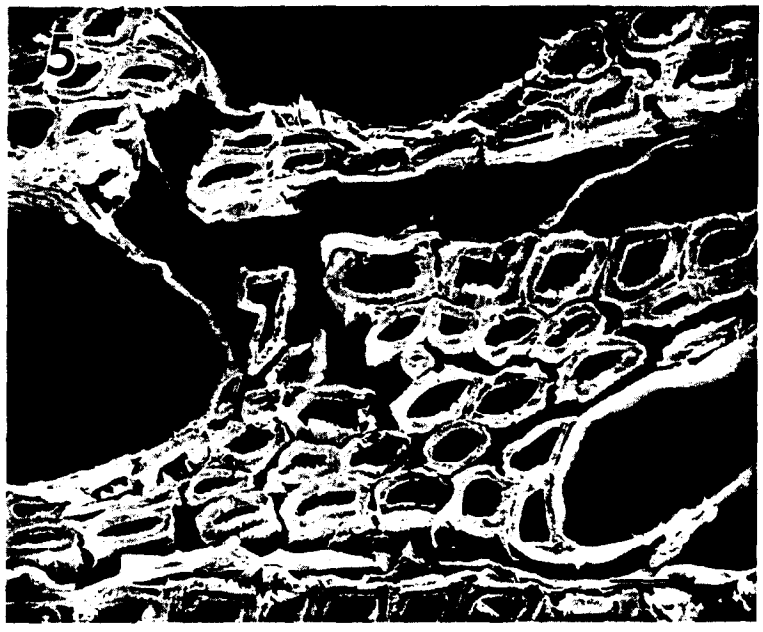


Figure 10.5 Scanning electron micrograph of transverse section of wood preferentially delignified by *Phlebia tremellosa* showing degraded middle lamellae and detached fibers. Bar = 50 mm.
Source: Blanchette, 1995.

energy savings (Akhtar et al., 1995). These techniques are now routinely used to help optimize the biopulping process as well as to select new species and strains.

Evaluation of Selected Fungi for Refiner Mechanical Pulping White-rot fungi screened in the wood decay test or with Simons stain were evaluated for their refiner mechanical pulping performance. The process involved the treatment of wood chips with the fungi in bioreactors on a bench scale at appropriate temperature and humidity, followed by mechanical pulping of control and fungus-treated chips in a single-disk atmospheric refiner, preparation of paper, and testing of paper for physical properties.

Three different types of bioreactors were designed and tested: a rotating drum bioreactor, a stationary tray bioreactor, and an aerated static-bed bioreactor (Kirk et al., 1993). In our studies, we employed the simple and inexpensive aerated static-bed bioreactor. Chips (1,500 g dry weight basis) were introduced into each 21 -liter bioreactor with water (usually containing nutrients) (Leatham, 1983), and the loaded bioreactors were usually sterilized by autoclaving. Chip moisture content was adjusted to 55 to 60% on a wet weight basis. The chips were then inoculated with the fungus and incubated at an appropriate temperature (39°C for *P. chysosporium* and 27°C for other fungi) for two to four weeks (usually two weeks) and aerated continu-

TABLE 10.1 Results of Lignin and Wood Sugar Analyses of Pine Wood Decayed for 12 Weeks by Various White-rot Fungi.[a]

	Loss (%)				
Fungus	Biomass	Lignin	Glucose (Glucan)	Xylose (Xylan)	Mannose (Mannan)
Ceriporiopsis subvermispora	25	50	3	48	13
Phanerochaete chrysosporium	20	31	4	44	0
Phlebia brevispora	27	49	11	60	16
Phellinus pini	27	45	8	55	40

[a]Composition of sound *Pinus strobus* = 33% lignin, 41% glucose, 8% xylose, and 11% mannose.

ously with humidified air (0.0227 vol/vol/min). Details are described in previous publications (Akhtar et al., 1992a,b, 1993; Kirk et al., 1993; Myers et al., 1988).

After harvest, the untreated control chips and the fungus-treated chips were fiberized by multiple passes through a Sprout-Waldron Model 105-A 300-mm-diameter single rotating disk atmospheric refiner. Energy consumed during fiberization and subsequent refining was measured using an Ohio Semitronic Model WH 30-11195 integrating Watt hour meter attached to the power supply side of a 44.8-kW electric motor. Pulps were then refined to Canadian Standard Freeness (CSF) values just above and just below 100 ml. (CSF is an arbitrary measure of the rate of water drainage from a pulp slurry). Handsheets (60 g m^{-2}) were made with control and fungus-treated samples and tested for physical properties using standard TAPPI methods. Energy values and physical properties were regressed to 100 ml CSF for comparison. Details on energy measurements, handsheet preparation, and testing methods have been described (Akhtar et al., 1992a,b, 1994; Myers et al., 1988).

Energy Savings and Physical Properties In early experiments, several white-rot fungi initially screened in wood decay tests were further screened for their biomechanical pulping performance using aspen (hardwood) wood chips (Akhtar, 1994, Leatham et al., 1990a,b.c; Myers et al., 1988). Based on energy savings and paper strength improvements, several fungi were initially selected: *Phanerochaete chrysosporium, Phlebia tremellosa, Phlebia subserialis, Phlebia brevispora. Dichomitus squalens, Poria medulla–panis, Hyphodontia setulosa,* and *Ceriporiopsis subvrmispora.* Of these, early emphasis was placed on *P. chrysosporium* because it was by far the most studied white-rot fungus and because it grows rapidly and competes well with indigenous microorganisms in wood chips. Occasionally, additional fungi identified for lignin selectivity, based on the wood decay test, were also evaluated for their biomechanical pulping efficacy.

Aspen was used for the initial research. As the research progressed, however, emphasis was given to screening the fungi on loblolly pine (softwood) chips because this species, together with other southern pines, is the major wood used in mechanical pulp mills in the United States. Some of the fungi that were effective on aspen wood chips saved energy and improved paper strength on loblolly pine as well, but

TABLE 10.2 Energy Savings and Changes in Physical Properties from Biomechanical Pulping of Aspen and Loblolly Pine Chips with Strain FP-90031-sp of *C.* subvermispora (4-Week Incubation).

	Savings/Changes Over Control (%)						
		Strength Properties			Optical Properties		
Wood	Energy	Burst Index	Tear Index	Tensile Index	Brightness	Opacity	Scattering Coefficient
Aspen	40	23	131	17	−18	−1.9	−33.8
Pine	42	32	67	−1	−22	−4.4	−30.9

Source: Akhtar et al., 1992a.

they adversely affected paper optical properties (Akbtar et al., 1993). Unfortunately, the most effective fungi on aspen were found to be relatively ineffective on loblolly pine chips. However, during the course of screening, we found that the relativetly uncommon fungus *C. subvemispora* was effective on both aspen and loblolly pine chips (Table 10.2). Although different strains of this fungus were found to vary in their effectiveness, several were effective on both aspen and loblolly pine chips (Blanchette et al., 1992). A U.S. patent was issued on the use of *C. subvermispora* for biomechanical pulping (Blartchette et al., 1991).

