BIOCHEMIMECHANICAL PULPING OF HORNBEAM CHIPS WITH *PHANEROCHAETE CHRYSOSPORIUM*

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The effect of fungal pretreatment of Hornbeam (*Carpinus betulus*) wood chips on the performance of treated pulps was studied. The chips were pretreated with *P. chrysosporium* BKM-1767 fungus at 1, 2, and 4 weeks using an inoculation temperature and relative humidity of 39 ^oC and 65%, respectively with two pulping times (80 and 90 min) and three sodium sulfite charges (14, 18, and 22%). The cooking temperature of 165 °C, and liquor-to-wood ratio of 7:1 were kept constant. Beating energy consumption showed a maximum savings of 43% for four-week treatment of wood chips with the fungus. The screen yield of the unbleached CMP ranged between 76 and 84% depending on the chip inoculation time and cooking conditions. A decreasing trend in screen yield of the pulp after chips incubation could be explained by the enzyme action on the lignin or polysaccharides. Pulp strengths including tensile, burst, tear, and fold declined with an increase in chip treatment time. Applying 3% H_2O_2, 4.2% NaOH, 3% $NaSiO_3$, and 0.3% DTPA for 1 hour in two similar stages and 2 weeks fungal pretreatment of chips showed the best optical properties of bleached pulp. After a two-stage H_2O_2-bleaching sequence, the maximal brightness value for the control and biopulps were 54.8% and 56.2%, respectively. Overall, two-week treatment showed the better performance of *P. chrysosporium* on Hornbeam chips.

Keywords: Hornbeam chips; Fungal pretreatment; P. chrysosporium; Saving energy; Biobleaching; Paper properties

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INTRODUCTION

White-rot fungi are the most efficient degraders of lignin (Kirk et al. 1980; Wolfaardt et al. 2004) and are probably also the most suitable organisms to be utilized in an industrial process that requires delignification (Messner and Srebotnik 1994). The selective lignin-degrading fungi are considered good candidates for biological pretreatment (Itoh et al. 2003; Taniguchi et al. 2005). As a result of about 200 tested fungal strains, *P. chrysosporium* was found to be the best biopulping fungus for pretreatment in hardwood mechanical pulping (Akhtar et al. 1992). Lin et al. (1990) reported that the primary interest stems from the ability of *P. chrysosporium* to degrade lignin and wood polymers by lignin-degrading enzyme systems.

During biopulping, this fungus naturally removes brown lignin from the pulp, alleviating the need for mechanical defibration. Incorporation of this natural alternative would limit the amount of energy consumed by refining equipment, and also decrease the amount of chemicals used during the bleaching of paper. Villalba et al. (2006) stated that *C. subvernmispora*, *P. brevispore*, and *P. chrysosporium* species are the most effective fungus for biopulping.

Pulping and papermaking processes consume high amounts of energy, which can account for 18 to 25% of the total cost of production (Bajpai et al. 2006). Treatment of wood chips with lignin-degrading fungi prior to pulping has been shown to have great potential for improvements in mechanical and chemical pulping (Breen and Singleton 1999). Recently, due to the shortage in energy availability and increasing cost in Iran, energy conservation has become a necessity in the paper industry.

Akhtar (1994) studied the inoculation of aspen wood chips with three different strains of the white-rot fungus *C. subvermispora* for four weeks prior to refiner mechanical pulping. All strains resulted in a lower brightness (18 to 21%) and light scattering coefficient (34 to 37%) than the untreated control. In addition, Scott et al. (1998) evaluated biopulping from a small laboratory scale to a 50-ton semi-commercial scale. The resulting biomechanical pulp was darker than pulp made from non-treated control chips, but was readily bleached to a satisfactory brightness using H_2O_2.

Guerra et al. (2005) treated *Eucalyptus grandis* wood chips with the white-rot fungus *C. subvermispora* in a 100-L bioreactor for 15 days. The biotreated alkaline sulfite pulps fibrillated more rapidly and contained lower amounts of rejects than the control. At low peroxide loads, the brightness increase for biopulps was lower than for the control pulps. Still, the bleachability of both pulps was similar for peroxide loads higher than 2%. After a two-stage H_2O_2-bleaching sequence, final brightnesses for the control and biopulps were 59.7+0.8% and 60.5+0.4%, respectively.

High brightness requires more than one bleaching stage; oxidative bleaching with peroxide and a reductive treatment with dithionite (hydrosulfite) are combined. Another option is two-stage peroxide bleaching. The higher the brightness target, the more complicated bleaching technology becomes. H_2O_2 will not react easily with the aromatic structure of lignin, making its level of removal moderate. In mechanical pulp bleaching this is an advantage, as yield and optical properties (opacity) are only moderately affected by a peroxide bleaching process (Suess 2010).

