ENZYMES IMPROVE ECF BLEACHING OF PULP

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The delignification efficiency of different laccase enzymes was examined on the eucalyptus Kraft pulp. The laccase enzyme from *Trametes versicolor* showing the highest delignification efficiency was selected and used in the elemental chlorine-free bleaching sequence for improving the pulp bleachability. An appreciable reduction in chlorine dioxide consumption was also obtained. Further reduction in chlorine dioxide consumption was obtained when the same laccase treated pulp was subjected to an acid treatment after the extraction stage followed by the DE_PD sequence. Elemental-chlorine free bleaching was also performed using the xylanase-laccase treated pulp. Xylanase treatment was incorporated to the laccase mediator system in the elemental-chlorine free bleaching both sequentially and simultaneously. The bleaching sequence DE_PD followed and in both the cases, the reduction in chlorine dioxide consumption was greater in comparison to the control. The chlorine dioxide consumption was reduced further when xylanase-laccase treated pulp was given an additional acid treatment. The final pulp properties of the treated pulps were comparable to the control pulp.

Keywords: Laccase, Mediator, Delignification, ECF bleaching, Xylanase, Chlorine dioxide

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INTRODUCTION

Increasing awareness about environmental concerns has led the paper industry to look for cleaner production options aimed at the reduced consumption of chlorine and its compounds in the bleaching sequences which thereby minimizes the discharge of chlorinated organics in the effluent, i.e., AOX. (Mishra et al. 2001). These organo-chlorine compounds are produced mainly by the reactions between residual lignin present in wood fibers and the chlorine used for bleaching. Some of these compounds are found to be toxic, mutagenic, persistent, bioaccumulating, and harmful to biological systems (Bajpai and Bajpai 1996).

Elemental chlorine-free (ECF) bleaching for the pulp and paper industry, based on chlorine dioxide, offers a number of fundamental benefits over the traditional methods. The U.S. EPA's Cluster Rule for the pulp and paper industry has ECF as one of its core Best Available Technology (BAT) elements (Pryke 1997). In addition to producing the highest pulp quality, ECF bleaching has proven itself to be a pollution prevention process for the pulp and paper industry. Perhaps most important is the fact that the use of chlorine dioxide in the first stage of chemical pulp bleaching virtually eliminates dioxins and 12

priority chlorophenols proposed by the U.S. Environmental Protection Agency (EPA) for regulation to non-detect levels. The other benefits of ECF bleaching are that it decreases chloroform formation and total chlorinated organic compound (AOX) formation by 90%; efficiently utilizes forest resources; contributes to eco-system recovery; and is compatible with emerging minimum-impact mill technologies (Pryke 1997).

The use of biotechnology in pulp bleaching has attracted considerable attention and achieved interesting results in recent years (Bajpai and Bajpai 1996; Bajpai et al. 2005; Call 1999; Sariaslani 1989 and Paice et al. 1995a). The incorporation of enzymes into the ECF technology can be of further benefit in terms of consumption of bleach chemicals followed by the amount of pollution generation. Enzyme prebleaching using xylanase enzymes offers a solution by improving the effectiveness of bleaching chemicals in removing lignin (Bajpai and Bajpai 1996; Viikari 1994; Roncero 2005; Kansoh 2004; Sudha 2003). Xylanase enzymes are reported to partially hydrolyze the hemicelluloses portion of pulp. It is presumed that the enzyme hydrolyzes xylan into smaller fragments allowing lignin associated with these short hemicelluloses chains to be more easily removed during subsequent extraction stages in bleaching. Xylanase-aided bleaching is an indirect method which does not directly degrade lignin and thus, has a limited effect (Bajpai and Bajpai 1996; Kansoh 2004; Sudha 2003).

