TRAMETES VERSICOLOR PRETREATMENT OF POPLAR CHIPS FOR UPGRADING KRAFT PULP

ESMAEIL RASOOLY GARMAROODY,[*] HOSSEIN RESALATI,[**] PEDRO FARDIM,[***] HOSSEIN JALALI TORSHIZI,[*] HAMIDREZA RUDI[*] and SEYED RAHMAN JAFARI PETROUDY[*]

[*]*Department of Cellulose and Paper Technology, Faculty of New Technologies and Energy Engineering, Shahid Beheshti University, Zirab Campus, Mazandaran, Iran*
[**]*Department of Pulp and Paper, Faculty of Natural Resources, Sari University of Agricultural Sciences and Natural Resources, Sari, Iran*
[***]*Laboratory of Fiber and Cellulose Technology, Department of Chemical Engineering, Faculty of Technology, Åbo Akademi University, Turku, Finland*
✉ *Corresponding author: Esmaeil Rasooly Garmaroody, e_rasooly@sbu.ac.ir*

Received February 27, 2015

Industrial poplar chips were pretreated for up to 3 weeks by *Trametes versicolor* and subsequently used for kraft pulping to achieve a constant kappa number of about 20. Pretreated wood chips were characterized using SEM and FT-IR analysis. The results showed that the lignin and carbohydrate structures were considerably degraded by fungal pretreatment after 3 weeks and had an influence on kraft pulping and paper properties. Higher chemical charge in pulping, lower fine and higher long fiber fraction, lower viscosity and increased drainage time were observed in pretreated pulp samples. Handsheet properties, such as density, tensile index, burst index and Scott band, were increased up to a 2 weeks pretreatment time and then decreased together with opacity, brightness and tear index with prolonged fungal pretreatment time. SEM images of handsheet cross-section showed more a homogenized profile with increasing pretreatment time.

Keywords: poplar chips, *Trametes versicolor*, SEM, FT-IR, bio-kraft pulping, fungal pretreatment

INTRODUCTION

Chemical pulps with good strength properties, such as kraft pulp, are used for a wide range of paper products, including corrugated containers, fine paper and tissue.[1] The goal in chemical pulping is to remove more than 90% of lignin from wood chips and leave the cellulose and hemicelluloses. Many paper grades demand the removal of residual lignin from pulp by bleaching with oxidizing chemicals. The chemical processes are not completely selective and they tend to degrade the polysaccharides to some extent and generate potentially hazardous effluents.[1] However, biotechnology offers the potential to produce higher quality products at reduced cost with less environmental impact for industry.[2]

Biopulping, defined as pretreatment of chips by fungi before actual pulping, is believed to save energy, improve product quality, increase production rate and reduce the environmental impact.[2-10] However, bio-kraft has not been investigated to the same extent as biomechanical processes.[2,10-16]

Lignin depolymerization was originally assumed to be responsible for biopulping, but the oxidative enzymes are too large to penetrate the secondary wall, even after 2 weeks of fungal treatment. Direct enzymatic action is thus unlikely. Alternatively, the production of low molecular weight diffusible oxidants, such as hydroxyl radical, has been proposed. The oxidant/radical mechanism is consistent with the chemical changes typically observed after biopulping. Typically, there is an increase in porosity and weight loss of all components, especially lignin and extractives. Rapid degradation of β-O-4 lignin linkages is observed during the first 30 days of fungal decay. Large numbers of metabolites have been isolated,

including oxalic acid, most of which are expected to participate as metal chelators or oxidation intermediates.[17]

White rot fungi, especially Basidiomycetes, are suitable microorganisms for biopulping. White rot fungi are not only capable of producing lignin-degrading enzymes, but also able to penetrate the substrate to transport these enzymes into materials such as wood chips.

In selective white rot, the wood secondary cell wall is delignified diffusively starting from the lumen, followed with the delignification of the middle lamella. As white rot fungi, which are capable of selective lignin degradation, prefer hemicelluloses as carbon source, so the wood cell walls are enriched with cellulose. Selective delignification can occur incompletely throughout the wood substrate or merely in small, localized areas of complete lignin removal. In late stages of decay, cellulose is also degraded and thus selective lignin degradation is usually limited to early stages of decay. Selectivity of white rot decay is also dependent on the physical and chemical environment in the wood, such as temperature, oxygen, nitrogen, and wood moisture content, and also varies between wood species.[18]

White rot fungi have all the necessary enzymes for the complete degradation of wood components. Some fungal species remove lignin more efficiently than other wood components; such a degradation pattern is known as selective lignin degradation or delignification. This effect decreases the dependence on chemicals in the pulping processes and it is the most useful for biopulping. The high capability of white rot fungi to degrade all wood components is based principally on the activity of different complexes of extracellular enzymes. These fungi secrete hydrolytic enzymes, such as cellulases, pectinases and xylanases, which are typically induced by their substrates. On the other hand, lignin is oxidized and degraded by a ligninase system made up of at least three enzyme activities: lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase. In addition, several findings provided information about low weight molecules that participate in the initial attack of lignocelluloses.[7]

Desirable characteristics of white rot fungi capable of delignifying wood include rapid growth, tolerance of high temperatures, asexual sporulation, and the ability to cause extensive selective delignification of substrates with minimal growth and loss of carbohydrates.[19]

Trametes versicolor is a basidiomycete fungus, which is widely spread in the northern forests of Iran and was used in the present study for pretreatment of poplar chips to investigate its effects on kraft pulping and paper properties.

