International Journal of Advanced Biotechnology and Research(IJBR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-6, Issue-3, 2015, pp320-326
http://www.bipublication.com

Research Article

Biobleaching of the Paper Mulberry Pulp Using White Rot Fungi

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[Received-16/06/2015, Accepted-27/06/2015, Published-21/07/2015]

ABSTRACT

Paper mulberry (*Broussentia papyrifera*) is an important alternative raw material for making handmade paper. With an aim to produce handmade paper from the bast fiber of paper mulberry in an ecofriendly way, we have evaluated biobleaching of mulberry pulp using white rot fungal cultures *Phanerochaete chrysosporium* and *Pleurotus ostreatus*. The fungal treated pulps were subjected to chemical bleaching utilizing hydrogen peroxide after an alkaline extraction stage. It was demonstrated that the white rot fungi could act as a 'bleach booster' that resulted in the brightness gains to the tune of 13-14 points in the treated pulps as compared to the control pulp.

Key words: Biobleaching, Paper Mulberry, White rot fungi, Handmade paper

INTRODUCTION

Biological bleaching occurs naturally in wood during its degradation by certain fungi. These fungi are called white rot fungi because of their characteristic bleaching effect as they decay wood [1]. This is due to the specific removal of lignin and is the basis of biobleaching efforts using whole cultures of white rot fungi. The process of fungal bleaching or *in vivo* biological bleaching includes pretreatment stage of the pulp with suitable strains of lignin degrading fungus before subjecting it to the normal bleaching process.

Various white rot fungi have been found to delignify kraft pulps effectively [2-5].Usually the pulp brightness after fungal treatment is reported to be 50-60% ISO, but it can be increased to 80-90%ISO by post treatment with chlorine dioxide [6] or peroxide [5,7]. The fungal bleaching of pulp can decrease both residual lignin color (thus increasing the brightness) and lignin concentration (kappa number) in hardwood (HWKP) and softwood (SWKP) kraft pulps. Biological bleaching thus offers a chemically milder and environmentally superior approach to pulp delignification than conventional bleaching processes [8]. The long times required for fungal delignification and the cost of preventing contamination with unwanted microbes have been prohibitive for the commercialization of a biological bleaching process in the paper industry but this may not be an issue for handmade paper

industry. Keeping in view the affordability of long incubation period of 2-3 weeks in handmade paper industry as compared to the mill sector due to the utilization of batch procedures at small scales, the present study was carried out to evaluate potential of the white rot fungal strains in bleaching of paper mulberry pulp.

MATERIAL AND METHODS

Mulberry Pulp

Mulberry pulp was produced from the bast fiber of paper mulberry utilizing the process of "Open hot digestion", commonly used pulping process in handmade papermaking. Briefly, the process included boiling of the chopped fiber for a period of three hours using sodium hydroxide (7g per 100g of oven-dried raw material) as a pulping chemical. The pulp obtained thus was washed thoroughly and beaten to a CSF (Canadian Standard Freeness) of about 350 ml.

Fungal Cultures

Two strains of white rot fungi viz. *Phanerochaete chrysosporium* (MTCC-787) and *Pleurotus ostreatus* (MTCC-142) were procured from Institute of Microbial Technology (IMTECH), Chandigarh. *Phanerochaete chrysosporium* (PC) was grown on malt extract agar –Blakslee's formula (malt extract: 20.0 g, glucose: 20.0 g, peptone: 1.0 g, agar: 20.0 g and distilled water: 1.0 L) and *Pleurotus ostreatus* (PO) was grown on yeast glucose agar medium (yeast extract: 5.0 g, glucose: 10.0 g, agar: 15.0 g, distilled water: 1.0 L, pH adjusted to 5.8) with an incubation period of seven and nine days, respectively. The freshly grown cultures were used as inoculum for the pulp treatment.