Another potential candidate is a laccase enzyme, which selectively decomposes the lignin in the fiber (Paice et al. 1995a; Call and Muck 1994; Call and Muck 1995a; Call and Muck 1995b; Paice et al. 1995b; Reid and Paice 1994; Kondo 1994; Kondo et al 1995; Bourbonnais and Paice 1996; Camarero et al. 2004; Fu et al. 2000; Kandioller and Christov 2001; Crestini et al. 2003; Nelson et al. 1998). Laccases belong to the multi-copper oxidases, which can reduce elemental oxygen to water in a four-electron step and simultaneously perform a one-electron oxidation of many aromatic substrates (Paice et al. 1995a; Reinhammer 1984). However, this enzyme alone is not able to delignify the pulp; it requires a mediator to become effective (Bourbonnais and Paice 1990). The mediator is a small redox molecule that acts as a "diffusible electron carrier" or "electron shuttle" between lignin and laccase. It is assumed that the mediator is needed because the large laccase molecule cannot enter the secondary cell wall and oxidize lignin directly. In the laccase mediator concept, the oxidized mediator acts directly on lignin and results in efficient delignification. The use of this enzyme is expected to have a lower impact on the environment by eliminating the use of chlorine and formation of organochlorine compounds (Call 2001). Its other expected benefits are a lower capital investment, a safe system for selectively removing lignin, and improved pulp yields (Reid and Paice 1994).

The successive combination of the two enzymatic methods, the hydrolytic xylanase and the oxidative laccase-mediator treatment, has previously been shown to increase the delignification efficiency (Herpoel et al 2002; Viikari et al. 1999). In light of the background cited here, attempts have been made to use xylanase and laccase enzymes in ECF bleaching for selective delignification of pulp for environment friendly bleaching.

EXPERIMENTAL

Wood samples, infected with fungi, were collected from wood yards of pulp mills and other places. The sample pieces were inoculated in sterilized potato dextrose medium containing bacterial inhibitors in 250 ml Erlenmeyer flasks. The flasks were kept under agitation in a shaking incubator at 150 rpm and 30°C for 3-4 days. The fungus mycelium was then streaked on PDA plates. Fungal patches were further streaked on PDA plates many times to get the pure fungal colonies. The pure colonies were inoculated on slants for screening of laccase producing strains. The PDA slants were stored at 4°C until used.

Fungal cultures were inoculated in 50 ml sterilized potato dextrose medium in 500 ml Erlenmeyer flasks, incubated at 27-30°C and 60-70% relative humidity in an incubator. The laccase activity in culture broth of each culture was determined by monitoring the oxidation of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) as increased absorbance at 420 nm. The reaction mixture contained 2.5 ml of 0.1M sodium acetate buffer pH 5.0, 0.17 ml of the enzyme sample and 0.33 ml 5mM ABTS, in a final volume of 3.0 ml. 1 U is the amount of enzyme that converts 1 μmol substrate in one minute under the described conditions at 25°C.

Eucalyptus wood chips were procured from a pulp and paper mill in North India. Pulping was done in a rotary autoclave digester by Kraft process. Numerous experiments were performed at different temperatures, time, and active alkali doses to optimize the pulping conditions to obtain a pulp of kappa number 18-19.

The efficacy of laccase enzymes for delignification of eucalyptus pulp was studied at a pulp consistency of 15%, temperature 45^{o}C, pH 4.0 for 5.5 hours at different dose levels using HBT as mediator. The enzyme showing maximum delignification was selected and used for further studies. The xylanase treatments were carried out at a pulp consistency of 10%, temperature 50^{o}C, pH 8, time 2 hours and enzyme dose 0.075% (Pulpzyme HC from Novozymes).

ECF bleaching of laccase and xylanase-laccase treated pulps was conducted using a DE_PD sequence. The bleaching sequence followed for the reference sample was DE_PDD. Acid treatment was conducted at pH 2, 90°C, for 2 hours at 10% consistency. Xylanase treatment was incorporated both sequentially and simultaneously. The effect of acid treatment in both the cases was also studied. The final pulps were characterized for optical properties.

Kappa number, a measure of residual lignin in the pulp, was determined as per Tappi test method T 236 om-99. The solution viscosity of a pulp gives an indication of the average degree of polymerization of the cellulose. The viscosity of pulp was determined by capillary viscometer method using Tappi test method T 230 om-99. Such a test gives a relative indication of the degradation (decrease in cellulose molecular weight) resulting from the pulping and/or bleaching process. The brightness and CIE whiteness of the pulp were measured using Technibrite TB 1c instrument as per Tappi test method T525 om-02 and T 560 pm-96, respectively. The brightness reversion of the pulp was estimated in terms of post colour (PC) number according to Tappi test method T 260 om-85.

RESULTS AND DISCUSSION

Eucalyptus pulp of kappa number 18.2 was prepared in the laboratory. The unbleached pulp yield was 42% with a pulp viscosity of 10 cp. Where the pulp yield is defined as the mass of pulp (oven dry basis) divided by mass of wood chips (oven dry basis) and expressed as percentage.