The genus *Carpinus Betulaceae* comprises approximately 35 woody species, which are widely distributed in Europe, eastern Asia, and North and Central America, although the greatest concentration of species diversity occurs in China (Hillier 1988). Hornbeam (*Carpinus betulus*) is a native diffuse porous hardwood species in Caspian forests and grows in mixed stands with oak and beech, and with iron wood in some areas. Hornbeam, having superior technological properties and having high usage potential, is an important species in the lumber industry. Mostly, it is used for tool handles, levers, fuel wood, furniture, and papermaking (Parsapajouh 1998). Hornbeam (*Carpinus betulus*) is one of the most important hardwood species among the broad leaf trees of Iran's Northern Forests, due to its vast distribution, large percentage of coverage (about 33%), and possessing of the longest fiber among the species used for pulping and papermaking applications in Iran (Sabeti 2002). Mazandaran Wood and Paper Company (MWPC) is the largest paper manufacturer in Iran, with a capacity of 175,000 tons per year. MWPC produces newsprint or printing paper on Paper Machine No. 1 and fluting paper on Paper Machine No. 2 using CMP and NSSC pulps, respectively. The CMP product line has been designed based on the use of 75% hornbeam and 25% beech wood.

Chemimechanical pulping of hornbeam chips with *phanerochete chrysosporium* has never been explored in the literature. Therefore, the present research studied the effects of fungal treatment on the refining energy savings, physical properties, and bleachability of chemimechanical pulp to produce newsprint. Furthermore, most of the previously studied softwoods and hardwoods were of low density, whereas hornbeam, as an appropriate species in papermaking, is a high-density hardwood.

EXPERIMENTAL

Materials

Hornbeam chip sampling was done using a randomized method at the MWPC yard in Iran. After washing and air-drying, the chips were placed in plastic bags to prevent growth of infectious microorganisms. The fungus used in this research was *P. chrysosporium* BKM-1767. *Phanerochaete chrysosporium* was obtained from the Iranian Research Organization for Science and Technology (IROST), and cultures were maintained on potato dextrose agar (PDA) slants at 4°C until used.

Methods

Biological pretreatment and inoculation

The bioreactor used in this study was an aerated, static-bed type reactor of cylindrical shape. It had a capacity of about 21 liters and was made of steel sheets. A pipe under the bioreactor allowed the passage of air into the reactor, with flow supplied by an aquarium pump (Fig. 1). Before inoculation, the bioreactors containing chips were decontaminated using atmospheric steam for 30 minutes in accordance with literature methods (Kirk et al. 1993; Akhtar 1997) to avoid contamination with microorganisms that could prevent or compete with the growth of the *Phanerochaete chrysosporium*. In accordance with methods described in the mentioned references, the fungus was first inoculated on a solid plate culture and stored at a temperature of 39 ºC for 5 days. Afterwards, it was inoculated in a liquid plate culture for an additional 5 days at a temperature of 39 ºC. In order to stop development after completing these preparation stages, the fungi were transferred to and stored in a refrigerator at a temperature of 4 ºC. After fungal inoculation of chips, moisture content was determined.

Under sterile conditions, about 1500 g of chips (on a dry basis) were poured into the bioreactor. The fungal inoculums, unsterilized Corn Steep Liquor (0.5% of dry weight), and sterile water required to bring the chips to between 55 and 60 % moisture were mixed with the chips, and the chips were placed in separated layers in the bioreactors. The chips were thoroughly mixed to ensure that the injection liquid adequately affected all chips.

The bioreactors were then placed in an incubation chamber at 39 ± 1ºC and a relative humidity of 65 ± 5% , moisture saturated (room temperature) air for 1, 2, and 4 weeks. After each treatment period, chip fractions were removed from the bioreactors, sealed in plastic bags, and frozen to stop fungal activity.

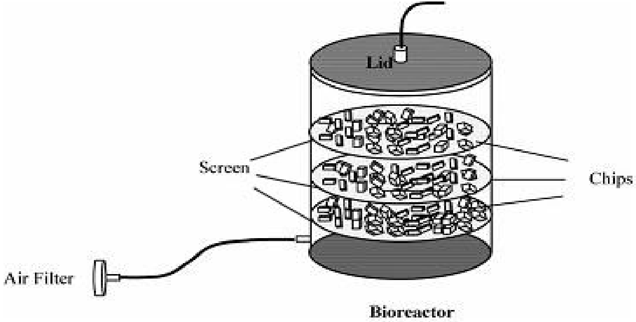

Fig. 1. Aerated static-bed bioreactor showing the layering of the chips to facilitate removal of samples at weekly intervals

Pulping

Chemimechanical pulping of the control and fungal-treated chips was carried out in a 10-liter M.K. digester. Time to cooking temperature, cooking temperature, and liquor-to-wood ratio remained the same for each experiment. Cooking liquor was prepared from MWPC. The chemimechanical pulps were produced from control and fungal-treated chips (BCMP) under the pulping conditions shown in Table 1.