Fungal Treatment of Pulp

The paper mulberry pulp was autoclaved in the Huffkin's flask (3L volume) along with the calculated amount of water to maintain 65% moisture.The pulps were then inoculated with the fungal culture by adding 40 discs of 7mm diameter/100 g of oven-dried pulp. An uninoculated sample of autoclaved pulp with 65% moisture was used as a control. All the flasks were then incubated at a temperature of 30 ºC for a period of two to three weeks.On completion of incubation period, pulps were harvested, washed and handsheets were made according to standard TAPPI method (T-205om-88).

Determination of Yield Loss and Kappa Number of the Pulp

The pulp yield was determined by weighing the pulp harvested after fungal treatment ('W' total wet weight of pulp) and taking two representative samples each of 2-3 gm in two clean Petri dishes for determining the dryness (dry content per 100g of Air Dried or AD weight). The weighted samples were then dried in a Hot Air Oven at 102 ± 2^0C for an overnight. The dried samples were then weighed and pulp yield was determined as follows:

$$\text{Dryness\%} = B/A \times 100, \quad \text{Pulp yield\%} = \frac{W \times \text{Dryness of pulp}}{\text{O.D. weight of the raw material taken}}$$

Where, A→Weight of wet pulp sample, B→Weight of dry pulp sample and W→ Total AD Weight of the pulp harvested after fungal treatment.

Kappa number of the treated and control pulps was determined by the standard TAPPI test method.

Chemical Bleaching of the Treated Pulps

The pulps harvested from the fungal treatment (F) after thorough washing were subjected to alkaline extraction (FE) as per the conditions given below: temperature: 60 ºC; time: 2 h; consistency: 8%, NaOH: 1.5 or 2% of oven-dried pulp. The alkaline extracted pulp was then washed and bleached with hydrogen peroxide (FEP) as per the conditions given below: temperature: 70 ºC; time: 2 h; consistency- 8%; NaOH: 1%; H_2O_2: 2 or 3%; initial pH >10.5, final pH->8.5.

Analysis of Pulp Filtrates: All the treated and control pulps were squeezed to collect filtrates of alkaline extraction before washing. The filtrates obtained were analyzed for lignin and color using the standard test methods. Lignin content was quantified according to TAPPI method T-222 by measuring absorbance at a wavelength of 280 nm using 20.2l/g/cm as the extinction coefficient [9]. Color of the filtrates was determined by measuring absorbance at 465nm and converting it into the Platinum Cobalt Units (PCU) using the conversion factor (500 PCU=0.41 Absorbance).

Analysis of Pulp Brightness: Pulp brightness was measured after making handsheets at all stages i.e. after fungal treatment (F), after alkaline extraction (FE) and after peroxide bleaching (FEP) using the standard TAPPI methods(brightness: ISO-2457, tensile strength: ISO-1924, Tear strength: ISO-1974).

RESULTS AND DISCUSSION

In vivo Bleaching

The yield loss and kappa number of the control and treated pulps were determined after fungal treatment and it was found that PC-treated and PO-treated pulps had 12% and 5% loss in yield respectively while control pulp showed no loss in yield. The yield loss in both the fungal treated pulps was because of fungal-removal of lignin from the pulp. Higher yield-loss in PC-treated pulp implies that certain amount of pulp-cellulose has also been metabolized. Kappa number of the fungal treated pulps were also found reduced (13.6 for PC-treated and 15.1 for PO-treated pulp) in comparison to the control pulp (33.6) indicating the removal of lignin which was more in the case of PC-treated pulp(table-1). This kind of kappa number drop has been reported [4] earlier also (40 to 21.7 on 5-days incubation and to 6.9 on 12 days incubation of SWKP with the fungus IZU-154). Similarly, Nezamoleslami *et al*,1998 [10] have shown that six-day incubation of the soda-AQ pulp of kenaf bast fiber with PC could reduce kappa number by about $2/3^{rd}$ and $1/3^{rd}$ depending upon the quality of the fiber used.