Four enzymes, two of Trametes species (Laccase-1 & Laccase-2) and other two of Aspergillus species (Laccase-3 & Laccase-4) were selected. To determine the delignification efficiency of laccase enzymes, the treatment of eucalyptus pulp was done in a rotary autoclave digester with different laccase enzymes and mediator (HBT). Laccase-1 and Laccase-2 showed maximum delignification with 60 U enzyme/g of pulp whereas Laccase-3 and Laccase-4 showed maximum delignification with 120 U and 400 U enzyme/g pulp, respectively. Delignification efficiency was 48%, 50%, 15% and 27% in case of Laccase-1, Laccase-2, Laccase-3 and Laccase-4, respectively (Table 1). Laccase-1 was selected for the bleaching studies, as it showed the highest delignification rate. With Laccase-1 enzyme, maximum delignification occurred at 45°C, pH 4.0-5.5 (adjusted with sodium acetate buffer), retention time 3.0-5.0 hours, pulp consistency 15.0%, oxygen pressure 10 kg/cm^2, enzyme dose 60 U/g and mediator dose 3% (Table 2). Laccase-mediator treated pulps were then alkali extracted using hydrogen peroxide. The brightness and kappa number of the pulp were 48% ISO and 6.3, respectively. To know the effect of E_P alone and E_P followed by acid treatment, experiments were conducted without any enzyme. The kappa number of pulp dropped to 12.0 on extraction alone (E_P), in the beginning, which reduced further to 10.8 (results not shown in the Table) on acid treatment (after EPA).

Table 1. Delignification of Eucalyptus Kraft Pulp* with Different Laccase Enzymes

Parameter	Laccase-1[a]	Laccase-2[b]	Laccase-3[c]	Laccase-4[d]	Control
LE_P stage Kappa no.	6.3	6.1	10.3	9.0	12.1
Reduction in kappa no. (%) by laccase treatment	48.0	50.0	14.9	27	-
LE_P stage brightness (% ISO)	48.0	48.9	35.2	34.5	31.5

*Kappa number 18.2; Kappa number after only alkaline extraction (EP) 12.0
Conditions:
Laccase-1 & Laccase-2: Enzyme dose 60 U/g; HBT (mediator) dose 3%; pH 4.0; temp. 45 °C; residence time 5.5 h; consistency 15%; O_2 pressure 10 kg/cm^2
Laccase-3: Enzyme dose 120 U/g; HBT dose 3%; pH 5.5; temp. 45 °C; residence time 5.5 h; consistency 15%; O_2 pressure 10 kg/cm^2
Laccase-4: Enzyme dose 400 U/g; HBT dose 3%; pH 4.5; temp. 45 °C; residence time 5.5 h; consistency 15%; O_2 pressure 10 kg/cm^2
E_P stage: NaOH 1.5%; H_2O_2 0.5%; temp. 70 °C; residence time 2 h; consistency 10%
[a]Laccase-1: from *Trametes versicolor* TCIRD-2 [b]Laccase-2: from *Trametes versicolor* TCIRD-6, [c]Laccase-3: from *Aspergillus niger* TCIRD-10, [d]Laccase-4: from *Aspergillus niger* TCIRD-15

The results of ECF bleaching of laccase treated pulps are shown in Table 3. An appreciable reduction in chlorine dioxide consumption was observed. Chlorine dioxide demand reduced to 45.6% in comparison to the reference sample (Tables 3 and 4). When the same laccase treated pulp was subjected to an acid treatment (pH 2, temperature 90°C, time 2 h, consistency 10%) after the extraction stage followed by DE_PD sequence, there was a remarkable effect of the acid treatment on the same pulp in the same conditions. The reduction in the chlorine dioxide dose increased to 58.1% in comparison to the control (Tables 3 and 4). This reflected the encouraging role of acid treatment on the laccase-mediator bleaching system.