Pattern of Energy Savings As mentioned in the preceding discussion, a large amount of refining energy is required in mechanical pulping. The refining process consists of two phases: fiberization of the wood chips and refining of the fibers. Kurdin (1979) and Kano and co-workers (1982) showed that only a small portion of the total energy applied in mechanical pulping is used for fiberization (fiber separation). The major portion of the energy is used in refining, which breaks up the fiber walls and peels off the outer layers. We were interested in determining in which refining phase most of the energy is saved by fungal pretreatment.

Our results indicated that during biomechanical pulping of wood chips with different white-rot fungi, energy savings are rendered in the fiberization process. The most effective fungal treatments were estimated to save up to two-thirds of the energy required for fiberization. After fiberization, the energy required to reline pulps to a given freeness was the same for the controls and the fungus-treated chips (Leatham et al., 1990b). A notable exception was the treatment of aspen and loblolly pine with *C. subvemispora,* which gave significant energy savings during both fiberization and subsequent relining (Akhtar 1994).

Optimization for Refiner Mechanical Pulping Many variables can affect biopulping. In our initial work, we simply made best guesses based on the literature, knowledge of fungal growth, and past experience. However, as in any industrial microbial process, the opportunity to increase the effectiveness and efficiency of biopulping and decrease its cost through optimization of variables is great. Consequently, we

selected certain variables for initial optimization studies. Some of these results are described in the following text.

Wood Chip Sterilization In early studies, sterilization or partial decontamination of wood chips prior to fungal inoculation was required because biopulping fungi were not able to outcompete indigenous microorganisms in unsterilized wood chips. A notable exception was the fungus *P. chrysosporium,* which did colonize unsterilized wood chips and perform biopulping at its optimum growth temperature (39°C). Biopulping with unsterilized wood chips looks attractive; however, achieving reproducible results on a routine basis might be a problem.

Chemicals have also been used for decontamination, but in most cases they are expensive and reduce the growth of the desired microorganism. We discovered some relatively inexpensive chemicals that not only decontaminate wood chips but also stimulate the growth of the blopulping fungus. Of the various chemicals tested sodium bisulfite, sodium meta-bisulfite, and sodium hydrosulfite were found to be effective (600-1,200 ppm on a dry weight basis).

Subsequently, unsterilized aspen wood chips were treated with sodium bisulfite (1,200 ppm) solution and then inoculated with *C. subvermispora* (strain CZ-3) to determine biopulping efficacy. Sodium bisulfite-treated chips gave comparable results to those obtained with autoclaved chips. We also found that sodium bisulfite actually stimulates the growth of several biopulping fungi: *P. chrysosporium, P. tremellosa, P. brevispora, P. subserialis, H. setulosa,* and *C. subvermispora.* Amendment of a modified chemically defined medium (Leatham, 1983) with sodium bisulfite (500 ppm) increased radial growth significantly, as compared with a medium not amended with sodium bisulfite. A U.S. patent was obtained on the use of sodium bisulfite in biopulping (Akhtar et al., 1995).

Although the bisulfite is effective, we subsequently found that simple steaming is equally so. Thus, decontamination of the wood chip surfaces is sufficient and is easily achieved with atmospheric steam. Ten minutes of steaming of wood chips gave results comparable to those obtained with autoclaving. Subsequent results indicated that atmospheric steaming of wood chips for as little as 15 seconds is enough to allow the fungus to dominate the chips (unpublished data).

Inoculum In any industrial microbial process, the inoculum is of key importance. A number of inoculum variables are involved, including level, physical form, age, and viability. In a series of experiments we examined some of these variables.

The effect of different inoculum levels (2.%, 5.0%, 10%, and 20%, dry weight basis), using precolonized chips as inoculum, was studied with *P. chrysosporium* on aspen wood chips. The lowest inoculum level (2.5%) gave slightly lower energy savings than the other three treatments (Kirk et al., 1993).

We postulated that the addition of nutrient nitrogen to the inoculum would help build fungal biomass and vigor, which, in turn, should improve biopulping performance of *P. chrysosporium.* To test this hypothesis, two glutamic acid concentrations (500 ppm N and 5,000 ppm N) were added to the 5% wood chips inoculum prior

TABLE 10.3 Typical Composition of Corn Steep Liquor.

Dry substance	50.7%
pH	3.9
Protein	40.8% (dry basis)
Lactic acid	16.0% (dry basis)
Reducing sugars	12.8% (dry basis)
Miscellaneous compounds	30.4% (dry basis)

to introducing the fungus. The results suggest that increased inoculum nitrogen has beneficial effects in terms of energy savings. However, the weight loss stimulated by the high nitrogen (5,000 ppm N) concentration offset the benefits (Kirk et al., 1993).

In initial experiments such as those described in the preceding text, precolonized wood chips were used as an inocuium. In other experiments, we evaluated a liquid inoculum of *C. subvermispora* on loblolly pine chips. Because this fungus does not produce spores, fragmented mycelium was used (Fischer et al., 1994). At a level of 3 kg/ton (dry weight basis) of chips, the fungus reduced refiner electrical energy consumption by 19% and improved tear index by 28%, as compared with the control (Akhtar et al., 1997b). We estimated that this level of inoculum was too high to be practical and therefore sought ways to reduce it. A remarkable reduction was eventually achieved by adding corn steep liquor to the inoculum suspension. Corn steep liquor was selected because it is relatively inexpensive ($55/ton of semisolid liquid) and commercially available throughout the United States and in other countries.

Corn steep liquor is a condensed fermented corn extract that is produced in the corn wet-milling process when the dry corn is soaked (steeped) in a warm sulfurous acid solution. During the process, the grain solubles are released and undergo a mild lactic acid fermentation by naturally occurring bacteria. Corn steep liquor is mainly used as feed supplement for ruminants, a nutrient source for poultry, and a nutrient in industrial fermentation processes. The composition of corn steep liquor varies: a typical analysis is shown in Table 10.3.

The miscellaneous fractions of corn steep liquor contain metal ions, amino acids, vitamins, and other compounds in small quantities (parts per million basis). When 0.5% sterilized corn steep liquor was added to *C. subvermispora* mycelium (strain CZ-3), an inoculum level as low as 5g/ton gave energy savings equivalent to 3 kg inoculum without the added liquor. In subsequent studies, two strains (L-14807 SS-3 and SS-5) were more effective than CZ-3 at the lower inoculum level, with 0.5% sterilized corn steep liquor (Table 10.4). Interestingly, augmentation of strain L-14807 SS-3 inoculum (5g/ton) with 0.5% unsterilized corn steep liquor led to 34% savings in energy (Table 10.4). With aspen chips, L-14807 SS-3 inoculum (5g/ton) without corn steep liquor did not show any energy savings, whereas with 0.5% unsterilized corn steep liquor 38% energy savings was realized in two weeks (unpublished data).