Table 1. Chemimechanical Pulping Conditions of Hornbeam Fungal-treated and Control Chips

Pulping Condition	Values
Chemical charges (Na_2O based on o.d. fiber), %	14,18, 22
Na_2SO_3, g/l (Based on Na_2o)	100
Free SO_2, g/l	115
Liquor-to-wood ratio	7:1
Time to maximum temperature, min	70
Time at maximum temperature, min	80, 90
Cooking temperature, °C	165
pH of white liquor	7.3

After cooking, pulps were washed to remove the pulping liquor. The pulp was dispersed in a water solution before screening using a standard pulp disintegrator. The disintegrated pulps were then washed and screened in a 14-mesh screen on top of a 200-mesh screen. Accept and reject diffractions were determined and the accept diffraction was used for further analysis. Percent screened yield was determined using the following equation:

$$\text{Screened yield} = (\text{accept} / (\text{total wood chips taken}) \times 100\% \qquad (1)$$

Beating

The pulp freeness was measured according to TAPPI Standard T 227 om-04. Pulp beating was performed using a PFI mill beater according to T248sp-00 method, calculating the energy required to reach a freeness of approximately 300 CSF. All of the data was regressed to a CSF of 300 to make comparisons between treatments. Handsheets were then made from the refined pulps with a CSF of approximately 300. The amount of energy consumed during beating of each pulp was measured directly. Finally, percentages of beating energy saving of the pulps were calculated.

Bleaching

Application of 14 and 22% chemical charges with two-stage peroxide bleaching yielded pulp with a brightness of 60% ISO for newsprint. The bleaching stages were performed twice at 70ºC for 1 h using 3% (w/w) H_2O_2 and 4.2% (w/w) NaOH (Table 1). All tested pulps were bleached under equal conditions in each stage. All bleaching stages were performed in plastic Ziploc bags in a water bath with intermittent kneading. In mechanical pulp bleaching, metals are incapacitated by “caging” them into strong complexes, i.e. chelation. Metals are collected by tree roots with water and other nutrients. The usually applied compound for sequestering metal ions is DTPA (Suess 2010). This compound forms extremely stable complexes. Thus, metal elements were removed by treatment with DTPA at 0.3% (w/ w) for 30 min at room temperature. Prior to use and after each bleaching steps, the pulp was thoroughly washed with distilled water (Shatalov and Pereira 2005, 2007).

Table 2. Bleaching Conditions with $H_2O_2^*$

Bleaching Condition	Values	Bleaching Condition	Values
Consistency (%)	12	Temperature, °C	70
H_2O_2 (%)	3	Time, min	60
$NaSiO_3$ (%)	3	NaOH (%)	4.2

*All pulps were bleached using two stages with H_2O_2

Paper characterization

Handsheets with a basis weight of 60 $g.m^{-2}$ were made in a conventional sheet former according to TAPPI T 205 sp-02. The strength properties of the sheets were tested in accordance with the following TAPPI test methods: Handsheet preparation, T 205 sp-02; Tear strength, T 414 om-04; Tensile strength and breaking length, T 494 om-01; burst strength, T 403 om-02; folding endurance, T 423 cm-98; brightness, T 452 om-02; Opacity, T 425 om-01; and yellowness T524 om-02.

Statistical analysis

The paper property testing results were subjected to analysis of variance (ANOVA, $P< 0.05$) for the control and pretreated pulps. The variance and Duncan Multiple Range tests were conducted to show the difference between the trials. Statistical analysis was carried out using SPSS software. Error bars in all graphs refer to 95% Just Significant Confidence Intervals. This approach provides a more accurate picture of the standard error and allows a direct visual comparison of means for analyses within each trial.

RESULTS AND DISCUSSION

CMP Pulping

Table 3 shows the loss of screen yield due to *P. chrysosporium* action. Screen yield loss is dependent on chemical charge and cooking, but in this study, fungal treatment time may have significant effect on the yield variation. The highest screen yield was measured at 84.3%, belonging to the control pulp, and conversely at 76.7% for $BCMP_4$ pulp. Screen yield decreased by 1.69, 2.7, and 6.3% with an increasing fungal pretreatment time.

