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Assessment of wood-inhabiting Basidiomycetes for biokraft pulping of softwood chips

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Abstract

Wood-inhabiting Basidiomycetes have been screened for various applications in the pulp and paper industry and it is evident that different fungi need to be used to suit the specific requirements of each application. This study assessed the suitability of 278 strains of South African wood-decay fungi for the pre-treatment of softwood chips for kraft pulping. The influence of these fungi on kappa number, yield and strength properties of pulp was evaluated. A number of these strains were more efficient in reducing kappa number than the frequently used strains of *Phanerochaete chrysosporium* and *Ceriporiopsis subvermispora*. Six strains of *Stereum hirsutum* and a strain of an unidentified species were able to reduce the kappa number significantly without a significant influence on the pulp yield. Treatment of wood with two strains of *S. hirsutum*, one strain of *Peniophora* sp. and a strain of an unidentified species resulted in paper with improved strength properties.

Keywords: Biopulping; Kraft pulping; Lignin degradation; Screening; Softwood; Solid-substrate cultivation; White-rot fungi

1. Introduction

White-rot fungi are the most efficient degraders of lignin (Kirk et al., 1980) and are probably also the most suitable organisms to be utilized in an industrial process that requires delignification (Messner and Srebotnik, 1994). White-rot fungi are not only capable of producing lignin-degrading enzymes, but are also able to penetrate the substrate to transport these enzymes into materials such as wood chips (Messner and Srebotnik, 1994). A variety of possible applications for these fungi exist, including that of biochemical pulping. The potential benefits of biochemical pulping include decreased lignin content of pulp, reduction of pulping time, reduced consumption of bleaching chemicals (Bajpai et al., 2001; Messner and Srebotnik, 1994; Wall et al., 1996) and an increase in the strength properties of pulp (Bajpai et al., 2001; Oriaran et al., 1990, 1991). The biological treatment of wood, prior to chemical pulping, has not been investigated to the same extent as biomechanical pulping (Messner and Srebotnik, 1994) and biokraft pulping has been investigated only for hardwood (Bajpai et al., 2001). A small number of publications deal with kraft pulping of decayed wood (Hunt, 1978; Hunt and Hatton, 1979; Oriaran et al., 1990, 1991; Wall et al., 1996), although kraft pulping accounts for more then 80% of the world's annual pulp production (Sjöström, 1981). To our knowledge, no results on the evaluation of fungi for biokraft pulping of softwood have been published.

Different biotechnological applications have specific requirements (Job-Cei et al., 1991) and it is difficult to predict the effect of biological treatment on pulping (Messner and Srebotnik, 1994). Our approach was, therefore, to evaluate the potential of a South African collection of wood-inhabiting Basidiomycetes

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as biopulping organisms. By testing the ability of strains to produce lignin-degrading enzymes (Stalpers, 1978) and by means of tests for oxidase reactions (Davidson et al., 1938), fungi with the ability to degrade lignin were selected for further screening (Wolfaardt, 1999).

Many different screening procedures have been developed to select organisms with appropriate characteristics. These methods include differential staining and microscopy (Otjen et al., 1987), scanning and transmission electron microscopy (Job-Cei et al., 1991), determination of weight loss (Job-Cei et al., 1991, Blanchette et al., 1992) and analysis of degradation products (Otjen et al., 1987, Bechtold et al., 1993). Unfortunately, none of these methods can be used to accurately predict the effect of fungal treatment on chemical pulping; possibly because an improvement in pulping could simply be ascribed to an improvement in cooking liquor penetration (Oriaran et al., 1991).

The purpose of this study was to evaluate newly isolated strains of lignin-degrading fungi from South Africa for potential application as a pre-treatment of softwood chips prior to kraft pulping. We chose to do mini pulping trials and to rank strains according to their improvement of the resultant pulp. A number of criteria routinely used in the pulping industry were applied to determine the effect of fungal treatment. These parameters included kappa number, pulp yield and paper strength.