The enhanced effect of unsterilized corn steep liquor (Table 10.4) could perhaps

TABLE 10.4 Energy Savings from Biomechanical Pulping of Loblolly Pine Chips with *C. subvermispora* Strains and Sterilized or Unsterilized Corn Steep Liquor.[a]

Corn Steep Liquor	Strains	Energy Savings over Control (%)
Sterilized	CZ-3	18
	FP-105752 SS-4	22
	L-14807 SS-1	18
	L-14807 SS-3	29
	L-14807 SS-5	28
	L-14807 SS-10	22
Unsterilized	L-14807 SS-3	34

[a]Chips inoculated with 5 g/ton of wood (dry weight basis); 0.5% corn steep liquor (dry weight basis); 2-week incubation.

Source: Akhtar et al., 1996, 1997b.

be due to denaturation of some proteins in the corn steep liquor by autoclaving, destruction of other compounds, or volatilization of compounds stimulatory to the fungi. The composition of corn steep liquor varies from one source to another, as well as from one batch to another. Unsterilized corn steep liquor obtained from different sources (Table 10.5) and batches (Table 10.6) did not seem to affect biopulping efficacy to a significant extent.

Unsterilized corn steep liquor (0.57% on a dry weight basis) was also found to be effective with other fungi on both loblolly pine (Table 10.7) and aspen chips (Table 10.8). Subsequent work indicated that an inoculum of lignin-degrading fungi could be further reduced to as low as 0.25g/ton with 0.5% unsterilized corn steep liquor

TABLE 10.5 Composition of Corn Steep Liquor from Various Sources and Effect on Energy Savings from Biomechanical Pulping of Loblolly Pine Chips with Strain L-14807 SS-3 of *C. subvermispora.*[a]

Composition or Energy Savings	CPC International Incorporated Argo, Illinois	American Maize-Products Company Hammond, Indiana	ADM Corn Processing Cedar Rapids, Iowa
Dry substance (%)	51	42	54
pH	3.9	4.0	4.2
Protein (%)	41	45	26
Lactic acid (%)	16	24	2.5
Sugars (%)	13	3	2.5
Energy savings over control (%)	32	36	30

[a]Chips inoculated with 5 g/ton of wood (dry weight basis); 0.5% unsterilized corn steep liquor (dry weight basis); 2-week incubation.

Source: Akhtar et al., 1997b.

TABLE 10.6 Composition of Various Batches of Corn Steep Liquor and Effect on Energy Savings from Biomechanical Pulping of Loblolly Pine Chips with Strain L-14807 SS-3 of *C. subvermispora.* [a,b]

Composition or Energy Savings	Batch 1	Batch 2	Batch 3
Dry substance (%)	56.1	50.7	53.0
pH	4.0	3.9	4.1
Protein (%)	41.2	40.8	39.8
Lactic acid (%)	17	16	16.8
Sugars (%)	15	12.8	11.7
Energy savings over control (%)	32	34	33

[a]Corn steep liquor batches (E802) were obtained from CPC International Incorporated, Argo, Illinois.
[b]Chips inoculated with 5 g/ton of wood (dry weight basis); 0.5% unsterilized corn steep liquor (dry weight basis); 2-week incubation.
Source: Akhtar et al., 1997b.

without sacrificing biopulping efficacy (Akhtar et al., 1997b). This amount of inoculum should be well within a commercial range.

Our ability to obtain reproducible results on a routine basis with the use of unsterilized corn steep liquor means that sterilization and the associated costs can be avoided, making the process more economically attractive. As anticipated, incubating unsterilized corn steep liquor on potato dextrose agar plates at 27–32°C for three to four days revealed the presence of a few bacteria and fungi (unpublished data), but at these low levels they obviously did not interfere with the biopulping performance of the fungi.

Inasmuch as corn steep liquor is produced throughout the United States and its composition (despite the variation) from one source to another or from one batch to another does not seem to affect the biopulping efficacy of the fungus, pulp and paper companies should be able to obtain a regular supply from the nearest location and minimize transportation costs. The component or components of corn steep liquor responsible for the beneficial effect are not known. **A** U.S. patent has been allowed on the use of corn steep liquor in biopulping (Akhtar, 1996).

Addition of Nutrients to Chips The effect of adding nitrogen and carbon sources to wood chips was studied with *P. chrysosporium* on aspen chips. Two different levels each of nitrogen (25 or 250 ppm N as glutamic acid, ppm, wood dry basis) and glucose (4,000 or 40,000 ppm) were added to bioreactors. The combination of 25 ppm nitrogen and 40,000 ppm glucose produced the best results in terms of energy savings. Subsequently, the effect of another nitrogen source (ammonium tartrate) (0, 36, 108, and 324 ppm N) was examined. We found that 324 ppm nitrogen gave the best results (Kirk et al., 1993).

TABLE 10.7 Energy Savings and Tear Index Improvement from Biomechanical Pulping of Loblolly Fine Chips with Lignin-Degrading Fungi with and without Unsterilized Corn Steep Liquor.[a]

Fungus	Corn Steep Liquor	Savings or Improvemer over Control (%)	
		Energy	Tear Inde
Phlebia brevispora HHB 7099	−	12	5
	+	16	13
Phlebia subserialis RLG 6074-sp	−	25	36
	+	33	44
Dichomitus squalens MMB 10963-sp	−	0	0
	+	18	41
Perenniporia medulla-panis HHB 12172	−	10	16
	+	19	34
Phlebia tremellosa FP 102557-2p	−	17	ND
	+	21	ND

[a]Chips inoculated with 5 g/ton of wood (dry weight basis); ± 0.5% unsterilized corn steep liquor (dı weight basis); 2-week incubation. ND = not determined.

The use of a nonchemically defined nitrogen source, yeast extract, was also investigated with both *P. chrysosporium* and *C. subvermispora* on aspen wood chips. With *P. chrysosporium,* two levels of nitrogen (108 and 324 ppm N as yeast extract) on a dry weight basis, were tested with a uniform level of 40,000 ppm lactose (dry weight basis). With *C. subvermispora,* three levels of nitrogen (108, 324. or 976 ppm N) with a uniform level of 4,000 ppm lactose (dry weight basis) were used. Energy savings of 32% with significant strength improvements after two-week incubation were obtained-one of the best results ever achieved with *P. chrysosporium.* With *C. subvermispora,* energy savings of 52% with strength improvements for the highest nitrogen level (976 ppm) were obtained; this was much better than average for this fungus on aspen chips.