After the fungal treatment (F), unbleached pulp brightness was found to increase in both the treated pulps as compared to that of control pulp (figure 1). It was found to rise to 32.2% ISO brightness points in PC-treated pulp & to 30.7% ISO brightness points in the PO-treated pulp from the original brightness of 23.06% ISO brightness points (table-2). This suggests a good amount of pulp delignification carried out by the two fungal strains. Similarly, a brightness gain of 5.4 points was reported with *Phanerochaete chrysosporium* grown on *Eucalyptus globulus* oxygen-delignified kraft pulp (EDTA-extracted to remove Mn present in the pulp) after 14 days of incubation as compared to the abiotic control. This gain could be increased to 8.28 brightness points by adding optimized Mn (II) concentration (i.e. 33 μM) in the EDTA-extracted pulp [11]. Similarly, Fujita *et al*, 1993 [4] have also reported increase in brightness points after treatment of the softwood kraft pulp (SWKP) with the white rot fungus, IZU-154. In their case, the brightness increased from 23% ISO to 27% ISO after five days of incubation and to 52% ISO after 12 days of incubation. Therefore, the increase in mulberry pulp brightness obtained in the present study is in concurrence with the earlier reports available for other pulps. However, the extent of gain obtained is less, which might be due to the absence of any additional nutrient supplied to the fungus. It has been reported that the degree of delignification could be increased from 21.1 to 33.5% by increasing concentration of the exogenous glucose from 0 to 0.47 % [12].

Chemical Bleaching of the Fungal Treated Pulps

Fungal treated and control pulps were bleached with hydrogen peroxide after an alkaline extraction stage. Two doses were tried at both the alkaline extraction and peroxide bleaching stages. From table-2, it can be seen that on chemical bleaching through alkaline extraction and Hydrogen

peroxide, the PC-treated and PO-treated pulps reached to the brightness values as high as 63% ISO and 59% ISO brightness respectively as compared to the control pulp brightness of 47% ISO (Table-1). Maximum gain in brightness points was achieved with *Phanerochaete chrysosporium* after every stage i.e. after alkaline extraction (10.6 points) and after peroxide bleaching (17.8 points) on comparing it with the control pulp. While in the case of pulp treated with *Pleurotus ostreatus*, gain in brightness was 6.6 points after alkaline extraction and 13.1 points after peroxide bleaching. Figure-2 shows the hand-sheets of the three pulps (control, PC-treated and PO-treated) after fungal treatment and without bleaching (F), after alkaline extraction (FE) and after peroxide bleaching (FEP). Analysis of filtrates of alkaline extraction for lignin and color showed their remarkable increase in the fungal treated pulps compared to that of control pulp indicating the effect of fungus on extracting out more lignin from the pulp and so the colour (Table-1). It has been reported that the biological delignification of unbleached kraft pulps can perform the same function as chlorination i.e. degrading and solubilizing the bulk of the residual lignin, so that the pulp can be efficiently brightened with chlorine dioxide or hydrogen peroxide [13]. Since lignin degradation by white rot fungus is oxidative with oxygen as the source of oxidizing power [14], biological bleaching can be considered a form of oxygen delignification catalyzed by enzymes.

Physical strength properties of the bleached pulps were also evaluated and given in table-3.The strength parameters were found to be at par except a significant loss in the value of tear index for the fungal treated pulp as compared to the control. This kind of strength loss might be due to the absence of any other nutrient source (ready carbon source) like sucrose/glucose/corn steep liquor during fungal treatment of the pulp and there might be some attack on pulp cellulose as well. Because it has been proposed [2] that addition of exogenous glucose protects wood glucose during fungal attack. Paice et al,1996 [15] have proposed that genetic engineering of a fungus to produce a designed mixture of enzymes and their co-substrates *in situ* may be useful and cost-effective since the fungi have evolved to consume all the components of wood, not to selectively remove the lignin. On comparing the PC and PO-treated pulps together, PO-treated one showed somewhat better strength. It has been reported earlier that PC shows simultaneous (indiscriminate degradation of cell wall layers causing holes and erosions without previous removal of lignin) while *Pleurotus* species shows selective (separation and preservation of the integrity of cellulose fibers) mode of lignin degradation during fungal decay of wood [16].