2. Methods

2.1. Fungi and inoculum

Two hundred and seventy eight strains of wood-inhabiting Basidiomycetes representing 44 genera were collected in South Africa (Wolfaardt, 1999). The different strains were deposited in the culture collection of Bio/Chemtek at the CSIR. All of these strains, as well as strains of *Phanerochaete chrysosporium* (ATCC32629) and *Ceriporiopsis subvermispora* (CZ-3), were used in screening trials. Inoculum was produced by initially growing fungi on 1.5% malt extract agar (MEA) plates. From these plates, plugs overgrown with mycelium were used to start a pre-inoculum in 200 ml liquid medium containing 3% (v/v) corn steep liquor (CSL) and 1% (w/v) sucrose. The pre-inoculum was incubated in stationary culture for seven days at 28 °C before being homogenized with an Ultra Turrax (IKA-Werke GmbH & Co. KG, Staufen, Germany). Part of the homogenized culture (20 ml) was inoculated into 200 ml CSL and sucrose medium in 500 ml baffled flasks. Cultures were incubated at 28 °C on a rotary shaker at 100 rpm for seven days, after which they were again homogenized to produce inocula for wood chips.

2.2. Wood and solid-substrate fermentation

Mixed pine wood (40% *Pinus patula*, 40% *P. elliottii* and 20% *P. taeda*) was chipped at Sappi's Ngodwana mill (18 mm long and 6–10 mm thick). The chips were dried for five days at 50 °C to a moisture content of 3% and stored at room temperature. The dried wood was placed in 2-l bioreactors (100 g/reactor) and wetted with 160 ml of the CSL and sucrose medium to provide additional carbon and nitrogen sources (Kirk et al., 1976). The wood and medium was autoclaved for 15 min (121 °C, 100 kPa) and then inoculated with 20 ml homogenized inoculum, which brought the moisture content to 60%. The control treatment consisted of an additional 20 ml of the medium above, which was added to the wood instead of inoculum. The treated wood was incubated for three weeks at 28 °C and a relative humidity of 85–95%. In cases where fungal growth was insufficient, incubation continued for eight weeks. This experimental procedure allowed control of solid-substrate fermentation conditions such as temperature and moisture, wood sterilization, nutrient supplementation and a treatment period of at least three weeks. These factors would have important economic implications to be considered during scale-up.

2.3. Pulping conditions

Batches of up to 20 chip samples (50 g dry weight/sample) were cooked in stainless steel mesh bags in a mini (20 l) digester. In the present study, the effect of biopulping could be observed only when the active alkali charges and liquid to wood ratios were increased in comparison to mill conditions. This could possibly be ascribed to the heterogeneous composition of cooking batches, or to a greater consumption of cooking chemicals by decayed wood (Hunt, 1978; Hunt and Hatton, 1979). The active alkali charge and liquid to wood ratio were, therefore, increased to 32% and 10.0:1 respectively. The digester was charged with kraft cooking liquor (32% active alkali, 25% sulphidity) at a liquid to wood ratio of 10:1. The pulping conditions were: ambient to 170 °C in 90 min and 90 min at 170 °C.

2.4. Screening procedure

The screening process consisted of two steps: Initially, all of the fungal strains were subjected to screening in a duplicated experiment where the best 15% were selected for their ability to improve pulp quality. Due to the great variability of cooking, strains were selected that were able to cause noteworthy reductions of kappa number in one of the duplicate treatments.

In a second screening step, wood chips were treated for three weeks with 36 selected strains before pulping. Each treatment was replicated three times in random-

ized block experiments. Three different experiments were conducted (A, B and C), because of restricted space in the digester and a control sample included in each experiment as reference.

2.5. Pulp evaluation

To determine yield, pulp was dried at 55 °C until the weight remained constant. The mass balances for pulp yields were then calculated to incorporate the effects of fungal treatment as well as pulping. Kappa numbers were determined according to Tappi Test Method, T236 and the reduction in kappa numbers and pulp yields were calculated as a percentage of the controls of each experiment. Pulp from all three replications was bulked to obtain enough material for handsheets that were used to determine bursting strength (Tappi Test Method, T403) and tearing strength (Tappi Test Method, T220). Strength ratings were calculated by expressing the average of bursting and tearing strengths of the treated sample as a percentage of the average of bursting and tearing strengths of the control. The data from each experiment were statistically analysed in a two-way analysis of variance and multiple comparisons of treatment means were done with Tukey's method (Winer, 1971) at the 95% level of confidence.

