

13 Fungal Pretreatment for Organosolv Pulping and Dissolving Pulp Production

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INTRODUCTION

Biopulping is the fungal pretreatment of wood chips, designed as a solid-state fermentation process, for production of mechanical or chemical pulps (cf. Chapters 10 and 12). The concept of biopulping is based on the ability of some white-rot fungi to colonize and degrade selectively the lignin in wood, thereby leaving the cellulose relatively intact. This chapter presents an evaluation of biopulping and biobleaching with white-rot fungi as pretreatment steps in organosolv pulping and in dissolving pulp production. Fungal-organosolv pulping is focused mainly on chemical and top-chemical bases for the combination of biopulping and organosolv processes. Experimental data are presented to illustrate the potential of this fungal-chemical method to produce wood pulps. Biopulping and biobleaching with selected white-rot fungi are also evaluated as potential methods for production of dissolving pulp. A report in part has been submitted for publication in *Enzyme and Microbial Technology*.

This chapter is based mainly on the work from a collaborative project between the Department of Microbiology and Biochemistry, The University of Orange Free State, of Bloemfontein South Africa and the USDA Forest Products Laboratory in Madison, Wisconsin. Fungal-organosolv pulping methods are based mainly on the work developed at the Department of Biotechnology in Lorena, Brazil, and the Renewable Resource Laboratory in Concepcion, Chile.

ORGANOSOLV PULPING

Background

The need to find new technologies for pulp and paper production that cause less environmental impact has contributed to an increase in the number of studies on

Environmentally Friendly Technologies for the Pulp and Paper Industry, edited by Raymond A. Young and Masood Akhtar
ISBN 0-471-15770-8 © 1998 John Wiley & Sons, Inc.

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production in recent years. Energy savings and strength improvements in refiner mechanical pulping have been successfully demonstrated for the use of white-rot fungi in the pretreatment of wood chips (Akhtar et al., 1992, 1993, 1995; Leatham et al., 1990; Myers et al., 1988; Setliff et al., 1990). Kraft cooking of *Phanerochaete chrysosporium*-degraded aspen wood chips also showed increases in pulp yield and in tensile, burst, and fold properties, related to the incubation time in the fungal pretreatment (Oriran et al., 1990).

Another interesting aspect to be considered in combined fungal-chemical pulping is the delignification topochemistry. Since fungal biodelignification occurs mainly in the wood cell walls (Blanchette et al., 1987; Eriksson et al., 1990), a following chemical treatment should preferentially solubilize middle lamella lignin, which is achieved in some organosolv processes (Pazner and Behera, 1989; Bendzala and Kokta, 1995).

Chemistry and Topochemistry Aspects

Relatively few fungal-organosolv delignification methods have been studied. Most of the available literature is related to the chemical and topochemical aspects of wood biodegradation and organosolv pulping considered as separate treatments; however, a review of these concepts indicates that wood delignification by fungi and organosolv processes present complementary chemistry and topochemistry.

Wood Biodegradation

Lignin biodegradation has been studied exhaustively through several approaches, such as degradation of low molecular weight lignin-model compounds, isolated lignins, ¹⁴C-labeled lignins, and lignocellulosic materials. Typical reactions caused by basidiomycetes during lignin biodegradation, namely depolymerization, including carbon-carbon and beta-O-aryl-ether cleavages, oxidative degradation of side chain of the polymer, and oxidative ring opening, were demonstrated (Higuchi, 1990; Robert and Chen, 1989 and references therein). C-alpha-C-beta and beta-O-aryl-ether cleavage were also reported as the mechanisms for lignin biodegradation by ascomycetes (Ferraz and Durán, 1995; Rodriguez et al., 1996).

The topochemistry involved in lignin removal by white-rot fungi is an important aspect to be considered in combined fungal-chemical pulping processes. Fungal wood colonization occurs mostly by hypha penetration through the lumen of vessels and ray parenchyma cells (Eriksson et al., 1990; Fengel and Wegener, 1989). As a consequence, lignin biodegradation starts at the secondary walls and is progressive until reaching the compound middle lamella. A comprehensive study by Blanchette and co-workers (Blanchette et al., 1987), using EDXA-SEM to evaluate lignin distribution in the cell walls of decayed wood samples, showed that middle lamella lignin is significantly removed only at advanced stages (3-month periods) of wood biodegradation by *Phlebia tremellosus* and *Phellinus pini*. Cell corner-lignin was the most resistant to fungal delignification, as showed in Figure 13.1.

In some cases of selective lignin biodegradation, fiber cells lacking a middle

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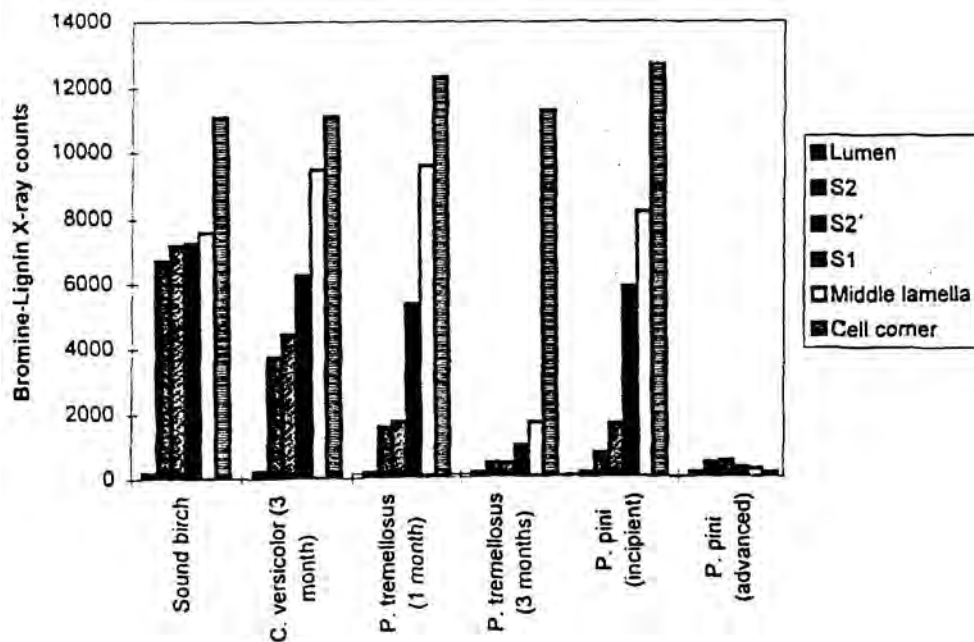


Figure 13.1 Topochemistry of wood delignification by white-rot fungi. (Redrawing with permission from Blanchette et al., 1987.)

lamella can be observed (Blanchette et al., 1987; Blanchette, 1984). An example of selective lignin biodegradation followed by fiber liberation is shown in Figure 13.2. Unfortunately, extensive lignin degradation as shown in Figure 13.2 is achieved only at advanced stages of wood decay produced after long biodegradation times.

Selective lignin biodegradation is essential in a combined fungal-organosolv pulping method. Fungal species should be selected based on their ability to degrade lignin, but minimal cellulose degradation is also necessary. Cellulose degradation in the fungal pretreatment means loss in yield and pulp quality in the subsequent chemical processing.

Acid-Organosolv Pulping

It is well established that hardwoods are easier to delignify with organic solvents than softwoods (Sarkanen, 1980; McDonough, 1993). Besides the differences in the total lignin content, the structural differences of lignins also play an important role. Hardwood lignins are richer in alpha-O-aryl-ether linkages that are easily split in acid-organosolv pulping. Moreover, the higher amount of syringyl units inhibits condensation reactions (McDonough, 1993).