EXPERIMENTAL

Materials

Trametes versicolor was collected from Naharkhoran natural forest in the northern part of Iran and was purified in the Laboratory of Plant Protection at Gorgan University of Agricultural Sciences and Natural Resources. The fungi mycelium was used as suspension for pretreatment of poplar chips.

Fresh industrial mixed chips of three poplar species with almost equal ratios (*Populus Euramericana*, *Populus nigra* and *Populus deltoids*) [(3-5) * (2.5-3) * (0.7-1) cm] have been randomly collected from a chips pile in a wood preparation plant at Mazandaran Wood and Paper Industries (MWPI). After quick washing by cold water to remove the surface dust and grits, the chips were air-dried and kept in propylene bags for subsequent uses.

Methodology

Culture conditions and chips incubation

Potato dextrose broth (24 g/l) and yeast extract (7 g/l) were used to prepare the fungal suspension at 27 °C for 12 days by 8 mm discs of fungal precultured solid medium. The nutrient solution including glucose 4%, mineral ($ZnCl_2$ 1.04%, $MnCl_2$ 3.6%, $CaCl_2$ 3.67%) and 0.5% Corn Steep Liquor (CSL) was added to 100 g chips. The inoculated chips were incubated under aseptic conditions (15 min at 121 °C), using 0.09-0.17 g oven dry (OD) mycelium slurry of fungus per 100 g OD chips, and placed under incubation conditions at 27 ± 1 °C and 65 ± 5% relative humidity and pH=5 for 1, 2 and 3 weeks. After being washed well with cold water to remove the residual fungus, the fungus pretreated samples were air-dried and the weight loss was determined according to Equation 1:[20,15,16]

$$\text{Weight loss } (\%) = [(W1-W2)/W1]*100 \quad (1)$$

where W_1: OD weight of initial chips, W_2: OD weight of pretreated chips.

SEM and FT-IR analyses of wood and pulp

Wood (as layers of 200-300 µm prepared by microtome after wetting the chips samples overnight in distilled water) and pulp samples were sputter-coated with 10 nm of platinum alloy and the images were recorded by SEM (JEOL JSM-6335F). Wood chips were converted to wood meal by a Wiley mill and the powder collected on mesh 60, after passing through mesh 80, was dried in a freeze-dryer overnight. 2 mg of screened wood meal was mixed with 200 mg KBr and homogenized, pressed into pellets and used for FT-IR analysis by Bruker IFS66S. IR spectra were

obtained over the wave number range of 500-5000 cm^{-1}.[5,21,8,22]

Kraft pulping

For kraft pulping, batches of 100 g chips (OD basis) were cooked in an oil bath rotating digester at fixed conditions as: 170 °C, 20-22% AA, 25% sulfidity, L:W= 6:1, and by controlling cooking time, to achieve a pulp kappa number of about 20. The pulps were disintegrated and washed by tap water and were air dried to determine the pulp yield and kappa number (T_{236} om-99).

Pulp analysis

Fiber classification of pulp was done according to SCAN 6:69 by a Bauer Mc Nett classifier. The viscosity of pulps was measured by a Digiterm 100 viscosity meter according to SCAN-CM 15:99. The pulp samples were refined to target 300 ml CSF freeness, according to ISO 5264-2 using a Mod. MK IV laboratory PFI mill. Disintegration of the refined pulps was done using a Mod. British Pulp disintegrator, according to ISO 5263-1. Freeness was determined according to ISO 5267-1. Drainage time was measured according to T 221 cm-99.

Handsheet analysis

Handsheets were made according to ISO 5269-1 by a British Sheet Mold handsheet maker. Test samples for physical and strength properties were prepared according to T_{220}sp-01 and the properties determined using the following standards: bulk: T_{426}wd-70; roughness: T_{538}om-01; tensile: ISO 1924-2; tear: ISO 1974; burst: ISO 2758; Scott band: T_{569} pm-00; opacity: ISO 2470-1 and brightness: ISO 2471.

SEM images of papers

The paper samples (3×8 mm) were located in an epoxy mold system (10×5×3 mm) and pre-polymer epoxy and hardener (in a 25:3 ratio) were added. Epoxy was hardened after leaving overnight and 2 mm thick slices were prepared from the cross section of paper samples by ultra-microtome. The slices were fixed on a copper plate and were sputter-coated with 10 nm of platinum alloy under vacuum. The images were recorded by SEM (JEOL JSM-6335F).

Statistical analysis

The results were subjected to the analysis of variance, followed by the Duncan test. Pulp and paper properties were compared statistically using a protected Duncan ($p \leq 0.05$), followed by on-factor analysis of variance (ANOVA), using IBM SPSS statistics by 21 procedures.

RESULTS AND DISCUSSION

Fungal pretreatment

The weight loss of poplar chips was increased by prolonging the pretreatment time (Table 1). There were significant differences between fungus pretreated samples, but no significant difference was observed between the control and reference samples (reference sample refers to ordinary mill chips with no treatment, the control sample is the chips with nutrients, but without fungal pretreatment and the pretreated sample is the chips with nutrients and fungal pretreatment). The weight increase in the control samples could be due to inefficient washing to remove the added nutrients. The weight loss in the treated wood samples from the present study was similar to the findings of Urzua *et al*.,[23] Akhtar *et al*.,[3] Ahmed *et al*.,[11] Scott *et al*.,[24] Van-beek *et al*.,[20] and Vicentim *et al*.[9]

As shown in Figure 1, fungus could only penetrate into some vessels and fiber lumens after 1 week of treatment, probably by spreading through natural pits and fungal bore holes.