3. Results

During the first screening step, 17 strains were discarded due to an apparent lack of growth on the wood. After pulping of the remaining treatments, 23 more strains were rejected, because pulp yields reflected cellulose degradation corresponding to brown rot. Thirty-six strains were selected for further evaluation and they were able to reduce kappa number by 13% after three weeks or by 23% after eight weeks. The reference strains, *P. chrysosporium* and *C. subvermispora* were not selected, since they caused only minor reductions in kappa number.

A number of the strains that were able to cause a notable reduction in kappa number in the first screening step, were not as effective in subsequent experiments. This could possibly be ascribed to some strains that were selected as result of incubation for eight weeks during the initial screening step (results not shown). The long incubation of some samples could, for instance, have been of benefit to wood colonization by slow-growing strains. Considerable variation between cooking batches were also observed in the initial screening, possibly because these batches were composed of a heterogeneous combination of treatments. All of the wood was not degraded to the same extent and it can be expected that this caused differences in alkali consumption and pulping efficiency in each batch. The variability in results obtained in the initial screening was to a large extent eliminated in the second screening step. The randomized block experimental design compensated for differences between cooking batches, and the composition of batches was less heterogeneous. In some cases no significant differences were found between the experimental blocks that represented different cooks.

Twenty-two of the strains tested in the second screening trial caused a significant modification of lignin, as reflected by reduction in kappa number of the pulp (Table 1). *Stereum hirsutum* was very efficient in reducing kappa numbers, with all nine tested strains causing significant ($p \leqslant 0.05$) reductions. *Pycnoporus sanguineus* was less efficient, with only two out of eight tested strains resulting in significant reductions. Two out of five strains of *Coriolus hirsutus* tested caused significant reductions. The most efficient strains, based on kappa numbers, were *P. sanguineus* (WR 398), *Pycnoporus* sp. (WR 270) and *S. hirsutum* (WR 310). Although these strains were able to reduce the kappa number by 18.1%, 18.5% and 18.5% respectively, compared to the control sample, they also produced significant losses in pulp yield (Table 1). The most promising results were obtained with *S. hirsutum* (WR 3), which reduced the kappa number by 15.2% without causing a significant reduction of pulp yield. Of the 22 strains that were able to cause a significant reduction in kappa number, only seven did not result in a significant loss in the yield (Table 1). These were six strains of *S. hirsutum* (WR 3, WR 9, WR 22, WR 25, WR 91 and WR 156) and a strain of an unidentified species (WR 251). The identity of strain WR 251 is in doubt, although it was identified as *Laetiporus sulphureus* (Fr.) Murr. *miniatus* (Jungh.) Imaz. on the basis of the dried fruit body. However, we regard this strain as an unidentified species, since these results indicated that it could be a white-rot fungus with some selective lignin-degrading potential. The pulp yields (Table 1) appeared to be low, compared with pulp yields obtained on a commercial scale. These yields should, however, be compared with those of the control samples that were also low and are possibly related to specific pulping conditions.

Handsheets were made from the control treatments, biopulp from the seven fungal strains that did not reduce the yield significantly, as well as four strains that reduced the pulp yield with large reductions in kappa number (Table 2). The strength of biopulp from the unidentified species (WR 251), *Peniophora* sp. (WR 286) and two strains of *S. hirsutum* (WR 156 and WR 310) was better than that of pulp from untreated wood. The strongest pulp was produced from treatment with *S. hirsutum* (WR 310), and this was 5% stronger than the control. This improvement in strength rating can be ascribed to the large improvement in the bursting strength and to a small increase in the tearing strength. In general, the bursting strength of handsheets from

Table 1
Influence of selected fungal strains on the lignin content and yield of kraft pulp from wood chips treated for three weeks