The chemistry of organosolv pulping has been evaluated by means of kinetic delignification studies and by lignin and lignin-model compounds acydolysis studies in nonaqueous media. Delignification kinetic studies were carried out for alcohols (mainly ethanol and methanol) (Pereira et al., 1986; Tirtowidjojo et al., 1988; Faass

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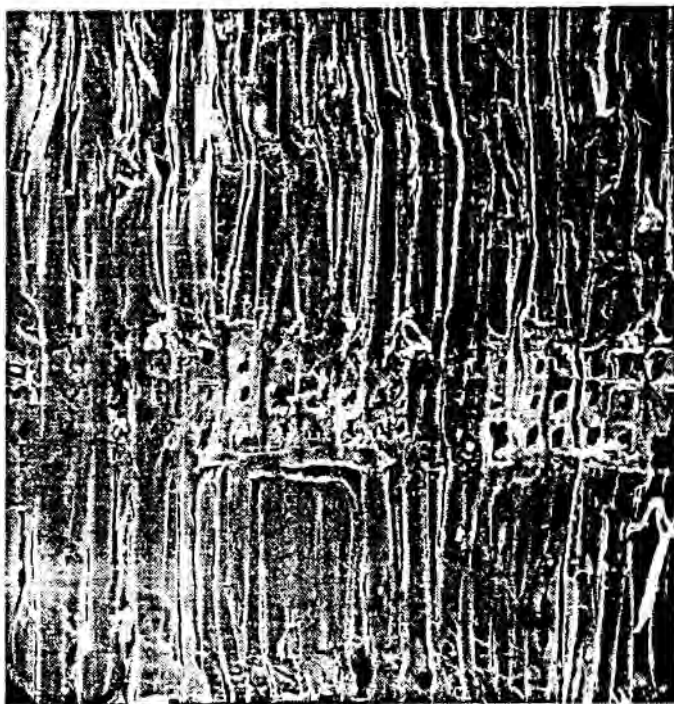


Figure 13.2 Scanning electron microscopy (155 \times) of a white pocket produced by *Ganoderma aplanatum* acting over *Pinus radiata* softwood for 140 days. (Photomicrography by A. Ferraz.)

et al., 1989), for organic acid solvents (Pascoal Neto and Robert, 1992; Young and Davis, 1986), and for acid-catalyzed organic solvents (Parajó et al., 1993; Erismann et al., 1994; Vazquez et al., 1995a). In most of these systems the delignification kinetics fit equations for two simultaneous first-order processes. The first one is the faster and corresponds to the bulk delignification phase, where most of the lignin solubilization occurs. The final phase corresponds to a slow-rate delignification. Table 13.1 shows some activation energy data for the bulk delignification phase. Although there is high discrepancy in the published results, the activation energy values are similar to, or lower than, the activation energy for acid-catalyzed cleavage of alpha-O-aryl-ether bonds in lignin model compounds, which were reported to fall between 80 and 118 kJ/mol depending on the substituent (Mesghini and Sarkanen, 1989). The cleavage of beta-O-aryl-ether bonds has higher activation energy, 150 kJ/mol (Sarkanen and Hoo, 1981), and seems not to occur in the bulk delignification phase of acid-organosolv pulping. Nevertheless, hydrolysis of beta-O-aryl-ether lignin model compounds by concentrated organic acids and beta-O-aryl-ether cleavage in acid-catalyzed organosolv delignification have been reported. Actually, the extent of cleavage of this linkage type is variable and clearly dependent on the reaction severity (McDonough, 1993).

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TABLE 13.1 Activation Energy for the Bulk Delignification Phase in Organosolv Processes.

Organosolv Process	Wood Species	Activation Energy (kJ/mol)	Reference
Methanol-water (7:3) and 0 to 0.05 M H ₂ SO ₄ catalyst	<i>Populus thioarpa</i> (Black cottonwood)	80.3	60
Ethanol-water (1:1) uncatalyzed	<i>Eucalyptus globulus</i>	26.0	59
Formic acid-water (49:1) and 1.2 M HCl catalyst	<i>Pinus radiata</i>	30.0	65
Acetic acid-water (7:3) uncatalyzed	<i>Eucalyptus globulus</i>	78.2	66
Acetic acid-water (7:3) and 0.027 M HCl catalyst	<i>Eucalyptus globulus</i>	66.5	66

Polysaccharide reactions during acid-organosolv cooking are also characterized by two parallel reactions that fit first-order kinetic models with distinct rates. In the first one, the most significant reaction is the hydrolysis of polyoses, whereas cellulose hydrolysis occurs only at elevated temperature or long reaction time (Vazquez et al., 1995b).

The papermaking properties of acid-organosolv pulps are lower than those of kraft pulps prepared from the same wood species (Johansson et al., 1987; Sundquist et al., 1988). The way chemical composition affects pulp papermaking properties is not completely understood, especially in considering organosolv pulps (Johansson et al., 1987; Sundquist et al., 1988; Young, 1985, 1994; Young and Rowell, 1996). However, low kappa numbers are obtained only under conditions where high amounts of polyoses are hydrolyzed (Johansson et al., 1987; Young and Davis, 1986; Parajó et al., 1993; Vazquez et al., 1995a). The lower content of polyoses may be one of the factors related to the inferior papermaking quality of organosolv pulps (Genco et al., 1990). Furthermore, at elevated temperature and high acid concentrations, cellulose also can be partially depolymerized. The degree of polymerization of cellulose has been shown to diminish as a function of the cooking time in organosolv processes (Pascoal Neto and Robert, 1992; Young and Davis, 1986).

It's clear that a compromise between lignin and polysaccharide hydrolysis occurs during acid-organosolv pulping. High temperature, long cooking time (before condensation reactions start to be significant), and high catalyst acid concentration facilitate delignification, but these conditions also cause the hydrolysis of polyoses and partial hydrolysis of cellulose. As a consequence, selective delignification under mild cooking conditions is feasible only if the lignin macromolecule is partially depolymerized in a previous step.

The topochemistry of delignification is also particular to acid-organosolv pulp-

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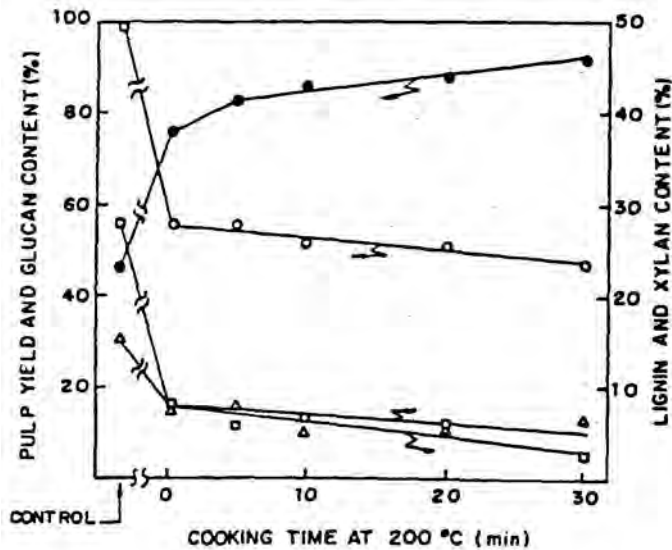


Figure 13.3 Pulp yield and chemical composition of unclassified pulps obtained after cooking undecayed *Eucalyptus grandis* wood in methanol/water at 200°C; (-O-) pulp yield, (-■-) lignin, (-●-) glucan, and (-triangle-) xylan content on pulp basis. Control shows the chemical composition of untreated wood chips (Ferraz and Durán, 1993).

occurred simultaneously. During the experiments carried out at 200°C with *G. itcpcfu* wood, a high delignification rate in this initialhulk-combined phase was also observed. The time required for the reactor to heat up to 200°C (16–18 min) and cool off again was practically enough for the delignification process. With additional reaction times of 10, 20, and 30 min, the residual lignin and xylan contents decreased slowly, as shown in Figure 13.3. Decayed wood samples were delignified in the same organosolv system by heating the reactor to 200°C (16–18 minutes) and then cooling it down to 30°C. The yield of residual delignified wood and the chemical composition of the residues are presented in Table 13.2. The chemical compositions of the pulps obtained from decayed and undecayed wood samples were similar, and only the sample decayed by *T. versicolor* for 1 month resulted in a lower residual lignin concentration. This was also the only sample selectively biodelignified during the fungal pretreatment (Ferraz et al., 1993).

The results indicate that one aspect that must be considered in combined fungal-chemical pulping methods is the severity of the reaction in the chemical process, which makes it difficult to observe differences in the chemical cooking behavior of sound and decayed wood samples. As a consequence, low severity in the chemical cooking is essential not only to prevent carbohydrate degradation, but also to show the actual effect of the fungal pretreatment.