As seen in Table 3, occasionally there is an increasing trend on screen yield, despite of increasing the chemical charge. This could be a result of different actions of the fungi on the chips. Saad et al. (2008) believed that the action of fungus during pretreatment depends on a good grown mycelium stage. Mycelium load- and pretreatment time, because of their difficult biological process reproducibility, especially when the fungal growth is heterogeneous, are responsible for the low action of fungus during evaluation of pretreatment time and pretreatment scale.

Beating Energy

A one-way ANOVA showed that there are significant differences among the means of beating energy consumption. The means were ranked in four independent groups by Duncan test (Table 1). In comparison with the BCMP pulp, the control pulp required more beating to reach the same freeness. In addition, the $BCMP_1$ required higher PFI revolutions than the $BCMP_2$ to reach a freeness of 300 mL CSF, and the $BCMP_4$ required lower beating than the $BCMP_2$ to reach a freeness level of 300 mL CSF. Fungal treatment of chips could have decreased the cell wall thickness but increase the cell lumen widths because the fungus attacks the cell walls through the lumens (Talaeipour et al. 2010; Sachs et al. 1989).

The described results indicate that cell wall thinning was a direct result of fungal activity, leading to lower energy consumption (Talaeipour et al. 2010). In this study, beating energy requirement was reduced by about 44% using $BCMP_4$ pulp; therefore, increasing cooking time and chemical charge would reduce the beating energy consumption. There is considerable energy saving with $BCMP_2$ and $BCMP_4$ at a chemical charge of 22% and cooking time of 90 min (Table 2).

Messner and Srebotnik (1994) reported a 23% reduction in the pulp-beating energy requirement after incubating spruce and pine chips for 2 weeks with the white-rot fungus *P. chrysosporium* between 35 and 40 ^{o}C, which is close to the mentioned results. In another experiment (Messner and Srebotnik 2002) it was found that *Ceriporiopsis subvermispora* is superior to other fungi when grown on aspen or loblolly pine for 4 weeks. Bio-mechanical pulping of aspen and loblolly pine showed energy savings of 47% and 37%, respectively; these results are higher and lower, respectively, than the results of the current study. Wood and fungus species are two important factors that could affect energy savings.

Table 3. Pulping Conditions, PFI Beating, and Paper Properties of BCMP and Control Pulps

Folding Endurance (number)	Burst Index (kPa.m^2.g^{-1})	Tear Index (mN.m^2.g^{-1})	Tensile Index (N.m.g^{-1})	Energy Saving (%)	PFI Revolution Reduction (%)	Beating Energy (kWh)	PFI Revolutions	Screen Yield (%)	Freeness (°CSF)	Chemical Charge (%)	Cooking Time (min)	Cooking Temp. (°C)
Control												
29	2.7	7.1	61	-	-	185	5200	83.8	300	14	80	165
20	2.7	6.6	57	-	-	165	4750	83.7	//	18		
93	3	6.3	64	-	-	160	4600	82.9	//	22		
32	2.6	6.3	54	-	-	180	5000	84.3	//	14	90	
47	2.8	6.8	58	-	-	175	4950	82.3	//	18		
86	3.1	6.4	61	-	-	160	4600	82	//	22		
51	**2.8**	**6.6**	**59**			**171** ^{a*}	**4850**	**83.2**				**Mean**
One Week Treatment (BCMP$_1$)												
14	2.2	5.7	47	5.41	5.77	175	4900	81.5	300	14	80	165
12	2.3	5.6	43	N	1.05	165	4700	81.4	//	18		
55	2.8	5.1	59	3.13	1.09	155	4550	82.3	//	22		
34	2.4	5.3	49	2.78	2	175	4900	80.8	//	14	90	
47	2.6	4.8	49	5.71	4.04	165	4750	82	//	18		
48	2.7	5.2	57	N	N	160	4600	82.8	//	22		
35	**2.5**	**5.3**	**51**	**4.26**	**2.79**	**166** ^{b}	**4733**	**81.8**				**Mean**
Two Weeks Treatment (BCMP$_2$)												
15	2.1	4.4	43	10.81	9.62	165	4700	80.7	300	14	80	165
25	2.3	4.1	47	N	1.05	165	4700	81	//	18		
13	2.5	4.5	45	N	N	160	4600	82.3	//	22		
19	2.4	4.3	47	16.67	10	150	4500	79.2	//	14	90	
27	2.4	4.2	51	17.14	12.12	145	4350	79.8	//	18		
44	2.8	4.2	59	21.88	14.13	125	3950	81.8	//	22		
24	**2.4**	**4.28**	**49**	**16.62**	**9.38**	**152** ^{c}	**4466**	**80.8**				**Mean**
Four Weeks Treatment (BCMP$_4$)												
15	2.2	4.3	44	29.73	22.12	130	4050	76.7	300	14	80	165
14	2	4.4	44	30.3	22.11	115	3700	79.2	//	18		
26	2.3	4.7	52	34.38	23.91	105	3500	80	//	22		
26	2.2	4.8	49	38.89	28	110	3600	76.7	//	14	90	
21	1.8	3.7	40	40	30.3	105	3450	76.8	//	18		
25	2	4.3	49	43.75	36.96	90	2900	78.7	//	22		
21	**2.1**	**4.3**	**46**	**36.17**	**27.23**	**109** ^{d}	**3533**	**78**				**Mean**