Treatment	Strain[a]	Kappa number[b]	Δ Kappa (%)[c]	Pulp yield (g/100 g wood)[b]	Δ Yield (%)[c]
Experiment A					
Control		31.3		42.2	
Coriolus hirsutus	WR 83	28.6	−8.6	39.6*	−6.2
Pycnoporus coccineus	WR 132	29.0	−7.5	42.1	−0.2
Pycnoporus sanguineus	WR 93	28.8	−8.1	39.9*	−5.5
	WR 114	27.5*	−12.2	37.8*	−10.3
	WR 130	29.2	−6.7	38.8*	−8.1
	WR 131	28.7	−8.3	38.3*	−9.2
	WR 170	29.0	−7.4	39.7*	−5.9
Stereum hirsutum	WR 3	26.5*	−15.2	40.8	−3.3
	WR 9	27.0*	−13.7	40.3	−4.5
	WR 25	27.6*	−11.7	40.9	−3.0
	WR 91	28.0*	−10.7	40.4	−4.3
	WR 95	26.5*	−15.3	40.2*	−4.7
	WR 156	27.7*	−11.5	40.7	−3.6
Trametes glabrescens	WR 120	29.8	−4.8	40.2*	−4.7
Experiment B					
Control		31.3		41.9	
Coriolus hirsutus	WR 61	29.7	−5.0	38.8*	−7.4
	WR 141	29.2	−6.8	40.8	−2.6
Lentinus stupeus	WR 24	29.5	−5.6	38.7*	−7.6
Pycnoporus sanguineus	WR 124	28.8	−7.9	39.0*	−6.9
	WR 146	29.4	−6.0	38.2*	−8.8
Stereum hirsutum	WR 22	27.8*	−11.3	41.0	−2.8
Stereum ostrea	WR 19	29.6	−5.3	39.0*	−6.9
Experiment C					
Control		32.1		41.8	
Coriolopsis polyzona	WR 308	29.6	−7.8	40.6	−2.9
Coriolus hirsutus	WR 407	27.7*	−13.9	36.1*	−13.6
	WR 255	27.7*	−13.8	36.7*	−12.2
Ganoderma curtisii	WR 349	28.3*	−11.9	36.6*	−12.4
Gymnopilus sp.	WR 351	27.8*	−10.6	35.5*	−15.1
Lentinus villosus	WR 339	28.1*	−12.5	37.2*	−11.0
Lenzites betulina	WR 402	27.7*	−13.9	37.2*	−11.0
Peniophora sp.	WR 286	26.7*	−17.0	36.7*	−12.2
Pycnoporus sanguineus	WR 398	26.3*	−18.1	35.8*	−14.4
Pycnoporus sp.	WR 270	26.2*	−18.5	34.9*	−16.5
Stereum hirsutum	WR 297	26.8*	−16.7	36.6*	−12.4
	WR 310	26.2*	−18.5	37.6*	−10.1
Trametes cingulata	WR 340	27.1*	−15.6	33.9*	−18.9
	WR 345	28.5*	−11.2	35.9*	−14.1
Unidentified sp.	WR 251	28.0*	−12.8	40.0	−4.3

* Significantly different from the control treatment at $p \leqslant 0.05$ by Tukey's test.
[a] Culture numbers of strains maintained in the collection of wood-inhabiting fungi at the CSIR.
[b] Average of three replications.
[c] Change calculated as the percentage reduction compared to the control treatment of the experiment.

biopulp was higher and the tearing strength lower than those of the controls.

4. Discussion

The strategy, to collect new strains with the aim of selecting superior ligninolytic strains, was followed by De Jong et al. (1992) and proved to be successful in the present study because the widely used biopulping species, *P. chrysosporium* (ATCC 32629) and *C. subvermispora* (CZ-3) (Akhtar et al., 1996), were not able to cause satisfactory reduction of the kappa number. A number of strains from species such as *P. sanguineus* and *S. hirsutum* were included in the screening trials because of the abundance of these species in nature and diversity within species (Otjen et al., 1987; Wolfaardt, 1999). Two strains, *S. hirsutum* (WR 156) and unidentified species (WR 251), were considered as potential biopulping fungi that could be tested in scale-up and optimization experiments.