A significant increase in the organosolv delignification rate was observed for the cooks of the 1-month-decayed wood samples (pretreated with *T. versicolor* as previously described) at 180°C as shown in Figure 13.4 (Ferraz et al., 1996a). A

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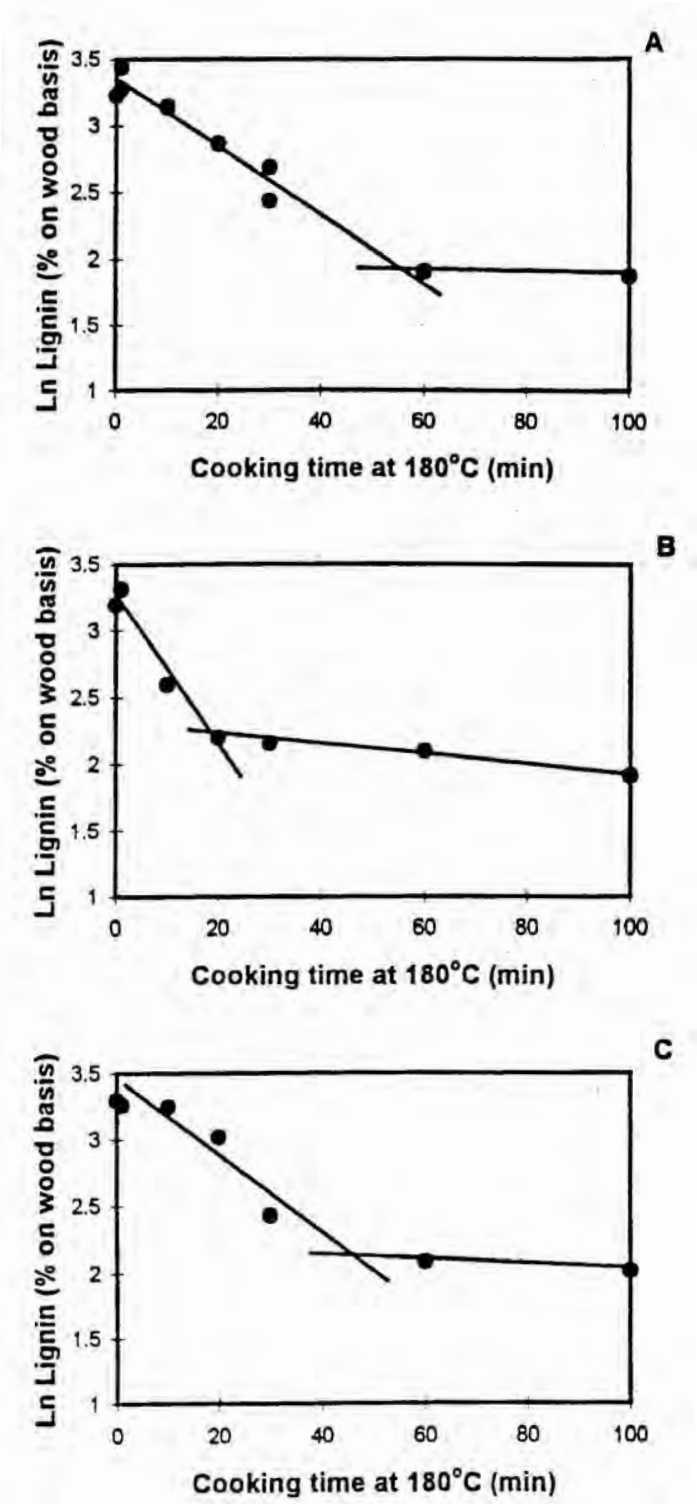
TABLE 13.2 Yield and Chemical Composition of *Eucalyptus grandis* Wood Samples Cooked for One Minute in Methanol/Water at 200°C.

Wood Sample	Yield (%)	Chemical Composition (%)		
		Glucan	Xylan	Total Lignin
Undecayed control	55.8	75.6	8.1	8.0
Decayed by <i>Trametes versicolor</i>				
1 month	57.8	77.9	7.6	6.1
2 months	78.0	75.7	7.1	8.0
4 months	55.0	78.8	9.1	9.1
Decayed by <i>Phanerochaete chrysosporium</i>				
1 month	64.2	68.8	9.1	11.3
2 months	54.9	76.0	8.6	9.3
4 months	56.3	74.9	8.6	13.4

comparison of the delignification kinetics showed that the same amount of residual lignin (6-8% on wood basis) was achieved by cooking both the wood samples, the undecayed and the 1-month decayed by *T. versicolor*; for 60 and 20 min, respectively (Figure 13.4). These results were the first indication that energy savings could be expected from organosolv pulping of samples previously biodelignified. Furthermore, pulps of increased strength properties could be expected by pulping wood chips at a shorter reaction time.

To evaluate how the fungal pretreatment affected the delignification rate constants of the organosolv process, a larger set of fungal-pretreated samples were delignified at 180°C in the same methanol/water solvent system previously described (Ferraz et al., 1996b). The biodegradation was carried out with three different fungal species at wood weight losses ranging from 1.5 to 27.1%, as showed Table 13.3. Organosolv delignification and xylan removal rate constants obtained from the bulk phases are also included in Table 13.3. Most of the decayed samples had higher delignification rates in the organosolv process. The maximal increase (3.9 times) was observed with the sample decayed by *T. versicolor* at 27.1% weight loss. Significant delignification rate increases were also obtained for samples decayed at low weight loss levels. An example is the sample pretreated by *Punularia artropurpurascens* at 1.5% weight loss that produced a 2.6 times delignification rate increase (Table 13.3). Faster delignification rates provided a shorter time for the change from bulk to the final delignification phase. As a consequence, the same residual lignin contents were achieved at shorter reaction times for the fungal-pretreated samples, meaning energy savings in the organosolv process. Nevertheless, in the final delignification phase the residual lignin decreased slowly from 8 to 6% (wood basis) for sound wood samples and for most of the fungal-pretreated wood samples. Residual lignin contents lower than 6% were obtained only in samples heavily degraded in

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the fungal pretreatment (weight losses higher than 15%). The xylan removal rate also increased significantly in samples pretreated for long biodegradation periods (Table 13.3). Extremely high xylan removal rate constants were observed in samples pretreated by *P. artropurpurascens*. From the pulping point of view, increases in the xylan removal rate constant should be avoided, because they mean decreases in the pulp yield and quality (Genco et al., 1990). Therefore, the choice of the best fungal pretreatment should be based not only on the delignification rate increase, but also on consideration of the xylan removal constant. Fungal species that provide increases in both lignin and xylan removal rates should be more suitable for pretreatment in organosolv pulping.

Figure 13.5 shows the delignification kinetic rate constants as a function of the weight and component losses caused by fungal pretreatment. These plots show that the delignification constants could not be correlated with weight, xylan, and glucan losses, because very low correlation coefficients were observed. Only the lignin loss seems to correlate with the organosolv delignification rate constant, inasmuch as the highest correlation coefficient was obtained ($r^2 = 0.70$). Multiple linear regression also did not provide statistically significant models ($P < 0.1$) to predict the delignification rate constant as a function of weight and component losses with fungal pretreatment.

The lack of correlation between delignification constants and the wood weight and component losses indicates that other wood modifications caused by the fungal pretreatment must be involved in the behavior of these wood samples in organosolv pulping. These results corroborate the initial idea that a partial lignin depolymerization with fungal pretreatment is enough to produce increases in the organosolv delignification rates. Actually, lignin loss represents the total lignin mineralization, and a better parameter to evaluate fungal pretreatment should be chosen. Perhaps estimation of the residual beta-O-aryl-ether linkages by acydolysis procedures would be suitable for this purpose. Moreover, no correlation between lignin loss and energy savings or increases in pulp strength properties in the biomechanical pulping process was observed (Leathan et al., 1990).

Morphological wood modifications such as ray parenchyma cell and pit opening biodegradation should provide easier penetration of the cooking liquor and, therefore, may also play an important role in the organosolv pulping behavior of decayed wood samples (Eriksson et al., 1990; Sachs et al., 1989).

The studies presented here showed that significant organosolv delignification rate increases are possible by means of fungal pretreatment of hardwood chips. Recently, additional positive results were obtained with the white-rot fungus *Cerioporiopsis subvermispota* for pretreatment of the softwood *P. radiata* in bioreactor experiments (unpublished results). Wood chips pretreated for 60 days in a bioreactor (2.5 kg of

Figure 13.4 First-order kinetic plot of residual lignin content in unclassified pulp obtained by cooking decayed and undecayed *Eucalyptus grandis* wood in methanol/water at 180°C. (A) Undecayed control; (B) decayed for one month by *Trametes versicolor*; (C) decayed for one month by *Phanerochaete chrysosporium* (Ferraz et al., 1995).