N- Negligible
*The superscript letters indicate the Duncan ranking of the averages at 5% level

Pulp Strength

The results of statistical analysis on the strength of the chemimechanical pulps showed that chemical charge and fungal treatment significantly influenced the strength at $\alpha = 0.05$. The Duncan rankings of the average values of strength measurement are shown in Table 4. All control pulp strengths were significantly higher than the BCMP pulps according to the results. In other words, the results indicated that increasing the fungal chip treatment time would cause a decrease in the strengths of the BCMP. Furthermore, the 4-week treatment with *P. chrysosporium* significantly reduced the tensile and burst indices of the pulps.

There are three independent groups (*a*, *b* and *c*) in the strength of the BCMP compared with the control pulp. There have been different reports on the effects of wood chip pretreatment with white rot fungi on strength properties of chemical and mechanical papers. Handsheets prepared from birch chips that had been incubated for 4 weeks and undergone 10 and 20 minutes of beating time showed a 10 percent reduction in tear and tensile indices (Messner and Srebotnik 1994). The tear index of handsheets made from pretreated aspen chips increased from 1.01 to 3.62 mN m^2 g^{-1} (Akhtar et al. 1992) but not for eucalyptus wood after fungal treatment (Setliff et al. 1990).

Villalba et al. (2006) reported that pretreatment of *Loblolly Pine* chips with *C. subvermispora* clearly increased the strength such as tear and tensile indices, whereas Akhtar et al. (1997) reported a decrease in the tensile and burst indices of incubated chips by the other fungi. Viikari and Lantto (2002) stated that there is a very significant increase in the strength properties of thermomechanical *eucalyptus* pulp, with the tear and tensile indices more than doubling at a comparable freeness. Tensile strength and burst index are dependent on fiber bonding (Dutt et al. 2009; Jahan and Rawshan 2009).

At the same freeness, the control pulp showed higher strengths than BCMP, which could be explained by better fibrillation and improvement in fiber bonding due to higher beating revolutions. Tear strength depends on fiber length, fiber bonding, and the total number of fibers that are involved in the sheet rupture. Fiber dimensions indicated that the fiber lengths in treated wood decreased (Talaeipour et al. 2010) and thus would decrease the tear index. Viikari and Lantto (2002) reported a greater reduction in fiber length by higher consumption the laccase dosage, which has a direct relation to fungal treatment term. On the other hand, some hemicellulose degradation occurred during fungal treatment of wood chips as mentioned above, having a negative effect on the pulp strength. Apiz et al. (2002) reported that some species of white rot fungi such as *Cyathus stercoreus* were preferential to degrade cellulose, which was not good for biopulping. The impact of pulping chemical charge revealed that if the variable increases, the strength properties, except tear index, all increased in control pulps and BCMP. Nevertheless, the tensile and burst indices of pulps produced using 14% chemical charge were almost similar to those of produced using 18%, but all of the strengths of BCMP pulps produced applying a 22% chemical charge were higher and are ranked as the independent group *a* using the Duncan test, except for tear index (Table 3). Hornbeam CMP produced applying a 22% chemical charge exhibited more fiber flexibility due to a lower lignin content, which caused higher fiber bonding than with the 18% and 14% chemical charges.

Pulp Bleachability

Figure 2 shows a comparison of the optical properties of the control pulp and BCMP. The results indicate that brightness did not changed significantly, whereas the opacity and yellowness averages put in different groups at $\alpha = 0.05$ (small letters) did experience significant changes (Table 4). The best bleached pulp resulted from $BCMP_2$, applying a 22% chemical charge and 90 minutes of cooking time to produce newspaper. Optical characteristics of the bleached pulp of pretreated chips were slightly better than that obtained for the bleached pulp of untreated chips, both pulped at the above conditions.