The mini pulping of the large number of samples was time consuming; however, it allowed us to screen strains

Table 2
Influence of treatment of wood chips with 11 selected fungal strains on bursting and tearing indices and the strength rating of kraft pulp

Treatment	Strain[a]	Bursting index (MN/kg)	Tearing index ($N m^2/kg$)	Strength rating[b] (%)
Experiment A				
Control		6.4	15.4	100
Pycnoporus sanguineus	WR 114	6.2	14.3	94
Stereum hirsutum	WR 3	6.2	13.8	92
	WR 9	6.5	14.6	96
	WR 25	6.7	14.4	97
	WR 91	6.5	14.4	96
	WR 95	6.3	15.5	100
	WR 156	6.7	15.6	102
Experiment B				
Control		6.4	15.4	100
Stereum hirsutum	WR 22	6.5	14.5	96
Experiment C				
Control		6.4	12.5	100
Peniophora sp.	WR 286	6.9	13.1	104
Stereum hirsutum	WR 310	6.9	12.7	105
Unidentified sp.	WR 251	6.8	12.4	101

[a] Culture numbers of strains maintained in the collection of wood-inhabiting fungi at the CSIR.
[b] Average of bursting and tearing strengths expressed as a percentage of the burst and tearing strengths of the controls.

for a specific application (kraft pulping) and on a specific substrate (pine wood). The treatment of the specific substrate is important, since different rates of delignification have been described for different substrates (Otjen et al., 1987; Homolka et al., 1994). Pulping conditions similar to those used at pulp mills have been used for the pulping of fungal treated hardwood (Oriaran et al., 1991; Wall et al., 1996). However, the present study required special pulping conditions to accommodate the large number of samples and it will be necessary to optimize conditions for the large-scale biopulping and to do an economic evaluation of the process.

Fungi capable of "selective delignification" are regarded as the most suitable organisms for biopulping (Otjen et al., 1987). This study showed that most of strains caused a reduction in pulp yield, possibly due to degradation of cellulose, as non-selective delignification took place. Several fungi are able to degrade lignin with some degree of selectivity (Blanchette et al., 1988), however, absolute selectivity as reported for *Ganoderma australe* (Bechtold et al., 1993) appears to be unattainable (Eriksson and Kirk, 1986). Selectivity of delignification has, for example, been shown to change, depending on the substrate and environmental conditions (Otjen et al., 1987). Pulp yields are often based on the oven dry weight of chips that are placed into the digester after fungal treatment (Oriaran et al., 1990) and it is then possible to observe an apparent increase in yield due to an increase in holocellulose to lignin ratio in the digester (Oriaran et al., 1991). However, mass balance calculations based on the weight of wood before fungal treatment showed a yield loss (Oriaran et al., 1990). The increased pulp strength observed in this study, was consistent with the results of kraft pulping of fungal treated hardwoods (Bajpai et al., 2001; Oriaran et al., 1990, 1991) and the increase in bursting strength and decrease in tearing strength indicated an improvement in fibre flexibility and a reduction in fibre length, respectively (Chen and Schmidt, 1995).

5. Conclusions

The results presented in this paper demonstrated the importance of screening to select superior fungal strains for use in biopulping. Under the specific pulping conditions of the screening trials, 38 strains of white-rot fungi were tested that were more suitable than the reference strains of *P. chrysosporium* and *C. subvermispora*. The importance of mini pulping experiments to select the strains with the greatest benefit in a biokraft pulping process was also evident.

References

Akhtar, M., Kirk, T.K., Blanchette, R.A., 1996. Biopulping: an overview of consortia research. In: Srebotnik, E., Messner K. (Eds.), Biotechnology in the Pulp and Paper Industry: Recent Advances in Applied and Fundamental Research. Proceedings of the 6th ICBPPI. Facultas-Universitätsverlag, Vienna, Austria, pp. 187–192.

Bajpai, P., Bajpai, P.K., Akhtar, M., Jauhari, M.B., 2001. Biokraft pulping of eucalyptus with selected lignin-degrading fungi. J. Pulp Paper Sci. 27 (7), 235–239.

Bechtold, R., Gonzáles, A.E., Almendros, G., Martinez, M.J., Martinez, A.T., 1993. Lignin alteration by *Ganoderma australe* and other white-rot fungi after solid-state fermentation of beech wood. Holzforschung 47 (2), 91–96.

Blanchette, R.A., Burnes, T.A., Leatham, G.F., Effland, M.J., 1988. Selection of white-rot fungi for biopulping. Biomass 15, 93–101.

Blanchette, R.A., Burnes, T.A., Eerdmans, M.M., Akhtar, M., 1992. Evaluating isolates of *Phanerochaete chrysosporium* and

Ceriporiopsis subvermispora for use in biological pulping processes. Holzforschung 46 (2), 109–115.