Table 1
Weight changes in poplar chips, based on original sample

Sample codes	Fungal treatment time, weeks		
	1	2	3
Reference	0	0	0
Control	+1.18	+0.23	+1.04
Pretreated	-1.00	-5.96	-10.80

The wood cell wall degradation can be seen in the 2 weeks treated sample, but by increasing the fungal pretreatment time to 3 weeks major degradation happened in the wood structure. Localized cell wall fragmentation or thinning is clearly visible in these samples. The physical basis for biopulping efficacy of the fungal treatment might involve the overall enzymatic

softening and swelling of the wood cell wall fibers, as well as thinning and fragmentation of the wood cell walls in localized areas.[25]

As seen in Figures 2-5, the complex bands between 900 and 1200 cm^{-1}, which represent mainly the polysaccharides, show only small deviations in the spectra of the wood chips degraded by the fungus. However, great deviations may be noted between 1200 and 1800 cm^{-1}. A little decrease in hemicelluloses at band 1740 cm^{-1} (non-conjugated carbonyl bands mainly originating from uronic acids of xylans) provides evidence that *T. versicolor* preferentially degraded the hemicelluloses fraction over cellulose.[5,8]

In this respect, no significant differences were found between 2 and 3 week pretreated poplar chips. A significant decrease at band 1648 cm^{-1} (due to conjugated carbonyl groups originating from lignin) and a little decrease at bands 1508 cm^{-1} and 1594 cm^{-1} (the aromatic skeletal vibration band) indicated that fungal treatment led to structural changes and degradation of aromatic units in lignin. The modification of the lignin structure was increased by prolonging the fungal pretreatment time.[8]

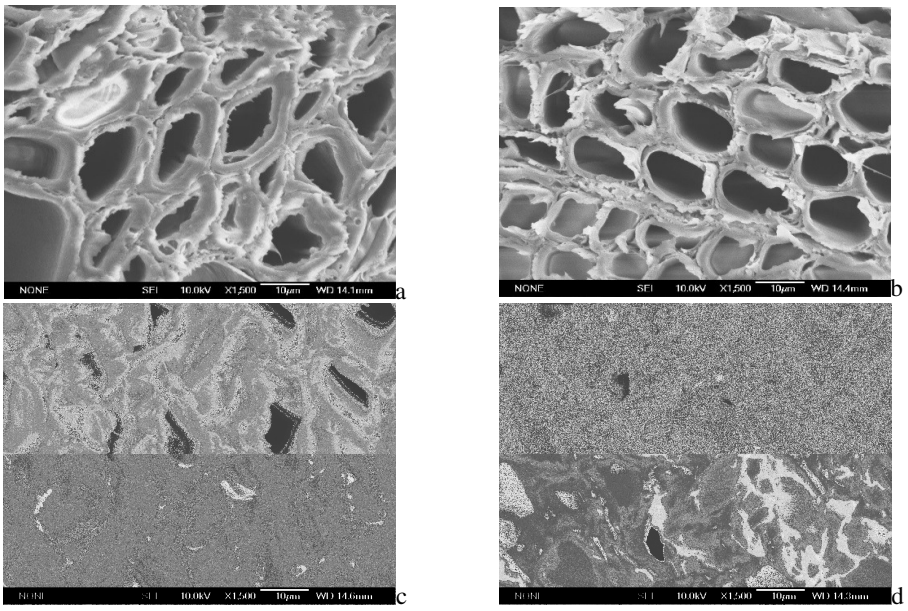

Figure 1: Cross section of reference and fungal pretreated poplar chips (a: reference, b: 1-week pretreatment, c: 2-week pretreatment, d: 3-week pretreatment)