Table 4. Strength Properties of Chemimechanical Pulps Produced Under Different Conditions

Variables		Folding Endurance (number)	Tensile Index ($N.m.g^{-1}$)	Burst Index ($Kpa.m^2.g^{-1}$)	Tear Index ($mN.m^2.g^{-1}$)
Fungal Treatment	Control	51 ^{a*}	59 ^{a}	2.80 ^{a}	6.58 ^{a}
	1 week	35.11 ^{b}	51 ^{b}	2.49 ^{b}	5.29 ^{b}
	2 weeks	23.89 ^{c}	49 ^{b}	2.40 ^{b}	4.36 ^{c}
	4 weeks	21.06 ^{c}	46 ^{c}	2.09 ^{c}	4.3 ^{c}
Chemical charge	14	23 ^{b}	49 ^{b}	2.36 ^{b}	5.28 ^{a}
	18	26.50 ^{b}	50 ^{b}	2.38 ^{b}	5.04 ^{b}
	22	48.79 ^{a}	56 ^{a}	2.62 ^{a}	5.08 ^{b}

*The superscript letters indicate the Duncan ranking of the averages at 5% level

Although both the control and biopulps had close brightness values, a two-fold bleaching step with 3% $H_2O_2$was enough to increase the brightness value by 56.2% using a two-week pretreatment (tCMP2) for newsprint according to Iran's national standard (ISIRI 1743). At higher peroxide loads, the brightness slightly increased for biopulps, and it seems that CMP pulps of Hornbeam wood are difficult to bleach to high brightness levels, even with an increased application of peroxide.

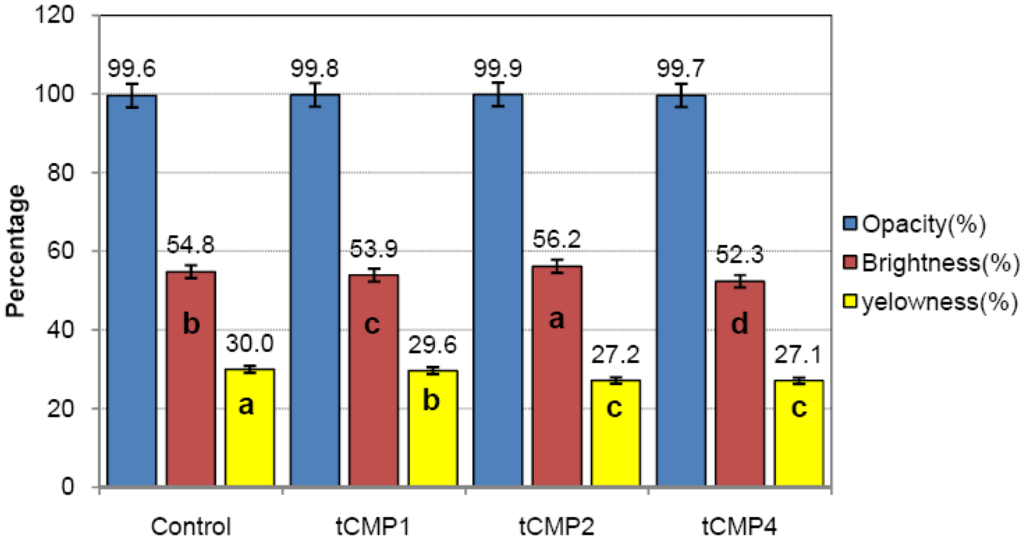

Fig. 2. Optical properties of chemimechanical pulps (Small letters indicate the Duncan ranking of the averages at a 5% confidence interval.)

CONCLUSIONS

1. Treating hornbeam wood chips with *P. chrysosporium* with the goal of obtaining bio-chemimechanical pulp resulted in substantial savings of defibrating energy, resulting in a 43 percent reduction in the energy requirement with respect to the process using untreated wood chips.
2. Increasing the time of fungal pretreatment of wood chips led to more dropping of screen yield, so that it declined at the maximum to 6.3% for four-week treatment time.
3. The CMP strength properties significantly declined with the chip bio-treatment, with the greatest reduction occurring in folding endurance.
4. Increasing the pulping chemical charge showed positive effects on the pulp strength. Conversely, increases in the terms of fungal treatment caused a strength reduction.
5. Pulps bleached with 3% H_2O_2, 4.2% NaOH, 3% $NaSiO_3$, and 0.3% DTPA for 1 hour in two similar stages and 2 weeks of chip pretreatment resulted in the best optical properties. It seems unlikely that more than 56% brightness is achieved using more H_2O_2 load.
6. Moderate periods of incubation (2 weeks) are required by *P. chrysosporium* for efficient pretreatment, but the cellulose weight loss and pulp strength losses could be considered as a negative factor for future industrial applications of the fungus pre-treatment on hornbeam wood chips.

REFERENCES CITED

Akhtar, M., Attridge, M. C., Blanchette, R. A., Myers, G. C., Wall, M. B., Sykes, M. S., Koning, Jr. J. W., Burgess, R. R., Wegner T. H., and Kirk, T. (1992). “Biotechnology in pulp and paper industry,” In: *Proceedings of the 5th international conference on biotechnology in the pulp and paper industry*, University Publishers Ltd., Tokyo, 3-8.