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TABLE 13.3 Fungal Pretreatment of *Eucalyptus grandis* Wood Chips and Kinetic Parameters of Its Organosolv Delignification at 180°C in a Mixture of Methanol/Water (78:22) Containing 25 mM Each of MgSO₄ and CaCl₂ (5L).

Fungal Species	Weight and Component Losses (%)				Delignification Constant (10 ⁻² min ⁻¹)	Xylan Removal Constant (10 ⁻² min ⁻¹)
	Weight	Lignin	Xylan	Glucan		
Undecayed control	0	0	0	0	2.6	2.6
<i>Trametes versicolor</i>	10 ± 1	19 ± 5	15 ± 2	4 ± 1	5.5	3.4
	17 ± 1	22 ± 2	17 ± 2	19 ± 3	7.3	9.0
	27 ± 1	31 ± 3	31 ± 3	25 ± 2	10.1	9.3
<i>Phanerochaete chrysosporium</i>	9 ± 1	4 ± 1	13 ± 4	11 ± 2	2.6	3.9
	16 ± 3	12 ± 4	22 ± 2	18 ± 2	6.2	5.5
<i>Punctularia arthropurpurascens</i>	2 ± 1	4 ± 1	6 ± 2	0 ± 2	6.8	98.7
	8 ± 1	14 ± 3	11 ± 6	4 ± 4	5.7	92.1

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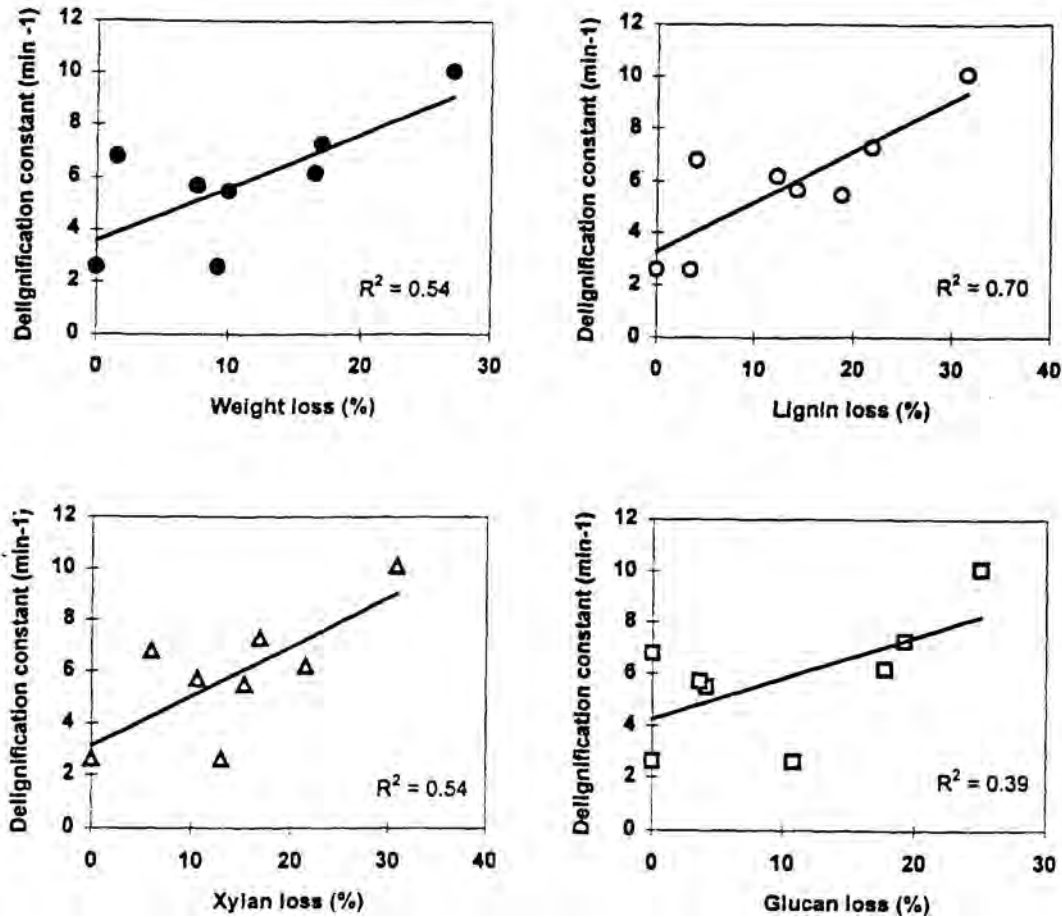


Figure 13.5 Relationship between organosolv delignification constants and weight and component losses in the fungal pretreatment of *Eucalyptus grandis* wood (Ferraz et al., 1996a,b).

fresh wood chips inoculated with 100 g of 1-month cultured wood chips) suffered a 9.6% weight loss. The sample was cooked at 150°C in a formic acid/etone (7:3) organosolv system (Erismann et al., 1994); the results are summarized in Figure 13.6. In addition to the increase in the delignification rate, the residual lignin in the pulp was significantly lower in samples pretreated with *C. subvermispora*. At the end of the cook, the fungal-pretreated sample yielded a pulp with 4% residual lignin (pulp basis) corresponding to a kappa number of 27. At the same cooking time the residual lignin in the undecayed control was 11% (pulp basis). The polyoses removal rate was similar for both decayed and undecayed wood samples, as showed in Figure 13.6. However, significant amounts of polyoses can be retained with a cooking time of only 30 to 50 minutes, which gives pulps with higher residual lignin.

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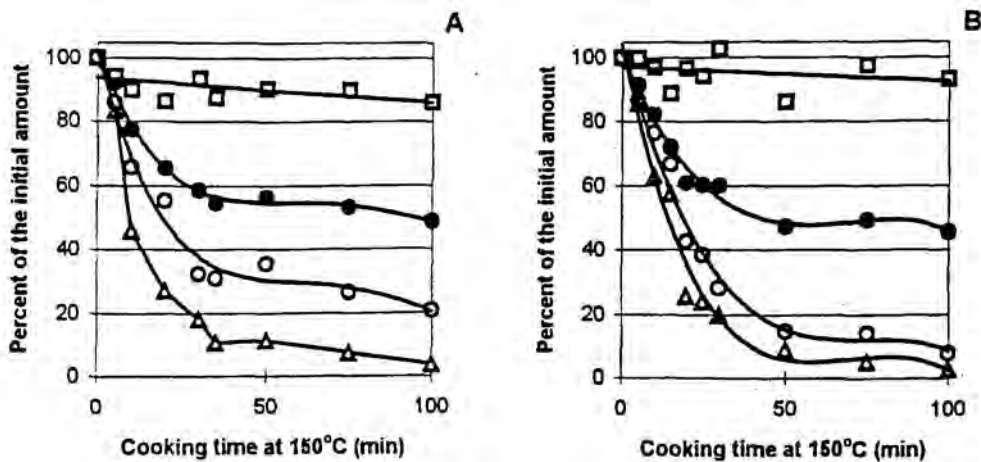


Figure 13.6 Formic acid/acetone (7:3) cooking of *Pinus radiata* at 150°C. (A) Sound wood; (B) wood chips pretreated by *Ceriporiopsis subvermisporea* for 60 days; (-O-) pulp yield; (-■-) lignin, (-●-) glucan, and (-triangle-) xylan.

DISSOLVING PULP PRODUCTION

Background

Dissolving pulp is a low-yield chemical pulp (30–35%) with a high cellulose content (95–98%) and relatively low hemicellulose (1–10%) and lignin (<0.05%) content. It is manufactured by both prehydrolysis-kraft and acid sulfite methods. The end uses of dissolving pulp include cellophane and rayon (after regeneration of cellulose from cellulose xanthate), cellulose esters (acetates, nitrates, propionates, and butyrates), cellulose ethers (carboxymethyl cellulose, methyl and ethyl cellulose), graft and cross-linked cellulose derivatives (Sjöström, 1981; Hinck et al., 1985; Biermann, 1993; Rowell and Young, 1978; Young, 1985). For instance, cellulose acetates are widely used in films, eyeglass frames, cigarette filters, and so forth, whereas carboxymethyl cellulose finds applications as a thickener, as a detergent, in cosmetics, and in other products.