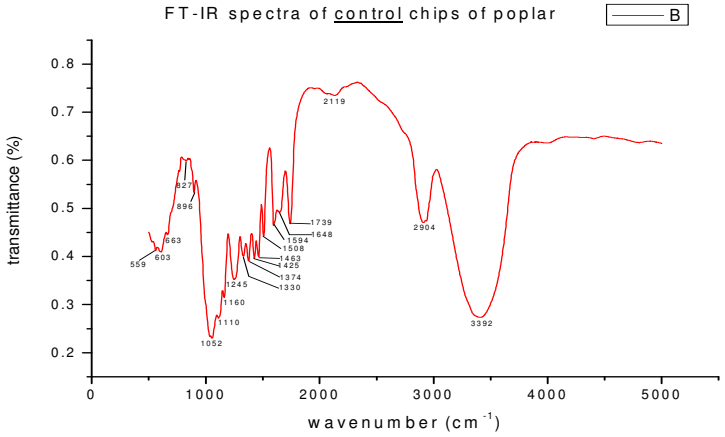

Figure 2: FT-IR spectra for untreated reference sample

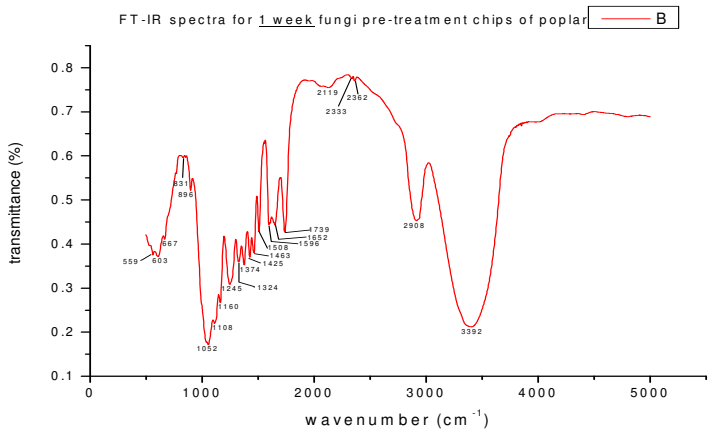

Figure 3: FT-IR spectra for 1-week fungus pretreated sample

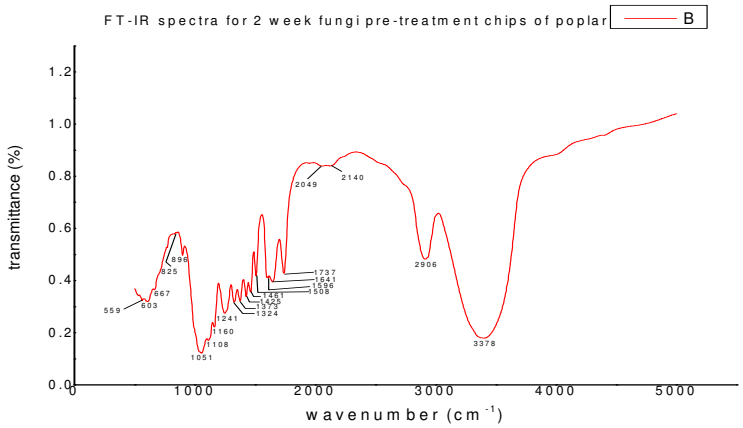

Figure 4: FT-IR spectra for 2-week fungus pretreated sample

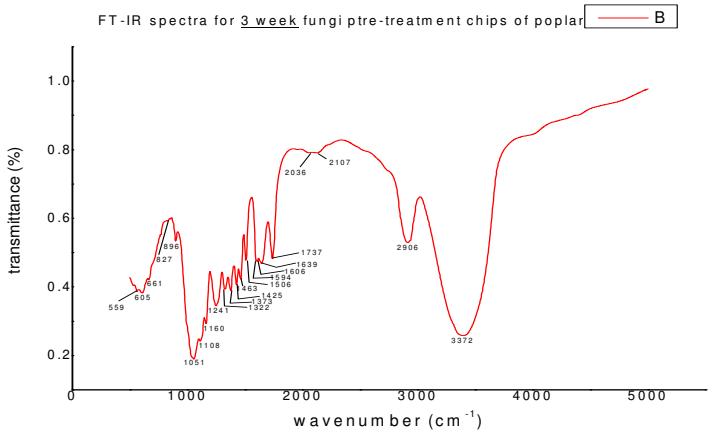

Figure 5: FT-IR spectra for 3-week fungus pretreated sample

The peak at 1330 cm^{-1}, for C-H vibration in cellulose and C_1-O vibration in syringyl derivatives, decreased by increasing fungal pretreatment time and was more remarkable in the 3-week treated sample. This indicated that at a higher degradation level of hemicelluloses, especially xylans, more damage was caused to cellulose by the fungi. Therefore, lower fiber

length, weaker fibers and, as a result, lower paper strength may be expected in the case of the 3-week fungal pretreatment.

Kraft pulping and pulp properties

The selected conditions for kraft pulping of different samples to achieve a kappa number of about 20 are shown in Table 2. The reference sample consumed 20% AA and other samples 22%. The results indicated that the kraft pulp of the untreated reference sample had a higher yield and final pH than the control and the fungal pretreated samples, at even lower chemical charge. The lowest yield and final pH belonged to the fungus pretreated sample at even higher active alkali charge. The higher chemical charge, lower pulp yield and final pH in kraft pulping of fungus pretreated samples may be explained by the fungal degradation of fiber cell walls and by the creation of new acidic groups in these chips prior to kraft pulping.[26,15]

The results of fiber classification in different pulp samples indicated that the pulp of the fungus pretreated samples had higher long fiber fraction (R_{15} + R_{30}) and lower fines fraction (P_{200}) with almost similar medium fiber fraction, in comparison with the reference sample (Table 2). In this respect, the control samples were located between the reference and fungus pretreated samples. It is well known that the fines fraction in unrefined hardwood kraft pulp is defined as primary fines and mainly consists from vessels and parenchyma cells.[27-29]

The lower level of fines fraction in fungus pretreated pulp samples, and consequent the higher percent of long fiber fraction with an almost constant level of medium fiber fraction, may be explained by partial elimination of these primary fines during fungal pretreatment. Improved quality paper may be produced from pulp containing lower fines fraction and higher flexible fiber with more collapsibility, such as fungus pretreated pulp.[30,31,9]

The results of viscosity for different pulp samples showed that pulp viscosity was decreased by prolonging fungal pretreatment time (Table 2). In this respect, no significant differences were observed between reference, control, and 1-week pretreated samples. However, 1- and 2-week pretreated pulp samples had similar viscosity, while the lowest viscosity was observed in the 3-week pretreated sample, which had a significant difference from the other samples. *T. versicolor* is not considered to be a fully selective fungus towards lignin, so reduction of viscosity with prolonged fungal pretreatment time was due to cellulose degradation and decrease in the degree of polymerization (DP) of cellulose molecules during the fungal pretreatment.

The refining response of the different pulp samples refined by the PFI mill to obtain a target freeness of 300 ml, CSF is shown in Table 2. It can be noted that not only the initial freeness of these samples were the same, but also the refining revolutions to reach a constant freeness was surprisingly almost similar in these samples. Leathman and Myers[32] showed that wood fungal pretreatment by *T. versicolor* had not tangible effects on refining revolutions.