Akhtar, M., (1994). “Biomechanical pulping of aspen wood chips with three strains of *Ceriporiopsis subvermispora*,” *Holz forschung J.* 48, 199-202.

Akhtar, M., Blanchette, R. A., and Kirk, T. K., (1997). “Fungal delignification and biomechanical pulping of wood,” *Adv. Biochem. Eng. Biotechnol.* 57, 159-165.

Akhtar, M., Lentz, M. J., Blanchette, R. A., and Kirk, T. K. (1997). “Corn steep liquor lowers the amount of inoculum for biopulping,” *Tappi J.* 80(6), 161-165.

Akhtar, M., and Swaney, E. S., (1999). “Biomechanical pulping: A mill-scale evaluation,” International Mechanical Pulping Conference,” TAPPI Proceedings, Atlanta, GA: TAPPI Press, 1-10.

Apiz, A., Fu, S., and Zhan, H. (2002). “Biochemical pulping of reed pretreated by white rot fungi,” *Guangdong Pulp & Paper* 5, 11-16.

Bajpai, P., Mishra, S. P., Mishra, O. P., Kumar, S., and Bajpai, P. K. (2006). “Use of enzymes for reduction in beating energy-laboratory studies,” *Tappi J.* 5(11), 25-32.

Breen, A., and Singleton, F. L. (1999). “Fungi in lignocellulose breakdown and biopulping,” *Current Opinion in Biotechnology* 10, 252-258.

Bruce, A. M., and Palfreyman, J. W. (1998). *Forest Products Biotechnology,* Taylor & Francis Routledge, p321.

Ek, M., Gellerstedt, G., and Henriksson, G. (2009). *Pulp and Paper Chemistry and Technology* 1, 219-245.

Dutt, D., Upadhyay, J. S., Singh, B., and Tyagi, C. H. (2009). "Studies on *Hibiscus cannabinus* and *Hibiscus sabdariffa* as an alternative pulp blend for softwood: An optimization of kraft delignification process," *Industrial Crops & Products* 29(1), 16-26.

Guerra, A., Mendonca, R., and Ferraz, A. (2005). "Bio-chemimechanical pulps from *Eucalyptus grandis*: Strength properties, bleaching, and brightness stability," *Journal of Wood Chemistry and Technology* 25, 203-216.

Hillier, J. (1988). *Manual of Trees and Shrubs*. Hillier Nurseries (Winchester), Ltd., Ampfield House.

Itoh, H., Wada, M., Honda, Y., Kuwahara, M., and Watanabe, T. (2003). "Bioorganosolve pretreatments for simultaneous saccharification and fermentation of beech wood by ethanolysis and white rot fungi," *J. Biotechnol.* 103, 273-280.

Jahan, M. S., and Rawshan, S. (2009). "Reinforcing potential of jute pulp with *Trema orientalis* (Nalita)," *BioResources* 4(3), 921-931.

Kamarei, M., Hamzeh, Y., Mahdavi, S., Azadfallah, M., and Hedjazi, S. (2009). "The surfactants effect on CMP pulp produced from poplar and eucalyptus," M.Sc. Thesis, Natural resources faculty, University of Tehran.

Kirk, T. K., Higuchi, T., and Chang, H. (1980). "Lignin biodegradation: Summary and perspectives," In: Kirk, T. K., Higuchi, T., and Chang, H. (eds.), *Lignin Biodegradation: Microbiology, Chemistry, and Potential Applications*, Vol. 2. CRC Press Inc, Boca Raton, USA.

Kirk, T. K., Koning, J. W., Burgess, R., Akhtar, M., Blanchette, R., Cameron, D. C., Cullen, D., Kerstin, P., Lightfoot, E. N., Mayers, G., Sykes, M. B., and Wall, M. B. (1993). *Biopulping A Glimpse of the Future?*, Madison: Wisconsin. Rep. FPL- RR-523.

Lin, J. E., Wang, H. Y., and Hickey, R. F. (1990). "Degradation kinetics of pentachlorophenol by *Phanerochaete chrysosporium,*" *Bio-technol. Bioeng.* 35(11), 1125-34.

Messner, K., and Srebotnik E. (1994). "Biopulping: An overview of developments in an environmentally safe paper-making technology," *FEMS Microbiology Reviews J.* 13(2-3), 351-364.

Parsapajouh, D. (1998). *Wood Technology*. 4th ed. Tehran University, No: 1851, Iran.

Pendy, A. (2004). *Concise Encyclopedia of Bioresource Technology*, Haworth Press, Inc., USA, p789.