Requirements

The hemicellulose in dissolving pulps is, in contrast to that in pulps used for papermaking, is undesirable and currently removed together with lignin during the pulping and bleaching stages. However, part of the hemicellulose (mainly xylan in hardwoods and mannan in softwoods) remains in the pulp after bleaching, causing certain problems in the viscose process (Hinck et al., 1985). The strength properties of the viscose end product are strongly influenced by the hemicellulose content of dissolving pulp. The higher the degree of polymerization (DP) of the pulp, the higher the end product strength. The efficiency of conversion of cellulose into the specific derivative is dependent on the xylan and mannan content of the dissolving

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pulp as well. High viscose yields require low hemicellulose levels in the cellulose. In addition, a reduction in the fiber swelling and penetration rate of NaOH into the cellulose fibers is caused by excessive amounts of hemicellulose present in pulp. The optical properties of cellulose acetates and nitrates can be affected negatively by the hemicellulose contaminants in cellulose (Rydholm, 1965). Thus, in dissolving pulp processing, the hemicellulose has to be removed or degraded substantially to ease the conversion of cellulose to its end-use product.

Bleaching of Pulps

The bleaching of both sulfite and prehydrolysis-kraft pulps is achieved by removal of residual lignin to increase the final brightness and the alpha-cellulose content of dissolving pulp. It involves the use of a number of bleaching chemicals containing chlorine or oxygen that are more specific to lignin removal than to carbohydrate removal. As a result, chlorinated lignin derivatives are formed and dissolved during the alkali extraction stages as degradation products from the reaction between residual lignin in the pulp and the chlorine-containing bleaching agents. Some of the chlorolignins of low molecular mass have mutagenic and toxic properties and are known to be highly resistant to biodegradation and to accumulate in aquatic organisms (Ander et al., 1977; Landner, 1979).

Biotechnological Approaches

The release of the bleach plant effluents with high absorbable organic halogen (AOX) levels into the receiving waters has become one of the major environmental problems for the pulp and paper industry in recent years. In response to environmental concerns and stringent emission standards, modifications of the production process at the pulping and bleaching stages have been developed. These imply extending the cooking time for additional lignin removal, introduction of oxygen delignification as a pretreatment step, or ECF and TCF bleaching (cf. Chapter 7).

A biological alternative to hemicellulose and lignin removal from dissolving pulp is to apply microorganisms (white-rot fungi) or enzymes (xylanases or lignin-degrading enzymes) to alleviate the heavy chemical loads during pulping and bleaching (cf. Chapter 6). Some of the advantages resulting from the possible implementation of the biotechnological methods in the manufacture of dissolving pulp would be (1) a savings in chemicals for pulping and bleaching, (2) improvement in the quality of the dissolving pulp, and (3) improvement in the effluent quality.

Biosulfite Pulping with White-Rot Fungi Because of the selective modification of lignin in wood by the white-rot fungi, its degradation and removal with pulping and bleaching chemicals become easier. This advantage of biopulping could be used not only for papermaking pulps but for dissolving pulp production as well. In fact, this is the first report on the application of biosulfite pulping to prepare dissolving pulp with improved properties.

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TABLE 13.4 Biosulphite Pulping of Eucalyptus Wood with Five Strains of *Ceriporiopsis subvermispota*.^a

Strains	S ₁₀ (%, w/w)	S ₁₈ (%, w/w)	α-Cellulose (%, w/w)	Kappa Number	Brightness (%, ISO)	Viscosity (cP)	Yield (%, w/w)
Control	9.2	6.8	90.7	8.9	51.5	48.5	47.4
SS-1	10.2	6.9	90.0	8.0	47.7	46.1	40.8
SS-3	9.2	6.7	90.6	8.3	49.3	47.5	47.1
SS-4	9.8	6.8	90.5	8.7	49.4	47.8	45.2
SS-5	9.4	6.7	90.7	8.9	48.6	47.1	46.9
SS-10	9.6	6.9	90.3	8.7	46.8	47.5	45.6

^aFungal pretreatment of wood chips: 2 weeks; pulping of chips: 405 min at 140°C with 8.25% SO₂ and 1.05% CaO.

grandis) for biosulfite pulping was run using *C. subvermispota* L-14807 SS-3 obtained from the Mycological Culture Collection, Forest Products Laboratory, in Madison, Wisconsin. All pulp characteristics were improved as compared with the control, especially kappa number (reduced by 31%) and pentosan content (reduced by 36%). The pulp yield was retained at the same level of 47%, indicating that it could be increased over the control at a given kappa number (Christov and Akhtar, 1996).

Tables 13.4 through 13.7 compile results from a second experiment on biopulping of eucalyptus wood chips in conjunction with bleaching/biobleaching of sulfite pulp to produce dissolving pulps. Five strains of *C. subvermispota* were used to decay the chips for 2 weeks prior to pulping. Table 13.4 shows the properties of pulp produced by fungal pretreatment and acid sulfite pulping of chips with a calcium base. In all instances, the brightness of the treated chips was reduced in comparison with the control. Yield-wise only SS-3 and SS-5 pretreated samples were comparable to the control level of 47%. Significant yield loss was observed in the SS-1 treated pulp (40.8%), which, however, correlates with the deterioration of the

TABLE 13.5 OD₁ED₂H-bleaching^a of Sulphite Pulp Produced by Biopulping of Wood Chips with Five Different Strains of *Ceriporiopsis subvermispota*.^b

Strains	S ₁₀ (%, w/w)	S ₁₈ (%, w/w)	α-Cellulose (%, w/w)	Brightness (%, ISO)
Control	7.9	4.7	92.8	93.1
SS-1	8.7	4.9	92.2	93.0
SS-3	7.8	4.8	92.8	94.2
SS-4	8.4	4.7	92.8	92.8
SS-5	7.9	4.7	92.9	93.1
SS-10	8.2	4.6	92.8	92.7

^aCharges at D₁ and D₂: 0.9 and 0.6% as act, Cl: H: 0.5% NaOCl.

^bFungal pretreatment of wood chips: 2 weeks.

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TABLE 13.6 OD₁E₀D₂P-Bleaching^a of Sulphite Pulp Produced by Biopulping of Wood Chips with Five Different Strains of *Ceriporiopsis subvermispota*.^b

Strains	S ₁₀ (%, w/w)	S ₁₈ (%, w/w)	α-Cellulose (%, w/w)	Brightness (%, ISO)
Control	7.7	4.5	92.7	93.2
SS-1	9.1	4.8	92.0	96.2
SS-3	8.3	5.4	92.6	92.9
SS-4	8.4	4.6	92.7	95.1
SS-5	8.5	4.6	92.2	93.6
SS-10	8.3	4.7	92.3	93.7

^aCharges at D₁ and D₂: 0.9 and 0.6% as act. Cl; P: 0.6% H₂O₂; 0.4% NaOH; 100°C.

^bFungal pretreatment of wood chips: 2 weeks.

S10 value (10.2%), indicating nonselective cellulose degradation. In most cases the alpha-cellulose content (the long DP fraction of cellulose) of the specimens was directly proportional to their yield (Table 13.4). There was no significant variation in the viscosity and alkali solubility S18 (hemicellulose content) of the samples. Kappa number was reduced by as much as 10% as compared with the control and did not correlate with the corresponding brightness of the samples.

Tables 13.5 through 13.7 present results from the bleaching of biosulfite pulp in three different bleaching sequences: OD₁E₀D₂H (Table 13.5), OD₁E₀D₂P (Table 13.6) and X-OD₁E₀D₂P (Table 13.7). The major difference in the results between the fungally pretreated samples and the respective controls was in the brightness of the handsheets, especially in the bleaching sequences OD₁E₀D₂P and X-OD₁E₀D₂P. In most instances a brightness gain over the control was observed. For example, in sequence OD₁E₀D₂P (Table 13.6) maximum brightnesses of 96.2 and 95.1% were obtained with the SS-1 and SS-4 pretreated sample. In sequence X-OD₁E₀D₂P (Table 13.7) the most successful treatments were the SS-3 and SS-10 strains, which

TABLE 13.7 X-OD₁E₀D₂P-Bleaching^a of Sulphite Pulp Produced by Biopulping of Wood Chips with Five Different Strains of *Ceriporiopsis subvermispota*.^b

Strains	S ₁₀ (%, w/w)	S ₁₈ (%, w/w)	α-Cellulose (%, w/w)	Brightness (%, ISO)
Control	8.1	4.5	92.5	93.2
SS-1	9.4	4.7	91.5	93.4
SS-3	8.1	4.5	92.7	95.5
SS-3	8.3	4.5	92.7	93.8
SS-5	8.4	4.5	92.3	94.0
SS-10	8.1	4.7	92.6	94.9

^aCharges at D₁ and D₂: 0.9 and 0.6% as act. Cl; enzyme pretreatment of sulphite pulp: 4 IU xylanase/g pulp using Canazyme HS-10; P: 0.6% H₂O₂; 0.4% NaOH; 100°C.