Chen, Y., Schmidt, E.L., 1995. Improving aspen kraft pulp by a novel, low-technology fungal pretreatment. Wood Fiber Sci. 27 (2), 198–204.

Davidson, R.W., Campbell, W.A., Blaisdell, D.J., 1938. Differentiation of wood-decaying fungi by their reactions on gallic or tannic acid medium. J. Agric. Res. 57, 683–695.

De Jong, E., De Vries, F.P., Field, J.A., Van der Zwan, R.P., De Bont, J.A.M., 1992. Isolation and screening of basidiomycetes with high peroxidase activity. Mycol. Res. 96 (12), 1098–1104.

Eriksson, K.-E., Kirk, T.K., 1986. Biopulping, biobleaching and treatment of kraft bleaching effluents with white-rot fungi. In: Moo-Young, M. (Ed.), Comprehensive Biotechnology in Industry, Agriculture and Medicine. Pergamon Press, Oxford, UK (Chapter 15).

Homolka, L., Nerud, F., Kofronova, O., Novotnà, E., Machurovà, V., 1994. Degradation of wood by the basidiomycete *Coriolopsis occidentalis*. Folia Microbiol. 39 (1), 37–43.

Hunt, K., 1978. How rot-decayed wood affects chipping and kraft pulping. Pulp Paper Can. 79 (8), 65–68.

Hunt, K., Hatton, J.V., 1979. Predicting the kraft pulping behavior of decayed pulpwood. Pulp Paper Can. 80 (3), 55–57.

Job-Cei, C., Keller, J., Job, D., 1991. Selective delignification of sulphite pulp paper: assessment of 40 white-rot fungi. Mater. Organ. 26 (3), 215–226.

Kirk, T.K., Connors, W.J., Zeikus, J.G., 1976. Requirement for a growth substrate during lignin decomposition by two wood rotting fungi. Appl. Environ. Microbiol. 32 (1), 192–194.

Kirk, T.K., Higuchi, T., Chang, H., 1980. Lignin biodegradation: summary and perspectives. In: Kirk, T.K., Higuchi, T., Chang, H. (Eds.), Lignin Biodegradation: Microbiology, Chemistry, and Potential Applications, vol. 2. CRC Press Inc, Boca Raton, USA.

Messner, K., Srebotnik, E., 1994. Biopulping: An overview of developments in an environmentally safe paper-making technology. FEMS Microbiol. Rev. 13, 351–364.

Oriaran, T.P., Labosky, P., Blankenhorn, P.R., 1990. Kraft pulp and paper making properties of *Phanerochaete chrysosporium*-degraded aspen. Tappi J. 73 (7), 147–152.

Oriaran, T.P., Labosky, P., Blankenhorn, P.R., 1991. Kraft pulp and paper making properties of *Phanerochaete chrysosporium*-degraded red oak. Wood Fiber Sci. 23 (3), 316–327.

Otjen, L., Blanchette, R., Effland, M., Leatham, G., 1987. Assessment of 30 white-rot basidiomycetes for selective lignin degradation. Holzforschung 41 (6), 343–349.

Sjöström, E., 1981. Wood Chemistry: Fundamentals and Applications, second ed. Academic Press, San Diego, USA.

Stalpers, J.A., 1978. Identification of wood-inhabiting fungi in pure culture. In: Studies in Mycology, 16. CBS, Baarn, Netherlands.

Wall, M.B., Stafford, G., Noel, Y., Fritz, A., Iverson, S., Farrell, R.L., 1996. Treatment with *Ophiostoma piliferum* improves chemical pulping efficiency. In: Srebotnik, E., Messner K. (Eds.), Biotechnology in the Pulp and Paper Industry: Recent Advances in Applied and Fundamental Research. Proceedings of the 6th ICBPPI. Facultas-Universitätsverlag, Vienna, Austria, pp. 205–210.

Winer, B.J., 1971. Statistical Principles and Experimental Design, second ed. Mc Graw-Hill Book Co., USA.

Wolfaardt, J.F., 1999. Utilization of wood-decay fungi for biokraft pulping of softwood. Ph.D. Thesis, Faculty of Science, University of the Orange Free State, South Africa.