Saad, M. B. W., Oliveira, L. R. M., Cândido, R. G., Rocha, G. J. M., and Gonçalves, A. R. (2008). "Preliminary studies on fungal treatment of sugarcane straw for organosolv pulping," *Enzyme and Microbial Technology* 43(2), 220-225.

Sabeti, H. (2002). *The Forest and Trees of Iran*, Yazd University.

Sachs, L. B., Leatham, G. F., and Myers, G. C. (1989). "Biomechanical pulping of aspen chips by *Phanerochaete chrysosporium*: fungal growth pattern and effects on wood cell walls," *Wood and Fiber Science*. 21(4), 331-342.

Scott, G. M., Akhtar, M., Lentz, M. J., and Swaney, R. E. (1998). "Engineering, scale up, and economic aspects of fungal pretreatments of wood chips," in: *Environmentally Friendly Technologies for the Pulp and Paper Industry*, John Wiley & Sons, Inc. New York, 341-383.

Setliff, E., C., Marton, R., Grancow, S. G., and Eriksson, K. L. (1990). "Biomechanical pulping with white-rot fungi," *Tappi J.* 73(8), 141-147.

Shatalov, A. A., Pereira, H. (2005). "*Arundo donax* L. reed: new perspectives for pulping and bleaching, Part 4. Peroxide bleaching of organosolv pulps," *Bioresour. Technol.* 96, 865-872.

Shatalov, A. A., Pereira, H. (2007). "Polysaccharide degradation during ozone based TCF bleaching of non-wood organosolv pulps," *Carbohydr Polym.* 67, 275-281.

Suess, H. U., (2010). *Pulp Bleaching Today,* Walter de Gruyter GmbH & Co. KG, Berlin/New York, p310.

Talaeipour, M., Hemmasi, A. H., Ebrahimpour Kasmani, J., Mirshokraie, A., and Khademieslam, H. (2010). "Effects of fungal treatment on structural and chemical features of hornbeam chips," *BioResources* (http://www.bioresources.com), 5(1), 477-487.

Taniguchi, M., Suzuki, H., Watanabe, D., Sakai, K., Hoshino, K., and Tanaka, T. (2005). "Evaluation of pretreatment with *Pleurotus ostreatus* for enzymatic hydrolysis of rice straw," *J. Biosci. Bioeng.* 100, 637-643.

TAPPI Test Methods T 227 om-04. (2007). Technical Association of the Pulp and Paper Industry, TAPPI Press, Atlanta, GA.

TAPPI Test Methods T 205 sp-02. (2007). Technical Association of the Pulp and Paper Industry, TAPPI Press, Atlanta, GA.

TAPPI Test Methods T T 414 om-04. (2007). Technical Association of the Pulp and Paper Industry, TAPPI Press, Atlanta, GA.

TAPPI Test Methods T 494 om-01. (2007). Technical Association of the Pulp and Paper Industry, TAPPI Press, Atlanta, GA.

TAPPI Test Methods T 403 om-02. (2007). Technical Association of the Pulp and Paper Industry, TAPPI Press, Atlanta, GA.

TAPPI Test Methods 423 cm-98. (2007). Technical Association of the Pulp and Paper Industry, TAPPI Press, Atlanta, GA.

TAPPI Standard, T 452 om-02. (2007). Technical Association of the Pulp and Paper Industry, TAPPI Press, Atlanta, GA.

TAPPI Standard, T 425 om-01, (2007). Technical Association of the Pulp and Paper Industry, TAPPI Press, Atlanta, GA.

TAPPI Standard, T 524 om-02, (2007). Technical Association of the Pulp and Paper Industry, TAPPI Press, Atlanta, GA.

Viikari, L. and Lantto, R. (2002). "Biotechnology in the pulp and paper industry," 8th ICBPPI Meeting. *Progress in Biotechnology 21*. Elsevier Science B.V., Amsterdam, p. 332.

Villalba, L. L., Scott, G. M., and Schroeder, L. R. (2006). "Modification of loblolly pine chips with *Ceriporiopsis subvermispora* Part 2: Kraft pulping of treated chips," *Journal of Wood Chemistry and Technology* 26(4), 349-362.

Wolfaardt, F., Taljaard, J. L., Jacons, A., Male, J. R., and Rabie, C. J. (2004). "Assessment of wood-inhabiting basidiomycetes for biokraft pulping of softwood chips," *Bioresour. Technol*. 95, 25-30.

Yu, H., Guo, G., Zhang, X., Yan, K., and Xu, C. (2009). "The effect of biological pretreatment with the selective white-rot fungus *Echinodontium taxodii* on enzymatic hydrolysis of softwoods and hardwoods," *Bioresour. Technol.* 100(21), 5170-5175.

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