^bFungal pretreatment of wood chips: 2 weeks.

short
normal
long

TABLE 13.8 Yield Loss (%) Due to Bleaching of Biosulphite Pulp Produced by Pretreatment of Eucalyptus Wood Chips with Five Different Strains of *Ceriporiopsis subvermispota*.^a

Strains	Bleaching Sequence		
	OD ₁ E ₀ D ₂ H ^b	OD ₁ E ₀ D ₂ P	X-OD ₁ E ₀ D ₂ P ^c
Control	14.1	13.2	13.4
SS-1	15.2	13.8	14.4
SS-3	14.3	14.0	14.1
SS-4	18.1	18.1	19.0
SS-5	11.6	10.3	10.4
SS-10	11.5	11.1	11.2

^aFungal pretreatment of wood chips: 2 weeks.

^bCharges at D₁ and D₂: 0.9 and 0.6% as act. Cl.

^cEnzyme pretreatment of sulphite pulp: 4 IU xylanase/g pulp using Cartazyme HS-10.

produced 1.7 and 2.3 brightness points increase over the control. The biobleaching effect due to xylanase was not observed in all instances, as shown by comparison of the results in Tables 13.6 and 13.7. For example, the brightness of the SS-1 pretreated sample bleached in the sequence OD₁E₀D₂P (Table 13.6) was almost 3 points higher than that of the SS-1 pretreated sample prebleached with xylanase (Table 13.7). No brightness increase was observed in the SS-4 pretreated samples, shown in Tables 13.6 and 13.7 as well. On the other hand, a biobleaching gain in brightness was detected in the SS-3 (2.6 points) and SS-10 (1.2 points) pretreated samples. This phenomenon might be explained as the result of the modifications in the composition and ultrastructure of the samples that took place during biopulping.

Table 13.8 compares the yield losses resulting from bleaching of the biopulped samples for dissolving pulp. The maximum yield loss (18.1–19.0%) was in the SS-4 pretreated dissolving pulp, whereas the minimum yield loss of 10.3–11.6% was in the SS-5 and SS-10 samples. If the bleaching sequences are compared, more pulp yield was lost in the OD₁E₀D₂H samples than in the other two. The yield loss due to xylanase prebleaching ranged from 0.1% (SS-3, SS-5, SS-10) to 0.9% (SS-4).

Figures 13.7 through 13.9 illustrate the final yields for dissolving pulp from *C. subvermispota*-decayed eucalyptus wood. The results are indicative of the different modes of action of the fungal strains on wood resulting in different pulp yields after bleaching. The highest yields of dissolving pulp, relative to the controls, were achieved by fungal pretreatment with the SS-5 and SS-10 strains. Although the yield of the SS-5 treated sample was lower than that of the control after biosulfite pulping (Table 13.4), it was highest after (bio)bleaching (Table 13.8). This resulted in a yield increase of 2% in the OD₁E₀D₂H, 2.4% in the OD₁E₀D₂P, and 1.5% in the X-OD₁E₀D₂P bleaching, respectively. Therefore, the fungal pretreatment of wood chips not only increases the extent of lignin removal during pulping and bleaching, resulting in higher brightness of the dissolving pulp, but also improves the selectivity of the bleaching process, thereby increasing the final pulp yield.

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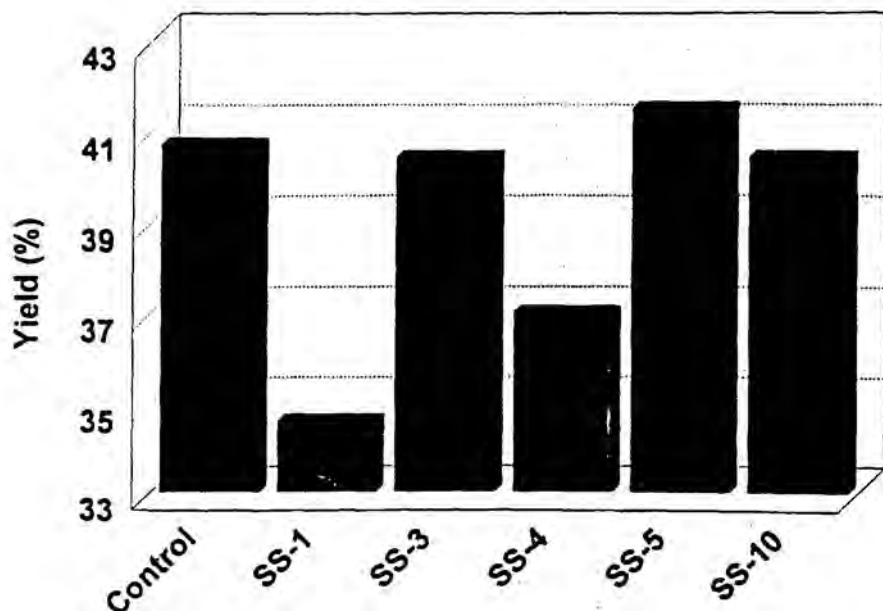


Figure 13.7 Yield of dissolving pulp produced by biosulfite pulping of eucalyptus wood chips with five strains of *Ceriporiopsis subvermisporea* and OD₁E₀D₂H bleaching.

A few South African isolates (*C. subvermisporea* WR 233, *Coriolus (Trametes) versicolor* WR 83, *Stereum hirsutum* WR 95, and *Pycnoporus sanguineus* WR 124) have also been evaluated in biosulfite pulping for dissolving pulp production (Christov and Wolfaardt, 1995). Superior brightness levels were produced when biosulfite pulp was bleached using *Aureobasidium pullulans* xylanase in sequence X-OD₁E₀D₂P (Figure 13.10). Once again, *C. subvermisporea* was most effective in biopulping to reach a 98% brightness after complete bleaching, followed by *C. versicolor* (95%). These brightness increases could be translated into a substantial savings in bleaching chemicals, especially chlorine dioxide, the amount of which was reduced by as much as 20%. However, because the fungal pretreatment time was as long as 3 weeks, an appreciable deterioration of S₁₀ and S₁₈ was observed as well, thus indicating the need for further optimization of the biotreatment conditions.

Biobleaching of Sulfite Pulps with White-Rot Fungi The first report on fungal pretreatment of sulfite pulps using white-rot fungi was delivered at the Sixth International Conference on Biotechnology in the Pulp and Paper Industry in Vienna in 1995 (Christov, et al., 1996a). Sulfite pulp was bleached effectively with two strains of *C. subvermisporea* (L-14807 SS-3 and CZ-3). Strain SS-3 was able to remove 85% of lignin (as kappa number), whereas CZ-3 reduced lignin by 88% (Table 13.9). In terms of pentosan removal from pulp, strain SS-3 was more successful

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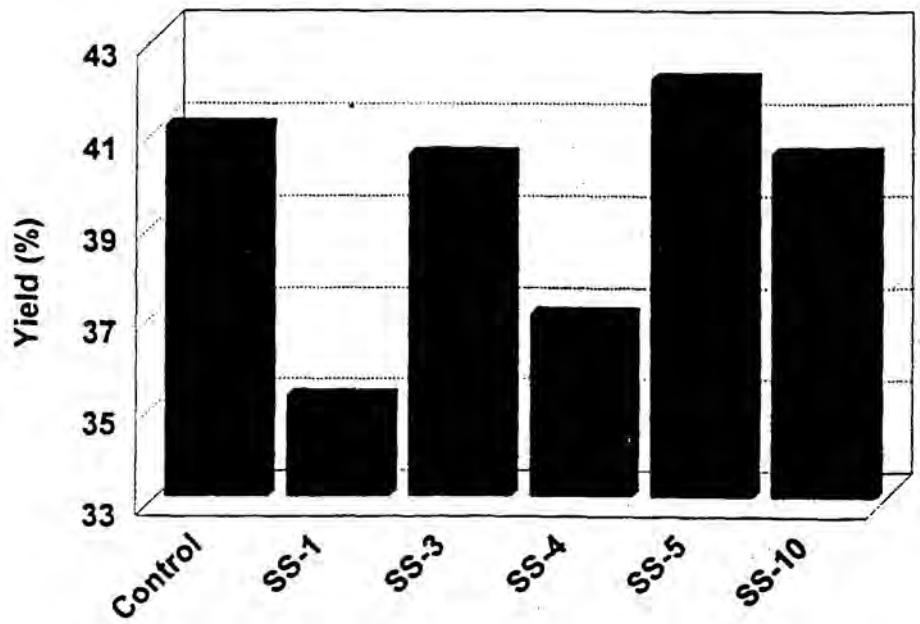


Figure 13.8 Yield of dissolving pulp produced by biosulfite pulping of eucalyptus wood chips with five strains of *Ceriporiopsis subvermispota* and OD₁E₀D₂P bleaching.