Table 2. Optimum Conditions of Delignification by Laccase-1

Parameter	Value
Enzyme dose (U/g)	60
Mediator (HBT) dose (%)	3
Consistency (%)	15
pH	4.0
Temperature (^oC)	45
Time (h)	5
O_2 pressure (kg/cm^2)	10

Table 3. Effect of Laccase-Mediator System on ECF Bleaching

Parameter	$D_OE_P*D_1D_2$	LEp**DOEp***D_1	$LE_P**AD_OEp***D_1$
Kappa factor	0.28	0.15	0.12
Reduction in ClO_2 Dose (%)	-	45.6	58.1
LEp** Brightness (%ISO)	-	46.4	-
LEp**A Brightness (%ISO)	-	-	47.0
D_O Brightness (%ISO)	42.0	66.8	63.5
E_P* Brightness (%ISO)	65.2	-	-
Ep***Brightness (%ISO)	-	79.4	78.9
D_1 Brightness (%ISO)	84.5	88.0	88.2
D_2 Brightness (%ISO)	87.8	-	-
Viscosity (cp)	7.2	7.1	6.9
Treatment conditions: L stage conditions: Laccase dose 60 U/g pulp, HBT dose 3%, pH 4.0, temp. 45 °C, consistency 15% and retention time 5 h A stage conditions: pH 2, temp. 90 °C, retention time 2 h, consistency 10% Ep* stage conditions: NaOH dose 0.85%, H2O2 dose 0.5%, temp. 70 °C, consistency 10% and retention time 2 h Ep** stage conditions: NaOH dose 1.5%, H2O2 dose 0.5%, temp. 70 °C, consistency 10% and retention time 2 h Ep*** stage conditions: NaOH dose 0.8%, H2O2 dose 0.5%, temp. 70 °C, consistency 10% and retention time 2 h D_O stage conditions: pH 3.5, temp. 55 °C, retention time 30 min, consistency 10% D_1 stage conditions: ClO_2 dose 0.8%, pH 3.5, temp. 75 °C, retention time 3 h, consistency 10% D_2 stage conditions: ClO_2 dose 0.4%, pH 3.5, temp. 75 °C, retention time 3 h, consistency 10%			

The results of ECF bleaching of xylanase-laccase treated pulp are shown in Tables 4 and 5. The bleaching sequence followed for the reference sample and the xylanase-laccase treated sample was the same as followed for the laccase mediator

bleaching system. It was observed that laccase treated pulp shows a cumulative effect along with xylanase treatment.

When only laccase treated pulp was used, the chlorine dioxide demand was reduced to 45.6% in comparison to the control sample, which increased to 55% when xylanase treatment was also included to the same laccase mediator bleaching system (Tables 4 and 5). Both the simultaneous and sequential combination of xylanases with laccase-mediator bleaching systems showed the same reduction in the bleach chemical consumption. Similar results were reported by Herpoel et al. (2002) with wheat straw pulp where up to 60% reduction in kappa number was obtained after xylanase and laccase sequential treatments, followed by alkaline extraction.

The addition of the acid treatment stage to the xylanase-laccase treated pulp led to further reduction in the chemical consumption to 67.4% rather than 55% when no acid treatment was involved. There was no difference in the results of sequential and simultaneous treatments of xylanase application (Tables 4 and 5). As a parallel experiment for the sake of comparison, the pulp was acid treated without any laccase treatment. There was a decrease in kappa number by 1.5 points (about 8.2%) only but the reduction in chlorine dioxide consumption in $AD_0E_PD_1D_2$ bleaching sequence was about 15% (results not shown in Tables), which indicated the potential of acid treatment.

Table 4. Bleach Chemical Requirements

Sample detail	Bleaching chemical (kg/TP)	
	ClO_2	Reduction in ClO_2 dose (%)
$D_OEp^*D_1D_2$	32.2	-
$LEp^{**}D_O\ Ep^{***}D_1$	17.5	45.6
$LE_P{}^{**}AD_OEp^{***}D_1$	13.5	58.1
$Sm.XLEp^{**}\ D_OEp^{***}D_1$	14.5	55.0
$Sm.XLEp^{**}\ AD_OEp^{***}D_1$	10.5	67.4
$Sq.XLEp^{**}D_OEp^{***}D_1$	14.5	55.0
$Sq.XLEp^{**}\ AD_OEp^{***}D_1$	10.5	67.4