Aeration Solid-substrate fermentations are known to be markedly affected by aeration. In addition, the ligninolytic activity of fungi depends on oxygen availability. Consequently, we evaluated the influence of three air flow rates on biopulping efficacy off? *chrysosporium* on aspen chips: low (0.001 vol/vol/min), medium (0.022 vol/vol/min), and high (0.100 vol/vol/min). The lowest flow rate was achieved using intermittent aeration and was suboptimal. The medium and high flow rates gave comparable energy savings and had similar effects on strength properties.

Wood Species As already mentioned, *C. subvermispora* was found to be effective on both aspen and loblolly pine chips. We recently evaluated this fungus on a mixture of softwood species and on spruce obtained from two paper companies in Wisconsin. In

TABLE 10.8 Energy Savings and Tear Index Improvement from Biomechanical Pulping of Aspen Chips with Lignin-degrading Fungi with and without Corn Steep Liquor.[a]

Fungus	Corn Steep Liquor	Savings or Improvement over Control (%)	
		Energy	Tear Index
Phlebia brevispora HHB 7099	−	0	0
	+	38	19
Phlebia subserialis RLG 6074-sp	−	0	0
	+	40	0
Phlebia tremellosa FP 102557-sp	−	0	0
	+	27	24
Hyphodontia setulosa FP 106976	−	0	0
	+	36	16

[a]Chips inoculated with 5 g/ton of wood (dry weight basis); ± 0.5% unsterilized corn steep liquor (dry weight basis); 2-week incubation.

both cases, fungal pretreatment saved substantial amounts of energy and improved the strength properties significantly (Table 10.9). It is important to emphasize here that the fungus, applied to spruce chips, saved energy and improved strength properties even in one week. In previous studies, this fungus did not show any appreciable biopulping effect in one week when applied to loblolly pine or aspen chips.

Wood Aging Wood batch and chip storage conditions (frozen, fresh, or aged for 3 to 5 days at room temperature) did not appear to affect biopulping results. However,

TABLE 10.9 Energy Savings and Strength Improvements from Biomechanical Pulping of Mixed Softwood Species and Spruce with Strain L-14807 SS-3 of C.subermispora.[a]

Wood/Incubation Time	Savings or Improvements over Controls (%)			
	Energy	Burst Index	Tear Index	Tensile Index
CONSOLIDATED PAPER COMPANY, INC.				
Mixed softwoods (2-week)	30	29	29	14
Spruce (1-week)	14	18	28	15
Spruce (2-week)	24	35	52	26
MOSINEE PAPER CORPORATION				
Mixed softwoods (2-week)	37	18	48	0

[a]Chips inoculated with 5 g/ton of wood (dry weight basis); 0.5% unsterilized corn steep liquor (dry weight basis); 1- or 2-week incubation.

TABLE 10.10 Energy Savings and Tear Index Improvement from Biomechanical Pulping of Fresh and Aged Loblolly Pine and Aspen Chips with *C. subvermispora* Strains.[a]

				Savings or Improvement over Control (%)	
Wood	Chip Condition	Strain	Incubation Time	Energy	Tear Index
Loblolly pine	Fresh	CZ-3	2-week	23	19
	Aged	CZ-3	2-week	16	0
	Fresh	SS-3	2-week	34	46
	Aged	SS-3	2-week	29	21
Aspen	Fresh	SS-3	2-week	38	0
	Aged	SS-3	2-week	13	0
	Aged	SS-3	4-week	37	48

[a]Chips inoculated with 5 g/ton of wood (dry weight basis); 0.5% unsterilized corn steep liquor (dry weight basis); 2- or 4-week incubation.

a high amount of inoculum was used in these studies. We were interested in determining whether aging of wood chips would affect biopulping if low inoculum were used. Our results indicate that the fungus performs significantly better on fresh chips (both loblolly pine and aspen chips) than on aged chips at a low inoculum level (Table 10.10).

Wood Chip Movement During Incubation In early experiments, fungus sensitivity to chip movement during treatment was studied by comparing stationary versus rotating drum bioreactors with aspen and *P. chrysopsporium.* Chip movement affected the extent of chip degradation, energy consumption during refining, and paper strength properties (Leatham et al., 1990a). Later studies, however, showed that shaking the chips once a week in the aerated static-bed bioreactors during a four-week incubation had no appreciable effect on energy savings or paper strength properties.

Superiority of Phlebia subserialis In previous studies, we identified *C. subvermispora* as the best biopulping fungus on both hardwood and softwood species. We recently discovered another fungus, *P. subserialis,* which gives results comparable to those obtained with *C. subvermispora.* Of importance is that *P. subserialis* has a broader temperature range than does *C. subvermispora.* For example, with pine, *P. subserialis* saved 17, 33, 34, 18, and 12% energy at 22, 27, 32, 35, and 39°C, respectively, in two weeks, as compared with the control, whereas *C. subvermispora* saved 18, 33, 32, and 0% energy at 22, 27, 32°C, and higher temperatures, respectively. *P. subserialis* also produces less aerial hyphae than does *C. subvermispora* on wood chips.

Interestingly, *P. subserialis* - treated chips are not as soft (and presumably not

TABLE 10.11 Bleaching Results with Aspen Control and Biopulps *(P. chrysosporium).*

Pulp	Chemical Charge	Brightness (%)
Control	Unbleached	62.2
	2%H_2O_2	80.0
	1%$Na_2S_2O_4$	71.2
Biopulps	Unbleached	51.8
	3%H_2O_2	76.0
	1%$Na_2S_2O_4$	59.3
	Two-step bleach (1) 3%H_2O_2 (2) 1%$Na_2S_2O_4$	78.0

as compressible) as those treated with *C. subvermispora.* These advantages of *P. subserialis* over *C. subvermispora* should reduce the cost of biotreatment significantly during scale-up (decreased need for temperature control and greater ease of aeration). These points are discussed in detail in Chapter 11. A U.S. patent has been filed on the use of this fungus in the biomechanical pulping process.

Bleaching Studies Fungal pretreatment significantly reduces the brightness of the resulting mechanical pulps. However, experiments showed that the pulps can be bleached with either alkaline hydrogen peroxide or sodium hydrosulfite. The biomechanical pulps gained more brightness points than did corresponding untreated control pulps under the same bleaching conditions. Even so, because the initial brightness values of the biomechanical pulps were lower than those of the corresponding untreated control pulps, the bleached brightness values were not as high at a given chemical charge as those of untreated contsol pulps. Biomechanical pulp was readily bleached to about 60% Elrepho brightness with 3% hydrogen peroxide and 1% sodium hydrosulfite—a brightness suitable for newsprint (data not shown); brightness values approaching 80% were achieved with a two-step bleach sequence (Table 10.11). Subsequent experiments demonstrated that aspen chips treated with *C. subvermispora* respond similarly to bleaching. Thus, bleachability of biomechanical pulps does not appear to be a problem. Details can be found in a previous publication (Sykes, 1993).