(13%) than strain CZ-3 (5%). The brightness of fungus-treated samples increased by 42% (strain SS-3) and 47% (strain CZ-3), respectively, as compared with the control samples. However, the alpha-cellulose content was reduced by 5 (SS-3) and 4 (CZ-3) points. In comparison, xylanase pretreatment lowered the pentosan content by 13% and improved the alpha-cellulose content by nearly a point without significantly affecting the brightness and kappa number (Table 13.9).

Bleaching of the pretreated pulp was carried out in the sequence OD₁E₀D₂H with reduced amounts (0-63%) of active chlorine (at D₁ and D₂ stages) (Table 13.7). The results indicated that the brightness of dissolving pulp pretreated with *C. subvermispota* strains was superior to the brightness of xylanase-pretreated dissolving pulp and the reference dissolving pulp, obtained without biotreatment. Even the elimination of the second chlorine dioxide stage (63% reduction of total active chlorine) did not reduce the brightness values, thus indicating that the charge reduction of total active chlorine within a certain range does not exert a direct influence on the final brightness of fungus-prebleached dissolving pulp. Similarly, Fujita and co-workers (1993) reported a 73% reduction of active chlorine at both C and D stages when softwood kraft pulp was prebleached with the white-rot fungus IZU-154. These results suggest that some selected strains of white-rot fungi are able to degrade and/or modify lignin during the prebleaching step to such an extent that the final delignification and bleaching of pulp can be accomplished with lower amounts of chemicals. Moreover, no correlation could be observed between the reduction of active chlorine, on one hand, and alpha-cellulose or pentosan content, on the other.

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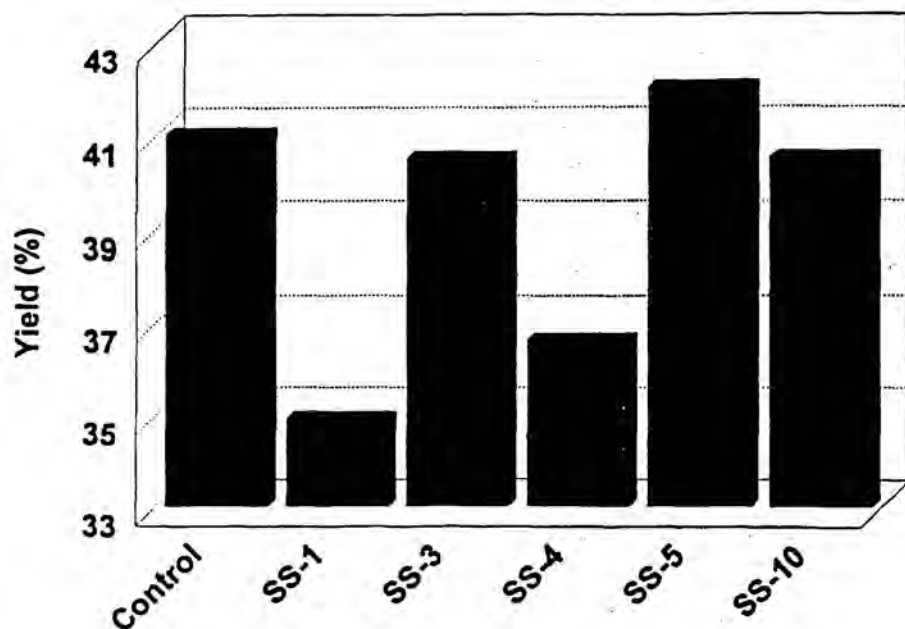


Figure 13.9 Yield of dissolving pulp produced by biosulfite pulping of eucalyptus wood chips with five strains of *Ceriporiopsis subvermispora* and X-OD₁E₀D₂P bleaching.

Apparently, more lignin is solubilized and removed during bleaching of the pulp when pretreated with strain SS-3 as compared with CZ-3. Although strain (22-3 bleached sulfite pulp to a greater extent than strain SS-3 (Table 13.9), chemical bleaching of these samples produced just the opposite effect: the brightness of the SS-3-pretreated dissolving pulp was higher than that of the CZ-3-pretreated dissolving pulp (Table 13.10). This might reflect the differences in the penetration and oxidation capabilities of the lignin-degrading enzymes (or their low molecular weight active compounds, which may be responsible for lignin oxidation), secreted by both strains: ligninases of strain SS-3 may be more capable of attacking and depolymerizing lignin localized in the secondary cell wall of pulp fibers than the CZ-3-secreted enzymes. Thus, the accessibility of lignin from the inner parts of the cell wall to the bleaching chemicals and the lignin diffusability out of the fibers should be greater when sulfite pulp is pretreated with the SS-3 strain of *C. subvermispora*. Strain CZ-3 proved to be more selective in lignin removal than strain SS-3 (Tables 13.9 and 13.10). More appreciable carbohydrate (cellulose and hemicellulose) degradation of the pulp was observed with strain SS-3 than with CZ-3 before (Table 13.9), as well as after, chemical bleaching (Table 13.10). This suggests that the cellulases and hemicellulases of strain SS-3 were more active than those of CZ-3 on sulfite pulp. Hence, the alpha-cellulose and pentosan contents of SS-3-treated and bleached pulp were lower by up to 3 points and 19%, respectively, as compared with those of CZ-3-pretreated dissolving pulp.

Pretreatment of sulfite pulp with *C. subvermispora* increased alkali solubilities

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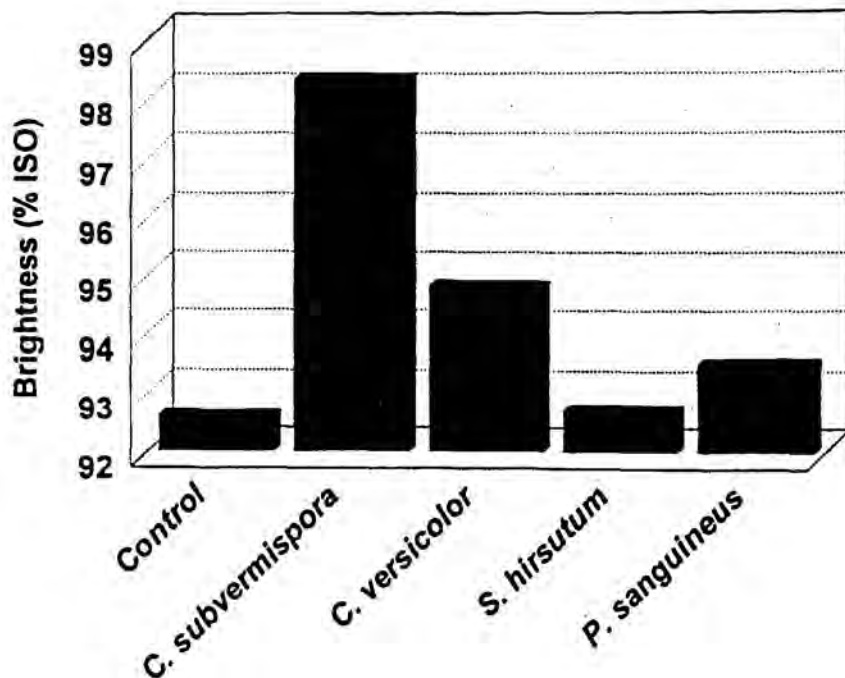


Figure 13.10 Brightness of dissolving pulp produced by biosulfite pulping with several white-rot fungi and biobleaching in sequence X-OD₁E₀D₂P.