There are reports available in the literature where the chemical bleaching can be shortened to a smaller sequence after the fungal treatment to get a brightness equivalent to the normal bleaching process. Brightness values of 79.5% ISO and 77% ISO were reportedly [10] obtained through FEP sequence in the soda-AQ pulps of Chinese and Japanese kenaf bast fiber respectively that were equivalent to 78.5 and 76% ISO obtained in the respective pulps using the conventional CEH (chlorine-alkaline extraction-hypochlorite) sequence so the chlorination stage could be substituted satisfactorily with fungal treatment stage. Similarly, brightness value of 86.3% ISO could be obtained [5] using FEP sequence to the oxygen bleached HWKP by totally eliminating the chlorination stage of the respective control pulp bleached using conventional CED (chlorine-alkali extraction-chlorine dioxide) sequence. Biological bleaching of soft wood kraft pulp by growing the fungus *Trametes (Coriolus) versicolor* for a period of 14 days followed by alkaline extraction and then DED (chlorine dioxide-alkaline extraction-chlorine dioxide) bleaching sequence has been reported to increase pulp brightness to 61% ISO as compared to the control which could be bleached to 33% ISO brightness only [2].

Thus the effect of fungus treatment in improving the brightness of the unbleached and bleached pulp in the present study is in concurrence with the previous reports. It can be concluded that the fungal treatment of mulberry pulp can prove to be a "bleach booster" because it could result into a brightness gain of up to 13-18 points as compared to the control pulp. It appears to be very feasible and easy for the handmade paper industry, which utilizes batch procedures at small-scales, to adopt the biobleaching process and become environmentally benign. However, it would be useful to study the effect of adding nutrients like glucose/sucrose/culture medium to the pulp during fungal treatment so as to have a better strength. Besides this, efforts can be made to substitute the autoclaving by steaming or by treatment of the fiber with certain disinfectants before fungal-inoculation so as to make the process cost-effective. Immobilization of the fungal cultures can also be useful.

ACKNOWLEDGEMENT

Authors gratefully acknowledge the facilities provided by the Director, Kumarappa National Handmade Paper Institute (KNHPI), Jaipur and the Executive Director, Birla Institute of Scientific Research (BISR), Jaipur. Our sincere thanks are due to Shri A. K.Sharma, Dy. Director, KNHPI for his support provided during the study. Thanks are also due to Prof. Bedwal, HOD, Dept. of Zoology, University of Rajasthan, Jaipur.

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Tables and Figures

Parameters	Control	PC-treated	PO-treated
Yield loss,%	Nil	12%	5%
Kappa number	33.6	13.6	15.0

Table 1: Yield Loss and Kappa Number of the Mulberry Pulp after Fungal Treatment

Parameters	Control				PC- treated				PO-treated			
Fungal Treatment (F)												
- Pulp brightness, % ISO	23.06				32.2				30.7			
-Gain in brightness points	-				9.2				7.7			
Alkaline extraction (FE)												
-NaOH (%)	1.5		2		1.5		2		1.5		2	
-Lignin (gpl) in filtrate	0.10		0.15		0.607		0.947		0.412		0.607	
-Colour (PCU)	405		485		2195		3232		915		1524	
- Pulp Brightness, %ISO	25.3		25.4		34		36		31.5		32	
-Gain in brightness points	-		-		8.7		10.6		6.2		6.6	
Peroxide bleaching (FEP)												
-H_2O_2, (%)	2	3	2	3	2	3	2	3	2	3	2	3
-Pulp brightness, %ISO	45	46	45	47	53	58	63	63	57	59	58	59
Gain in brightness points	-	-	-	-	8	12	18	16	12	13	13	12

Table 2: Control and Fungal Treated (PC & PO) Pulps of Paper Mulberry

Parameters	Control	PC- treated	PO-treated
Burst index (KPa m^2/gm)	2.10	2.17	2.10
Tensile index (Nm/gm)	23.05	21.3	22
Tear index (mNm^2/gm)	9.8	5.1	6.0