Brightness stability was evaluated by subjecting handsheets from bleached pulps to accelerated thermal- and photo-aging tests (Sykes, 1993). The stability of biomechanical pulps was slightly lower than that of control pulp. The ease of bleachability of the pulps suggests that the chromophores are in part quinonoid in nature.

Analyses of Effluents Samples of the wastewater from the first refiner passes of aspen chips treated with either *P. chrysosporium* or *C. subvermispora* were analyzed for biochemical oxygen demand (BOD), chemical oxygen demand (COD), and Microtox toxicity (Sykes, 1994). Fungal pretreatment decreased effluent toxicity sub-

TABLE 10.12 An Example of Wastewater Characteristics for First Refiner Pass Fffluents from Control and Fungus-treated Aspen Chips.

Treatment	BOD[a] ($g\ kg^{-1}$ pulp)	COD[b] ($g\ kg^{-1}$ pulp)	Toxicity ($100\ EC50^{-1}$)
Control	40	74	17
C. subvermispora	36	100	4

[a]Biochemical Oxygen Demand.
[b]Chemical Oxygen Demand.

stantially, decreased or increased BOD values slightly, but increased COD w-dues somewhat as compared with those of the control effluents (Table 10.12). Increased COD values may be due to the release of biorecalcitrant lignin-related products by fungal action.

Evaluation of Selected Fungus for Thermomechanical (TMP) and Chemithermomechanical (CTMP) Pulping We recently evaluated the effectiveness of *C. subvermispora* pretreatment for TMP and CTMP processes. Two strains, CZ-3 and SS-3, were evaluated on loblolly pine chips, and one strain, SS-3, was evaluated on lodgepole pine chips. A two-week treatment with 5 g/ton of inoculum containing 0.5% unsterilized corn steep liquor was used with strain SS-3, whereas 1 kg/ton of inoculum was used without corn steep liquor with strain CZ-3. Atmospheric refining, handsheet preparation, and testing were described earlier (Akhtar et al., 1992a,b, 1993; Myers et al., 1988). The percentage of energy savings or changes in physical properties reported here is based on their respective control values. Standard deviation values were taken into account for interpretation of results.

TMP Preparation At harvest, control and fungus-treated chips were passed once through an Andritz Sprout-Bauer model 12-1CP 305-mm-diameter pressurized refiner, fitted with plate pattern D2B505. Chips were steamed for 5, 10, or 20 min at 69 or 207 kPa before fiberization with a 0.254-mm plate gap; the treatments are described in Table 10.13. Chip feed rate through the pressurized refiner varied between 259 and 817 g of chips per minute (dry weight basis). Fiberized chips were then refined in a Sprout-Waldron model 105-A 300-mm-diameter atmospheric refiner, through multiple passes, to reach 100 ml freeness.

CTMP Preparation The pressurized and atmospheric refiners described here were also used for CTMP preparation. Chips were steamed for 5 or 10 min at 207 kPa before fiberization at 0.254- to 0.305-mm plate gap (Table 10.13). Chemical injection (alkaline peroxide (AP) CTMP) and chip impregnation (alkaline sulfite (AS) CTMP) procedures were used to produce two kinds of pulps. The chemical injection procedure utilized a positive displacement metering pump to deliver solutions composed of sodium hydroxide, hydrogen peroxide, sodium silicate, diethylenetria-

TABLE 10.13 Conditions Used for TMP and CTMP Processes.

Pulping Process/ Wood Species	Treatments[a]	Chip Steaming Pressure (kPa)	Steaming Time Before Refining (min)	Number of Replicates
		TMP		
Loblolly pine	Control	69	10	1
	CZ-3	69	10	2
	Control	207	5	2
	CZ-3	207	5	2
	Control	207	10	4
	SS-3	207	10	4
Lodgepole pine	Control	207	20	1
	SS-3	207	20	1
		AP-CTMP (2% NaOH and 3% H_2O_2 Applied by Injection into Refiner)		
Loblolly pine	Control	207	5	2
	CZ-3	207	5	2
	Control	207	10	4
	SS-3	207	10	4
		AS-CTMP (2% NaOH and 4.6% Na_2SO_3 Applied by Impregnating Chips)		
Loblolly pine	Control	207	5	2
	CZ-3	207	5	2
	Control	207	10	4
	SS-3	207	10	4

[a] Strains of *Ceriporiopsis subvermispora* were used in a 2-week treatment. Controls were noninoculated chips.

mine pentamethylene acetic acid, and magnesium sulfate to the pressurized refiner horizontal feed screw housing at a point approximately 152 mm before the refiner plates. The impregnation procedure involved steaming the wood chips for 10 min at 138 kPa, followed by immediate submersion in a sodium sulfite solution for a 30 min soak at atmospheric pressure. Excess solution was drained from the chips, which were placed in the pressurized refiner digester to start the fiberization procedure.

TMP and CTMP with Strain L-14807 SS-3

LOBLOLLY PINE The fungal pretreatment on loblolly pine saved 25, 29, and 32% energy during TMP, AP-CTMP, and AS-CTMP production, respectively. Density and burst index were relatively unaffected by fungal pretreatment. For TMP, tear index was essentially unaffected, whereas it was significantly increased for

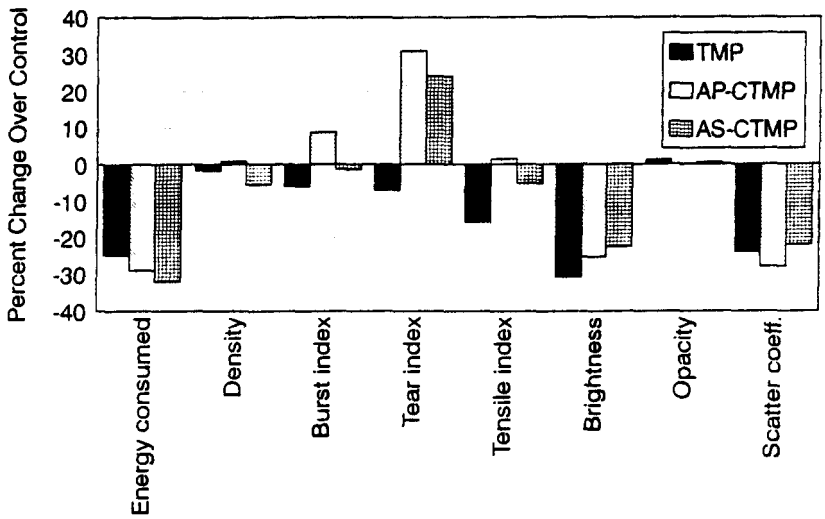


Figure 10.6 Change in different parameters resulting from fungal pretreatment (Strain L-14807 SS-3 of *Ceriporiopsis subvermispora)* of loblolly pine during thermomechanical pulping (TMP), alkaline peroxide chemithermomechanical pulping (AP-CTMP), and alkaline sulfite chemithermomechanical pulping (AS-CTMP) as compared with control.