Refined kraft pulp obtained from the fungus pretreated chips, appeared to be looser, bulkier, and similar to wool, in comparison with the reference chips, and had fibers with abundant fibrillation, which was enhanced by increasing fungal pretreatment time (Fig. 6). These changes may be due to fungal degradation of the residual lignin structure and removal of more hemicelluloses during kraft cooking. These results confirm the findings of Kirk *et al*.[6]

The refined pulp drainage time was increased by increasing fungal pretreatment time (Table 3). FT-IR analysis and SEM images showed that due to the degradation of carbohydrates by the fungi, the hydrophilic properties of fiber were increased by prolonged pretreatment time, which led to improved fibrillation in refining and increased surface area, which caused an increase in drainage time.

Retulainen *et al.*[33] and Norell *et al.*[34] stated that microfibril interactions made fibers approach and improved hydrogen bonding during the drainage process, so drainage time could be increased by the refining action. In addition, the water retention value (WRV) will be increased by refining due to internal fibrillation and high swelling,[35] which also lead to higher drainage time. The WRV of viscose pulp pretreated with cellulase was increased to 90%,[36] and that of deinked pulp pretreated by laccase was increased to 45%.[37]

Table 2
Selected conditions for kraft pulping and pulp properties after different treatments

Refining revolutions	Initial freeness ml, CSF	DP	Viscosity (ml/g)	Mesh size					pH of residual liquor	Pulp yield (%)	Sample code
				-200	200	100	30	15			
769	3200	792.3	997.4	16.53	8.9	64	10.4	0.17	12.31	53.71	R
744	3200	793.7	999.3	14.25	8.94	64	12.8	0.01	12.03	50.29	C1
744	3250	793.6	999.2	15.99	7.8	64	12.2	0.01	11.88	49.19	C2
744	3500	758.9	988.7	13.29	8.15	63.2	15.3	0.06	11.65	48.16	C3
744	3750	785.1	987.6	12.34	8.6	65	14	0.06	11.67	48.48	P1
769	3500	777.4	977.1	11.36	8.14	63. 6	16.7	0.20	11.43	47.89	P2
744	3300	746.2	959.2	12.28	7.32	64.5	15.7	0.2	11.24	47.53	P3

Abbrev: R: Reference sample; P: fungus pretreated; C: untreated control; 1, 2 and 3: treatment time (weeks)

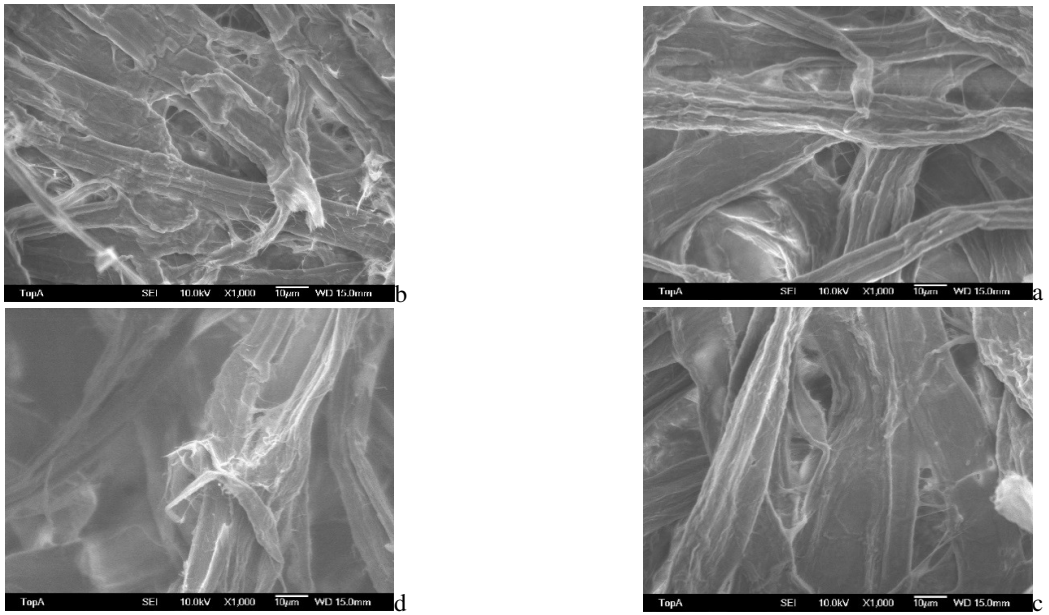

Figure 6: SEM images of kraft pulp from reference and fungus pretreated chips (a: reference, b: 1-week pretreatment, c: 2-week pretreatment, d: 3-week pretreatment)

Table 3
Effects of fungal pretreatment on kraft pulp drainage time in seconds

3 weeks	2 weeks	1 week	0	Sample code
-	-	-	9.4	Reference
9.6	9.5	8.7	-	Control
11.8	9.7	8.9	-	Fungi pretreated