Table 3: Physical Strength Properties of the Mulberry Pulp Bleached Through FEP Sequence

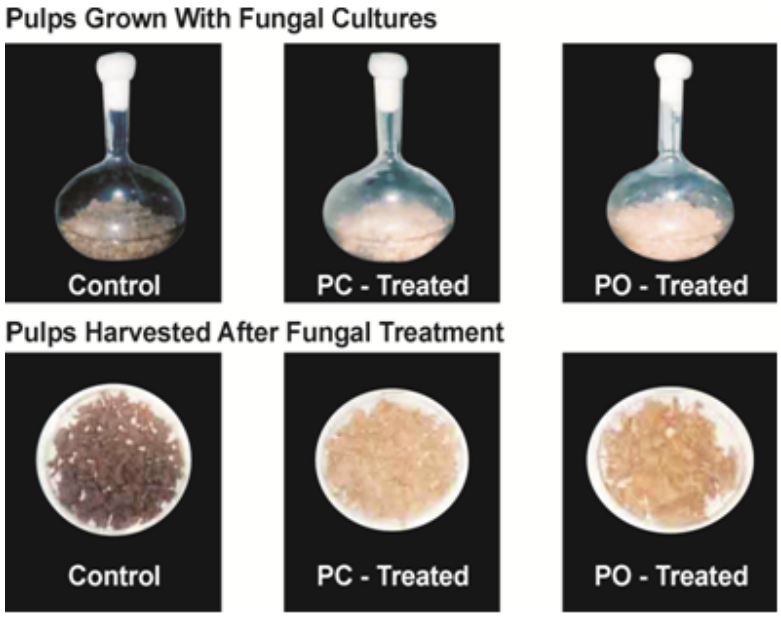

Figure 1. Control and fungal treated pulps of paper mulberry before & after harvesting

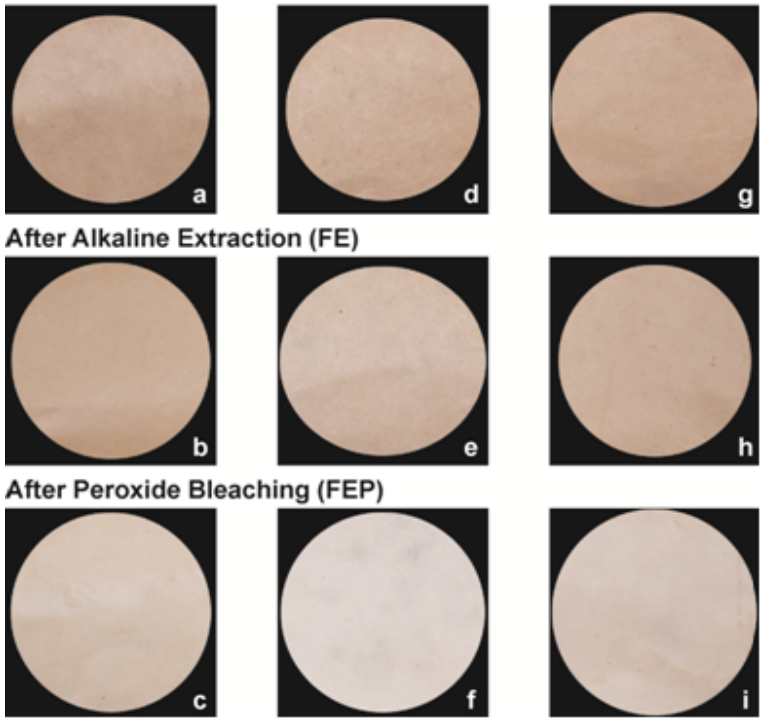

Figure 2. Hand sheets of pulps prepared after F, FE and FEP sequence of the PC and PO treated besides the control pulp of paper mulberry (a,b,c : control; d,e,f : PC-treated and g,h,i : PO treated)