AP-TMP and AS-CTMP pulps. Tensile index was reduced slightly for TMP. Brightness and light-scattering coefficients decreased, whereas opacity was not affected. These results are shown in Figure 10.6.

LOOGEPOLE PINE The fungal pretreatment on lodgepole pine saved 43% energy for TMP production and yielded handsheets with about the same strength properties as those of the control. Opacity was unaffected, whereas brightness and light-scattering coefficient decreased significantly. Additional tests revealed an increase in Pulmac shive values, which indicates that the fibers did not fully separate, leaving more fiber bundles. Average fiber length increased slightly, which indicates that more of the original fiber length was retained during fiberization and refining. The fines content also increased, probably as a result of the removal of material from the fiber surface rather than fiber breakage. Such surface removal was confirmed by the decrease in fiber coarseness. An increase in paper smoothness indicates that these longer, less coarse fibers probably bonded better. The increased fines content would also contribute to a smoother paper surface. These results are shown in Figure 10.7.

TMP and CTMP with Strain CZ-3 on Loblollv Pine During TMP, AP-CTMR and AS-CTMP production, fungal pretreatment on loblolly pine with strain CZ-3 saved 11, 6, and 18% energy, respectively (Figure 10.8). Burst index increased significantly for papers from TMP and AP-CTMP, but it was not affected for paper from AS-CTMP. The fungal pretreatment increased the tear index for papers from all

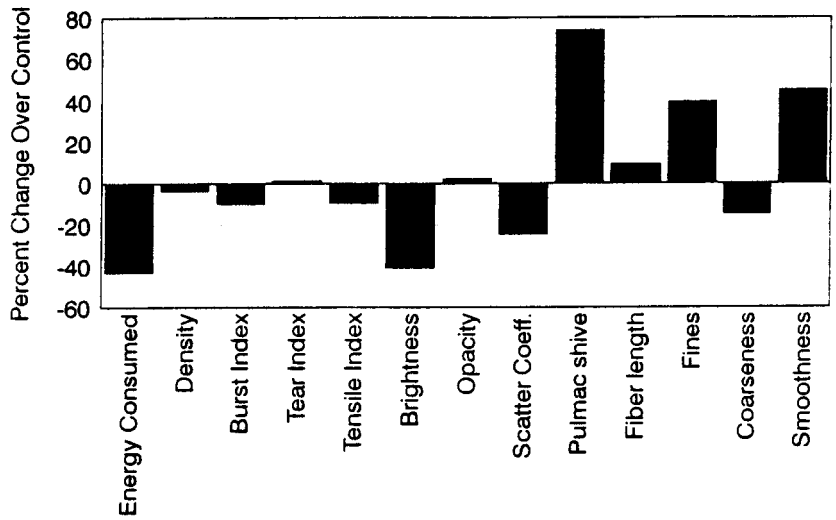


Figure 10.7 Change in different parameters resulting from fungal pretreatment (Strain L-14807 SS-3 of *Ceriporiopsis subvermispora)* of lodgepole pine during TMP as compared with control.

three pulps, especially TMP and AP-CTMP. Tensile index was also somewhat increased. There was a decrease in brightness of papers from the TMP and AP-CTMP processes; opacity was not affected. Light-scattering coefficient was decreased by fungal pretreatment for all three processes. These results were obtained with chips steamed at 207 kPa for 5 min prior to refining. Another experiment was performed with TMP, in which the chips were steamed at 69 kpa for 10 min prior to refining, to determine whether steam pressure and time together have some effect on biopulping performance of the fungus. The fungal pretreatment saved 37% energy over the contro1. However, no significant improvements in paper strength properties were noted, in contrast to results at the higher pressure. Brightness and light-scattering coefficient were decreased, and opacity did not change. Results from the two steaming conditions are shown in Figure 10.9.

The basis for the property changes resulting from fungal pretreatment has not yet been investigated. Where and how the fibers separate during pulp preparation have a major impact on paper performance (Salmen and Patterson, 1995). As mentioned earlier, a refiner mechanical pulping (RMP) procedure was used in previous biopulping research to prepare the pulp (Kirk et al., 1993). RMP separates the fiber within the cell wall, but it breaks fibers and generates a high percentage of fines. The TMP process also generally separates the fibers within the cell wall, but it retains more of the original fiber length and causes less fiber breakage and less tines content. Processing conditions can greatly affect the properties of TMP, as illustrated in Figure 10.9. Changing the temperature used in preparing TMP can shift the fiber separation zone and have a major impact on pulp and paper properties. Too high a temperature in the pressurized refiner will shift the separation zone into the

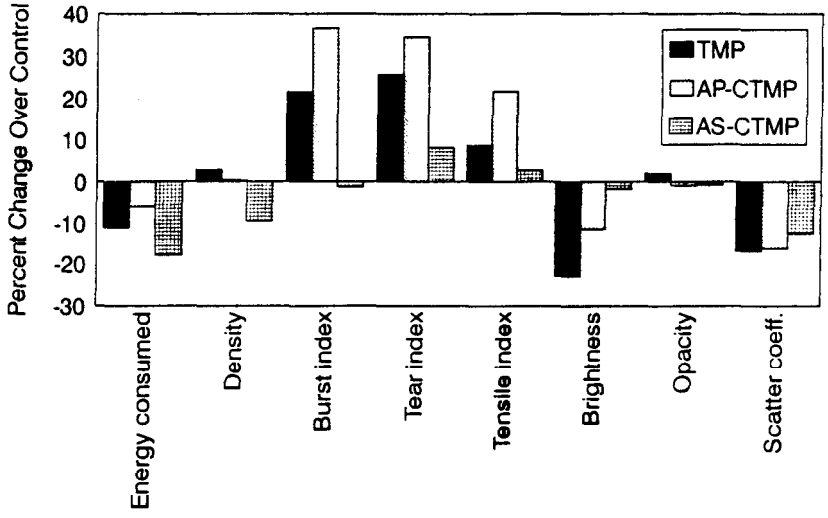


Figure 10.8 Change in different parameters resulting from fungal pretreatment (Strain CZ-3 of *Ceriporiopsis subvermispora)* of loblolly pine during TMP, AP-CTMP, and AS-CTMP as compared with control.

lignin-rich middle lamella, yielding a very smooth, lignin-encased fiber that defies refining for papermaking. Adding chemicals to the wood chips can increase energy consumption and will also lower the lignin softening point. The fungus used in this study is a lignin-degrading fungus, which either consumes and/or modifies the lignin. This also might change the lignin softening point, and each strain might have a different temperature preference. Depending on the interaction of fungus activity, fiberization temperature, and chemical addition, the lignin softening temperature and subsequent fiber separation point might be significantly modified. Whenever energy consumption was reduced and strength properties increased, the individual or combined effects were beneficial. Because the fungi used in biopulping alter the cell wall, in as yet ill-defined ways, it is to be expected that optimum conditions for producing TMP will differ from those for chips not treated with fungi.