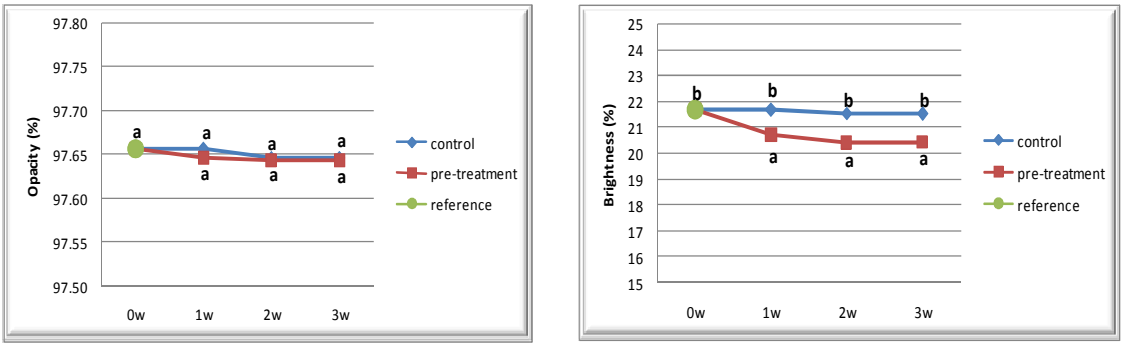

Figure 7: Effect of fungal pretreatment on kraft pulp brightness and opacity

Handsheet properties

No significant differences were observed in opacity among the reference, control and fungus pretreated samples (Fig. 7). However, brightness was decreased by increasing fungal pretreatment time and the difference was statistically significant. It may be due to the creation of chromophore groups during the incubation period, which could not be removed during subsequent kraft pulping. However, biological pulps appeared whiter due to their lower yellowness.[38] This finding confirms the results of Kirk *et al.*,[6] Fischer *et al.*,[39] and Ahmed *et al.*[11]

The effect of fungus pretreatment on the kraft pulp strength properties is shown in Figure 8. The results indicated that strength properties, such as tensile, burst and internal bonding, were increased by increasing treatment times, and the differences among the 2- and 3-week pretreated samples and the reference or control samples were statistically significant. However, tear strength was meaningfully decreased upon prolonged treatment time. The differences between the above mentioned strength properties were not statistically significant for the 2- and 3-week pretreated samples. The increase in fiber-to-fiber bonding strengths caused by fungus pretreatment was probably due to the decrease in fines content, the increase in long fiber fraction, improved fibrillation and higher fiber collapsibility, as indicated by the results of fiber classification, SEM and FT-IR. However, the reduction in tear strength after fungal pretreatment could be due to the increase in fiber-to-fiber bonding and decrease in single fiber strength, as indicated by the lower viscosity and DP. These results were similar to the findings of Leathman *et al.*,[32] Chen and Schmidt,[40] Bajpai *et al.*,[41] Walfaardt *et al.*,[15] and Sahin.[42]

SEM images of handsheet cross sections

SEM images of cross sections of the sheets made from the fungus pretreated samples, in comparison with the reference sample, are shown in Figure 9. As seen in these images, the fiber lumen in the cross section of the 3-week pretreated sheet can be hardly recognized, which is an indication that the prolonged fungus-pretreated fibers had better conformability, collapsibility, and compressibility than the reference sample. This may be due to an increase in hydrophilicity, an improvement in swellability and fibrillation of fiber cell wall caused by the fungal degradation. Our results confirm the finding of Saches *et al.*[33] and Kirk *et al.*,[6] who evaluated sheet cross sections of aspen (*Populus tremuloides*) and pine (*Pinus taeda*) by SEM and reported that the sheets made from mechanical pulps (SGW, TMP, CTMP) showed non-collapsed fibers, leading to poor conformability and reduced bonding. The NSSC- and kraft-processed pulps gave handsheets that exhibited fibers of enhanced compressibility and conformability.