S₁₀ and S₁₈ of dissolving pulp significantly, indicating nonselective cellulose degradation (Christov et al., 1996b). Cellulose was more affected when pulp was pre-bleached with strain SS-3 than with CZ-3: the degraded cellulose content (S₁₀-S₁₈) as well as S₁₈ increased almost 2.3 times and 41%, respectively, as compared with those of the control (Table 13.11). Apparently, some cellulose chains of pulp fibrils are exposed to a more severe dismemberment by the fungus than others, to yield glucopolysaccharides with a DP less than 50. This would cause an increase of S₁₈ when analyzed. It also confirms previous morphological and ultrastructural observations that some parts of the cell wall can be preferentially attacked and modified (Otjen et al., 1987). Furthermore, the selectivity of the white-rot fungi, in terms of lignin degradation, tends to decline as the incubation periods progress (Otjen and Blanchette, 1984).

When a combined fungal-enzyme pretreatment of sulfite pulp was coupled with a chemical bleaching, alkali solubility of pulp S₁₀ and S₁₈ did not undergo significant changes with the lowering of the active chlorine doses. In addition, the brightness values for the dissolving pulp improved only slightly owing to the xylanase treatment (Table 13.11).

Thus, biobleaching of sulfite pulp with the white-rot fungus *C. subvermispora* produced a dissolving pulp with a superior brightness of 93% using 63% less active chlorine. However, cellulose degradation also occurred, causing a decrease in the alpha-cellulose characteristics to below the required level for dissolving pulp pro-

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TABLE 13.9 Treatment of Sulfite Pulp with *Ceriporiopsis subvermispora* (L-14807 SS-3 and CZ-3) and *A. pullulans* Xylanases (X).^a

Pulp Treatment	Kappa Number	a-Cellulose (% w/w)	Pentosan Content (% w/w)	Brightness (% ISO)
Control	6.7	89.9	3.9	56.0
SS-3	10	84.9	3.4	79.4
CZ-3	0.8	86.0	3.7	82.2
Xylanase	6.5	90.7	3.4	56.5

^aFungal treatment time: 2 weeks; X: 15 IU xylanase/g pulp; 3 h; 55°C; pH 4.7; 9% pulp consistency.

duction. This detrimental effect can be minimized by optimizing fungal pretreatments expected in the near future.

Biobleaching of Sulfite Pulps with Enzymes Extensive research on biobleaching of sulfite pulps to produce dissolving pulp using xylanases has been conducted by Christov et al. (Christov and Prior, 1993, 1994, 1995, 1996; Christov and Akhtar, 1996; 1996a,b). It was reported that pretreatment of sulfite pulps with xylanases could lead to substantial savings of active chlorine (50%) for bleaching. Alternatively, the brightness of dissolving pulp could be improved by nearly 2 brightness points if no reduction of active chlorine takes place (Christov and Prior, 1996a,b).

CONCLUSIONS

Fungal-Organosolv Pulping

The chemistry and topochemistry of wood delignification by white-rot fungi and organosolv processes are complementary. Fungal pretreatment can partially depolymerize lignin to give 3 residual wood easier to delignify by organosolv processes.

TABLE 13.10 Biobleaching of Sulfite Pulp with *Ceriporiopsis subvermispora* (L-14807 SS-3 and CZ-3) or *A. pullulans* Xylanases (X)^a and Reduced Amounts of Active Chlorine in Sequence OD₁E₀D₂H.

Pulp Treatment	Total Active		Pentosan Content (% w/w)	Brightness (% ISO)
	Chlorine Reduction (%)	α -Cellulose (% w/w)		
Control	0	91.6	2.4	87.5
SS-3	0	85.9	1.8	94.0
	63	87.1	1.8	93.3
CZ-3	0	88.3	2.1	92.5
	63	88.5	2.0	93.3
Xylanase	0	92.4	1.9	88.5
	37	92.1	1.8	88.3

^aFungal treatment time: 2 weeks; X: 15 IU xylanase/g pulp; 3 h; 55°C; pH 4.7; 9% pulp consistency.

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TABLE 13.11 Bleaching of Sulphite Pulp in Sequence OD₁E₀D₂H Following Pretreatment with *Ceriporiopsis subvermispota* (F) and/or *A. pullulans* Xylanases (X).

Pulp Treatment	S ₁₀ (%, w/w)	s ₁₈ (%, w/w)	S ₁₀ -S ₁₈ (%, w/w)	Brightness (%, ISO)
OD ₁ E ₀ D ₂ H (control)	9.5	5.2	4.3	87.8
X-OD ₁ E ₀ D ₂ H ^a	8.5	4.7	3.8	88.6
FS-OD ₁ E ₀ D ₂ H ^b	17.2	7.2	10.0	94.0
FZ-OD ₁ E ₀ D ₂ H	13.7	6.4	7.3	92.5
FS-X-OD ₁ E ₀ D ₂ H ^c	15.1	6.1	9.0	94.2
FZ-X-OD ₁ E ₀ D ₂ H	12.4	5.6	6.8	93.0

^aX-OD₁E₀D₂H: Xylanase-pretreated and OD₁E₀D₂H-bleached sulphite pulp.

^bFS/FZ-OD₁E₀D₂H: Fungus strain SS-3-/CZ-3-pretreated and OD₁E₀D₂H-bleached sulphite pulp.

^cFS/FZ-X-OD₁E₀D₂H: Fungus SS-3/CZ-3-xylanase pretreated and OD₁E₀D₂H-bleached sulphite pulp.

Increases in delignification rates are obtained even at low weight loss values in the fungal pretreatment. Faster delignification rates provide a shorter time to change from bulk to the final delignification phase. As a consequence, the same residual lignin contents were achieved at shorter reaction time in fungal-pretreated samples, meaning energy savings in the organosolv process. Furthermore, pulps of increased strength properties can be expected by pulping wood chips at shorter reaction time. No direct relationship between lignin loss and delignification efficiency was demonstrated; however, selective lignin biodegradation is necessary to prevent cellulose degradation in the fungal pretreatment.

Fungal pretreatment also affects the polyoses removal rate in organosolv pulping. Increases in the polyoses removal rate means decreases in pulp yield and quality. Therefore, the choice of the best fungal pretreatment should be based not only on the delignification rate increase, but also on consideration of the xylan removal constant. Fungal species that provide increases in both lignin and xylan removal rates should be more suitable for pretreatment in organosolv pulping. Only limited work has been carried out to evaluate fungal treatment prior to organosolv pulping. Further work is necessary to study several white-rot fungi acting on different wood species, and fungal-organosolv pulping must be evaluated for a broad range of organic solvent systems. Moreover, additional work is necessary to increase the knowledge of the chemical and biochemical bases of the process and the technological feasibility.

Biopulping and Biobleaching for Dissolving Pulp Production

The fungal pretreatment of wood (biopulping) and pulp (biobleaching) with selected white-rot fungi (e.g., *C. subvermispota*) can offer some apparent benefits to the pulp and paper industry. In the sector of dissolving pulp production, these include the potential increase in pulp yield, higher brightness ceiling, reduced chemical loads for pulping and bleaching, improved effluent quality, smoother pulping, establishment of TCF bleaching, and increased pulp efficiency and mill capacity.

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However, optimization of the treatment conditions is required before this bio-process becomes economically feasible for dissolving pulp production. The nonselective cellulose degradation resulting from the presence of cellulase activities of the fungi is another problem for high cellulose content grades. Thus, there is a need for screening of and development of new strains that can aggressively colonize wood chips with almost no cellulose loss.

ACKNOWLEDGMENTS

A. Ferraz offers thanks for the financial support provided by FAPESP, CNPq, and SCTDE/SP in Brazil, and Fundacion Andes in Chile for the development of specific projects in the areas of fungal-organosolv pulping and wood biodegradation. Our work in this field is part of the research activities developed at the Lignocellulosic Chemistry Laboratory (Lorena, Brazil), and the other researchers of this group, Flavio Teixeira da Silva, Andre Ribeiro Cotrim, Adilson Golçalvez, and Regis Mendonça are acknowledged for their helpful discussion of this theme over the last years. Jaime Baeza, Jaime Rodriguez, and Juanita Freer from Renewable Resource Laboratory (Concepcion, Chile) are also acknowledged for their helpful discussion and critical reading of this chapter on fungal-organosolv pulping.

L. Christov wishes to thank Sappi Saiccor for its continuous financial support and Sappi Management Services for giving permission to publish this work. We are grateful to Ron Braunstein (Sappi Management Services) for the brightness tests and C. Yunnice, J. Thubron, and D. Weightman (Sappi Saiccor) for assisting in pulping the samples and viscosity measurements. The excellent technical assistance of D. van den Berg, M. Cawood, H. Borchers, and M. Lentz is gratefully acknowledged. We thank B. A. Prior of the University of the Orange Free State for the critical review of this manuscript.

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