The results described here clearly demonstrate that two strains of the same fungus affect the three pulping processes differently. Moreover, the two wood species respond differently. These various results show that fungal pretreatment and pre- and postrefining conditions must be optimized for further improvements.

Microscopy Studies To gain insight into the mechanism of biopulping, we examined at the microscopic level the fungal growth patterns of *P. chrysosporium* and *C. subvermispora* in aspen wood chips. *P. chrysosporium* grew well both across the chip surfaces and throughout the cell walls (Figure 10.10). The hyphae penetrated the chips through the lumens of wood vessels and fiber cells, as well as through natural wood cell pits and fungal bore holes. Partial degradation of the cell lumen walls was evident. Erosion troughs and localized wall fragmentation or thinning

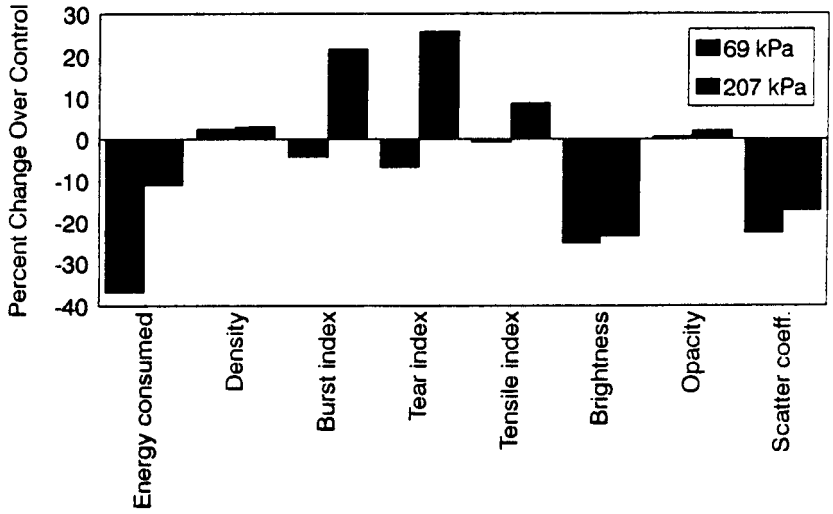
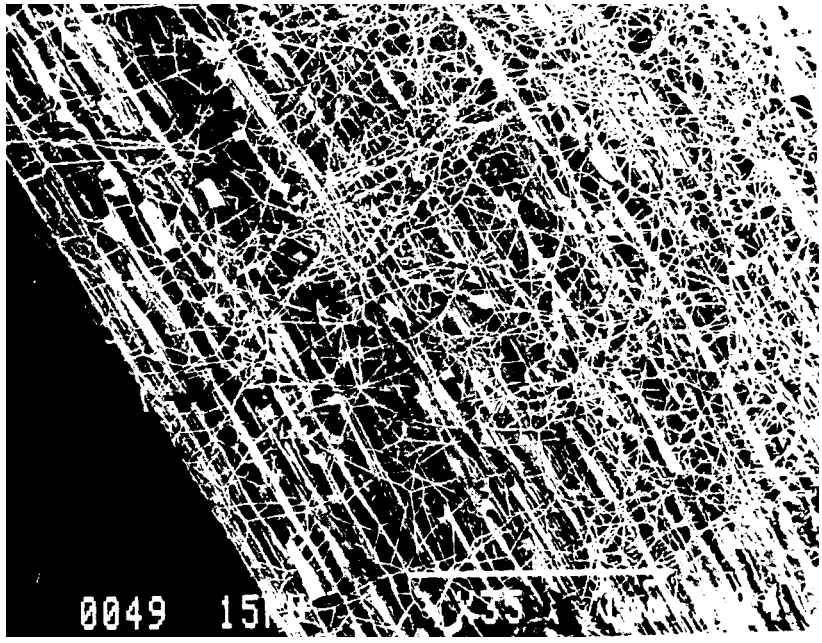


Figure 10.9 Change in different parameters resulting from fungal pretreatment (Strain CZ-3 of *Ceriporiopsis subvermispora)* of loblolly pine during TMP using two steaming pressures (69 and 207 kpa).

were clearly visible, as was a generalized swelling and relaxing of the normally rigid wood cell wall structure. *C. subvermispora* - treated aspen chips showed packed hyphae within the ray cells. Numerous crystals of calcium oxalate were found on the hyphae, during both the incipient and advanced stages of growth (Figure 10.1). Our observations suggest that the physical basis for the biopulping efficacy of the fungal treatment is likely to involve an overall softening and swelling of the cell walls, as well as thinning and fragmentation in localized areas (Sachs et al., 1989) (Figure 10.11).

Scanning electron microscopy was used to compare qualitatively biomechanical pulps with conventional mechanical and chemical pulps. We observed increased fibrillation on biomechanical pulp as compared with refiner mechanical pulp (control). The biomechanical pulp fibers appeared more woolly, looser, and more uniform in length than the conventional mechanical and chemimechanical pulp fibers. Fiber bonding in handsheets produced from biomechanical pulp fibers appeared to be similar to that observed in handsheets produced from chemical pulps (Sachs et al., 1990b). To gain insight and to assess visually how fiber morphology may have contributed to handsheet properties, we studied cross sections of handsheets. Handsheets made from mechanically processed pulps showed uncollapsed fibers, leading to poor conformability and reduced bonding. The kraft pulps yielded handsheets that exhibited fibers of enhanced compressibility and conformability. Handsheets prepared from biomechanical pulps visually resembled the kraft handsheets, exhibiting good compressibility and conformability of the fibers (Sachs et al., 1990a). We also learned that chip size has only a minor effect on the biopulping efficacy of the fungus on aspen wood chips (Sachs et al., 1991).