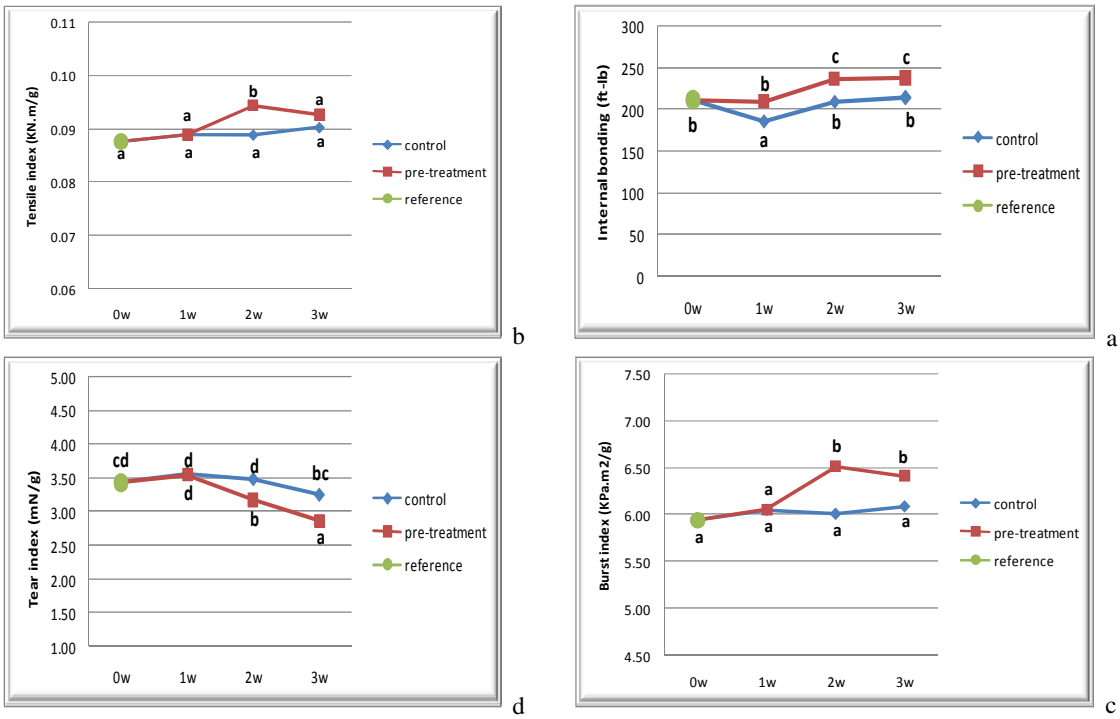

Figure 8: Effect of fungal pretreatment on kraft pulp strength properties

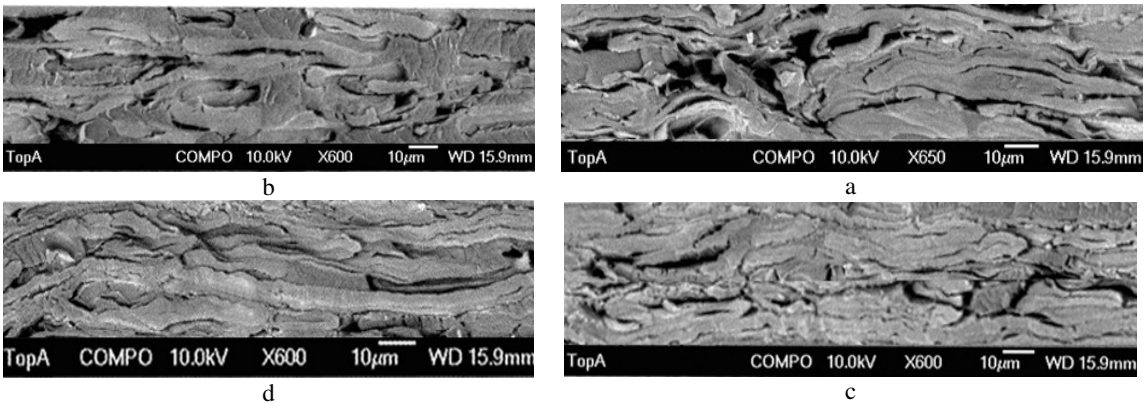

Figure 9: SEM images of handsheet cross sections (a: reference, b: 1-week pretreatment, c: 2-week pretreatment, and d: 3-week pretreatment)

CONCLUSION

The SEM images of cross sections of the fungus pretreated chips showed that the fungus could only penetrate into some vessels and fiber lumens after 1 week of treatment. However, the wood cell wall degradation can be seen in the 2-week treated sample, but by increasing the pretreatment time to 3 weeks, major degradation happened in the wood structure and localized cell wall fragmentation or thinning was clearly visible in these samples.

The results of kraft pulping indicated that the untreated reference sample had higher yield and final pH than the control and fungus pretreated samples, at even lower chemical charge. The pulp of the fungus pretreated sample, in comparison with the control and reference pulps, had a higher long fiber fraction and lower fines fraction, lower viscosity, especially for prolonged fungal

pretreatment time, similar initial freeness and refining revolutions to reach a constant freeness, higher drainage time, similar opacity, lower brightness, higher burst, tensile and internal bonding, and lower tear strength.

FT-IR and SEM analyses showed that due to the degradation of carbohydrates by the fungi, the hydrophilic properties of fiber increased with prolonged pretreatment time, which led to improved fibrillation in refining and increased surface area.

The increase in fiber-to-fiber bonding strengths caused by the fungal pretreatment was probably due to the decrease in fines content, increase in long fiber fraction, improved fibrillation and higher fiber collapsibility, as indicated by the results of fiber classification, SEM and FT-IR. However, the reduction in tear strength after fungal pretreatment could be due to the increase in fiber-to-fiber bonding and decrease in single fiber strength, as indicated by the lower viscosity and DP.

SEM images of the cross sections of the handsheets made from the fungus pretreated samples, in comparison with the reference sample, showed that the fiber lumen in the cross section of the 3-week pretreated sheet could be hardly recognized, which is an indication that the prolonged fungus pretreated fibers had better conformability, collapsibility, and compressibility than the reference sample.

To sum, the positive effect of fungal pretreatment on fiber structure not only contributes to pulp processing and paper quality, but also results in saving energy and reducing chemical consumption.

ACKNOWLEDGMENTS: The authors thank the Wood and Paper Laboratory and Plant Protection Laboratory of Gorgan University of Agricultural Sciences and Natural Resources in Iran for their friendly cooperation. Special thanks to Åbo Akademi University and to the Laboratory of Fiber and Cellulose Technology in Finland for their kind collaboration. Also, the authors thank the RAIZ Laboratory of Aveiro University, Portugal, especially Prof. Dmitry Evtuguin. Finally, thanks are due to Top-Analytica Co. in Finland for performing the SEM.