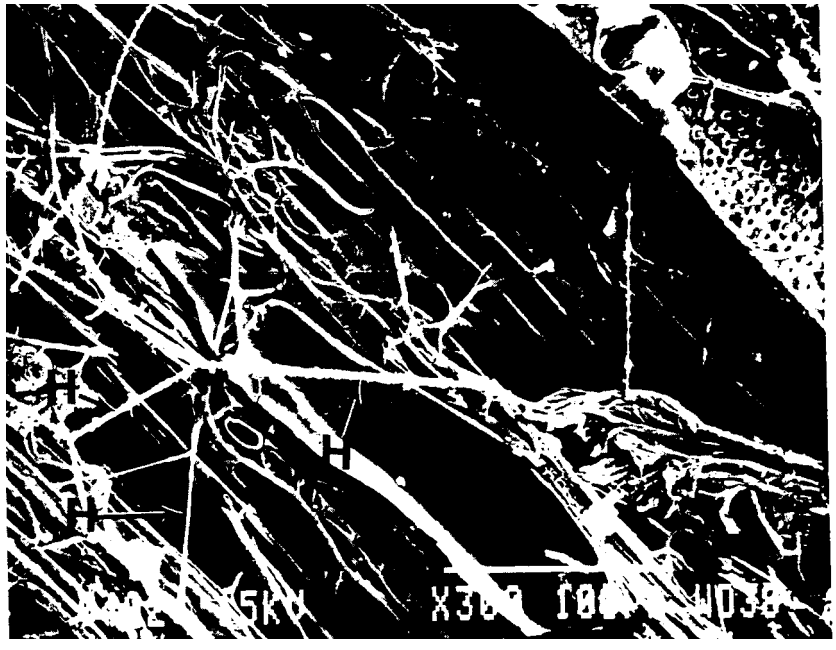


Figure 10.10 Weblike hyphal network on surface of nutient-supplemented aspen wood chip during 3-week treatment by *Phanerochaete chysosporium (top)* and hyphae (H) bridging vessles or fiber bundles on wood chip surfaces with little contact with wood *(bottom)*. Bar = 1mm.
Source: Sachs, et al., 1989.

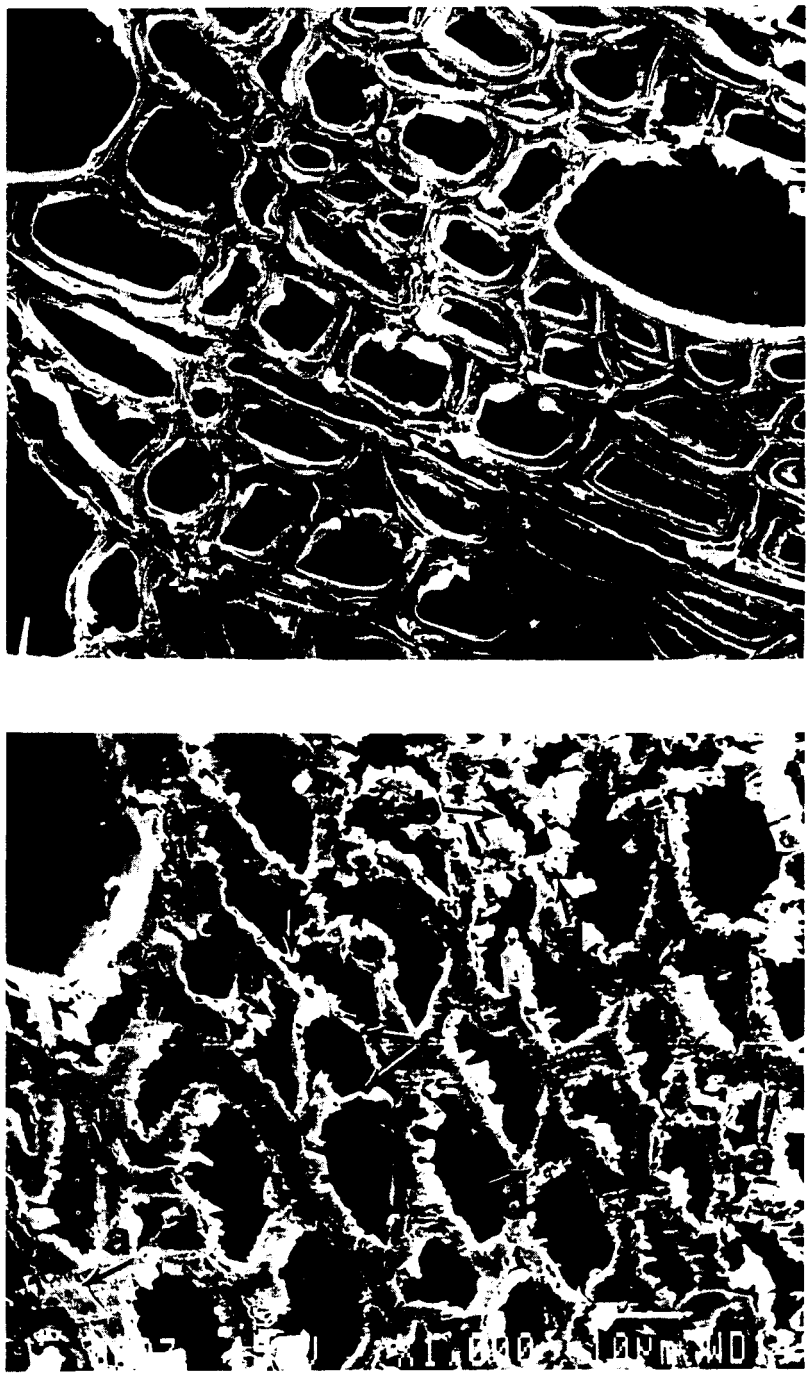


Figure 10.11 Normally rigid cell wall structure within aspen chips *(top)* was modified by 3-week treatment with *P. chrysosporium (bottom).* Modifications included cell wall swelling *(a)*, enzymatic softening or relaxing resulting in partial collapse of tubelike structure *(b)* and localized areas of wall thinning *(c)* or fragmentation *(d).* Bar = 10 mm.
Source: Sachs et al., 1989.

CONCLUSIONS

Some selected lignin-degrading fungi can alter cell walls of wood in a short period after inoculation. These changes are beginning to be identified and understood through the use of various histological and ultrastructural techniques. Results demonstrate that lignin modification, rather than removal, is involved during biopulping. A relatively rapid screening method has been identified (Simons staining method) that predicts biopulping efficacy of the fungus. Pretreatment of wood chips with lignin-degrading fungi for refiner mechanical pulping increases mill throughput or saves a substantial amount of electrical energy during refining at the same throughput, results in stronger paper, and lowers the environmental impact of pulping. Optical properties are diminished; however, brightness can be restored readily with peroxide bleaching. Pretreatment of wood chips with a lignin-degrading fungus shows promise for thermomechanical and chemithermomechanical pulping, but fungal pretreatment and pre- and postrefining conditions have to be optimized for improvements in physical properties. Although previous work on biopulping had been minimal, a comprehensive evaluation of biopulping at the Forest Products Laboratory showed that the fungus can be economically grown on wood chips in an outdoor chip pile-based system. Current research is focused on the use of fungal pretreatment for nonwoody plants and kraft pulping.

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Edited by

Raymond A. Young and Masood Akhtar

University of Wisconsin
Madison, Wisconsin

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