REFERENCES

[1] M. Bergbauer and C. Eggert, *Biotechnol. Lett.*, **14**, 869 (1992).
[2] Y. Copur and A. Tozluoglu, *Int. Biodeter. Biodegrad.*, **60**, 126 (2007).
[3] M. Akhtar, R. A. Blanchette and T. K. Kirk, in "Advanced Biochemical Engineering Biotechnology", edited by T. Scheper, Springer-Verlag, 1997, pp. 159-195.
[4] J. Buchert, M. Tenkanen, A. Kantelinen and L. Viikari, *Bioresour. Technol.*, **50**, 65 (1994).
[5] O. Faix, J. Bremer, O. Schmidt and T. J. Stevanovic, *J. Anal. Appl. Pyrol.*, **21**, 147 (1991).
[6] T. Kirk, J. W. Koning, R. R. Burgess, M. Akhtar, R. A. Blanchette *et al*., "Biopulping: A Glimpse of the Future?" FLP-RP-523, U.S. Department of Agriculture, Forest Service, Forest Products Laboratory, Madison, WI, 1993, pp. 74.
[7] L. Levin, L. Villalba, V. D. Rea, F. Forchiassin and L. Papinutti, *Process Biochem. Int.*, **42**, 995 (2007).
[8] K. K. Pandey and A. J. Pitman, *Int. Biodeter. Biodegrad.*, **52**, 151 (2003).
[9] M. P. Vicentim, R. A. Faria A. Ferraz, *Holzforschung*, **63**, 408 (2009).
[10] M. B. Wall, G. Stafford, Y. Noel, A. Fritz, S. Iverson *et al*., in *Procs. The 6th International Conference on Biotechnology in the Pulp and Paper Industry*, edited by E. Srebotnik and K. Messner, Facultas-Universitätsverlag, 1996, pp. 205-210.
[11] A. Ahmed, G. M. Scott, M. Akhtar and G. C. Meyers, in *Procs. North American Nonwood Fiber Symposium*, Georgia, 1996, pp. 231-238.
[12] M. Akhtar, G. M. Scott, R. E. Swaney and T. K. Kirk, "Overview of Biomechanical and Biochemical Pulping Research", Forest Products Laboratory, U.S. Department of Forest Service, Madison, U.S.A., 1998.
[13] R. Mendonca, A. Guerra and A. Ferraz, *J. Chem. Technol. Biotechnol.* **77**, 411 (2002).
[14] K. Messner, K. Koller, M. B. Wall, M. Akhtar and G. M. Scott, in "Enzyme Applications in Fiber Processing", edited by K. E. L. Eriksson and A. Cavaco-Paulo, American Chemical Society, Washington DC, 1998.
[15] F. Walfaardt, J. L. Taljaard, A. Jacobs, J. R. Male and C. J. Rabie, *Bioresour. Technol.*, **95**, 25 (2004).
[16] K. Yaghubi, M. Pazouki and S. A. Shojaosadati, *Bioresour. Technol.*, **99**, 4321 (2007).
[17] C. Hunt, W. Kenealy, E. Horn and C. Houtman, *Holzforschung*, **58**, 434 (2004).
[18] T. K. Hakala, Academic Dissertation, University of Helsinki, Finland, 2007.
[19] J. E. Adaskaveg, R. L. Gilbertson and M. R. Dunlap, *Appl. Environ. Microbiol.*, **61**, 138 (1995).
[20] A. B. Van-beek, B. Kuster, F. W. Claassan, T. Tienvieri, F. Bertaud *et al*., *Bioresour. Technol.*, **98**, 302 (2007).
[21] K. K. Pandey, *J. Appl. Polym. Sci.*, **71**, 1969 (1999).
[22] M. Talaeipour, A. H. Hemmasi, K. J. Ebrahimpour, S. A. Mirshokraie and H. Khademieslam, *Bioresources*, **5**, 477 (2010).

[23] U. Urzua, L. F. Larrondo, S. Lobos, J. Larrain and R. Vcuria, *Fed. Eur. Biochem. Soc.*, *Lett.*, **371**, 132 (1995).
[24] G. M. Scott, M. Akhtar, R. E. Swaney and C. J. Houtman, "Recent Development in Biopulping Technology at Madison WI. Biotechnology in Pulp and Paper Industry", Elsevier Science B.V., 2002, pp. 61-71.
[25] L. B. Sachs, G. F. Leatham and G. C. Myers, *Wood Fiber Sci.*, **21**, 331 (1989).
[26] K. Hunt and J. V. Hatton, *Pulp Pap. Can.*, **80**, 55 (1979).
[27] D. O. Adams, in *Procs. The Third International Fundamental Research Symposium*, Montreal, Sep. 15-17, 1958.
[28] G. Haywood, *Tappi J.*, **33**, 370 (1950).
[29] F. A. Simmonds and A. Hyttinen, "Strength of Some Hardwood Pulps and Their Fiber Fractions", United States Department of Agriculture, Forest Service, Forest Products Laboratory-Madison, FPL-023, 1964.
[30] F. Masarin, P. C. Pavan, M. P. Vicentim, P. B. Souza-Cruz, C. Loguercio-Leite *et al.*, *Holzforschung*, **63**, 259 (2009).
[31] I. Pulkkinen, K. Ala-Kaila and J. Aittamaa, *Chem. Eng. Process.*, **45**, 546 (2006).
[32] G. F. Leathman, G. C. Myers, T. H. Wegner and R. A. Blanchette, *Tappi J.*, **73**, 249 (1990).
[33] I. B. Saches, G. F. Leatham, G. C. Myers and T. H. Wegner, *Tappi J.*, **73**, 249 (1990).
[34] M. Norell, K. Johansson and M. Persson, in "Papermaking Science and Technology", Book 4 Papermaking Chemistry, edited by L. Neimo, Fapet Oy, Helsinki, 1999, pp. 43-81.
[35] H. Sixta, "Handbook of Pulp", Wiley-VCH Verlag GmbH &Co. KGaA, Weinheim, 2006.
[36] C. B. Schimper, C. Ibanescu and T. Bechtold, *Cellulose*, **16**, 1057 (2009).
[37] Y. Chen, J. Wan, Y. Ma and H. Lv, *Bioresour. Technol.*, **101**, 7052 (2010).
[38] C. J. Behrendt, R. A. Blanchette, M. Akhtar, S. A. Enebak, S. Iverson *et al.*, *Tappi J.*, **83**, 1 (2000).
[39] K. Fischer, M. Akhtar, K. Messner, R. A. Blanchette and T. K. Kirk, in *Procs. 16th International Conference on Biotechnology in the Pulp and Paper Industry*, 1994, pp. 193-198.
[40] Y. Chen and E. L. Schmidt, *Wood Fiber Sci.*, **27**, 198 (1995).
[41] P. Bajpai, P. K. Bajpai, M. Akhtar and M. B. Jauhari, *J. Pulp Pap. Sci.*, **27**, 235 (2001).
[42] H. T. Sahin, *J. Appl. Biol. Sci.*, **1**, 63 (2007).