Treatment conditions:
X stage conditions: Xylanase dose 0.075%, pH 8.0, temp. 50°C, retention time 2h and consistency 10%
L stage conditions: Laccase dose 60 U/g pulp, HBT dose 3%, pH 4.0, temp. 45°C, consistency 15% and retention time 5 h
A stage conditions: pH 2, temp. 90°C, retention time 2 h, consistency 10%
Ep* stage conditions: NaOH dose 0.85%, H_2O_2 dose 0.5%, temp. 70°C, consistency 10% and retention time 2 h
Ep** stage conditions: NaOH dose 1.5%, H_2O_2 dose 0.5%, temp. 70°C, consistency 10% and retention time 2 h
Ep*** stage conditions: NaOH dose 0.8%, H_2O_2 dose 0.5%, temp. 70°C, consistency 10% and retention time 2 h
D_O stage conditions: pH 3.5, temp. 55°C, retention time 30 min, consistency 10%
D_1 stage conditions: ClO2 dose in 1st, 2nd, 3rd sequence 0.8% & in others 1.1%, pH 3.5, temp. 75°C, retention time 3 h, consistency 10%
D_2 stage conditions: ClO_2 dose 0.4%, pH 3.5, temp. 75°C, retention time 3 h, consistency 10%

Table 5. Effect of Xylanase Treatment on the Laccase-Mediator Bleaching System

Parameter	$D_OEp^*D_1D_2$	Sm.XLEp** $D_OEp^{***}D_1$	Sm.XLEp**A $D_OEp^{***}D_1$	Sq.XLEp** D_O $Ep^{***}D_1$	Sq.XLEp**A $D_OEp^{***}D_1$
Kappa factor	0.28	0.12	0.09	0.12	0.09
Reduction in ClO_2 dose	-	55.0	67.4	55.0	67.4
Ep* Brightness (%ISO)	65.2	-	-	-	-
XLEp** Brightness (%ISO)	-	49.5	-	49.2	-
XLEp**A Brightness (%ISO)	-	-	50.3	-	50.0
D_O Brightness (%ISO)	42.0	67.8	62.9	68.1	62.7
Ep***Brightness (%ISO)	-	79.7	77.5	77.0	77.3
D_1 Brightness (%ISO)	84.5	88.3	88.0	88.0	87.9
D_2 Brightness (%ISO)	87.8	-	-	-	-
Viscosity (cP)	7.2	7.0	6.8	7.0	6.8

Treatment conditions:
X stage conditions: Xylanase dose 0.075%, pH 8.0, temp. 50 °C, retention time 2h and consistency 10%
L stage conditions: Laccase dose 60U/g pulp, HBT dose 3%, pH 4.0, temp. 45 °C, consistency 15% and retention time 5 h
A stage conditions: pH 2, temp. 90 °C, retention time 2 h, consistency 10%
Ep* stage conditions: NaOH dose 0.85%, H_2O_2 dose 0.5%, temp. 70 °C, consistency 10% and retention time 2 h
Ep** stage conditions: NaOH dose 1.5%, H_2O_2 dose 0.5%, temp. 70 °C, consistency 10% and retention time 2 h
Ep*** stage conditions: NaOH dose 0.8%, H_2O_2 dose 0.5%, temp. 70 °C, consistency 10% and retention time 2 h
D_O stage conditions: pH 3.5, temp. 55 °C, retention time 30 min, consistency 10%
D_1 stage conditions: ClO_2 dose in 1st sequence 0.8% and in others 1.1%, pH 3.5, temp. 75 °C, retention time 3 h, consistency 10%
D_2 stage conditions: ClO_2 dose 0.4%, pH 3.5, temp. 75 °C, retention time 3 h, consistency 10%

The final pulps were characterized and it was found that the pulp properties were comparable to the reference sample, which shows that the laccase-mediator system does not affect the properties of the pulp (Table 6). Poppius-Levlin et al. (1999) have also reported that although laccase treatment temperature, laccase charge and mediator charge had pronounced effect on lignin reactions, their effect on pulp properties was insignificant.

About 43% reduction in kappa number of Eucalyptus kraft pulp after laccase treatment and alkaline extraction has been achieved by Fu et al. (2000), whereas bleaching of high kappa kraft pulps of different raw materials with a laccase mediator system provided 43-61% reduction in kappa number after E+P stage, using violuric acid as mediator (Chandra et al. 2001). Kandioller and Christopher (2004) have also reported various degrees of delignification depending on the pulp type, enzyme and mediator charge using laccase mediator treatment followed by alkaline extraction. By repeated laccase treatment and alkaline extraction (reinforced with oxygen and hydrogen peroxide), up to 80% reduction in kappa number was obtained by Sealey et al (1997).

This indicates that the laccase mediator treatment is capable of reacting with vestiges of residual lignin, which are typically very unreactive.

Further work on the effects of initial alkali extraction (E/ E_P/ E_{OP} stage) and /or acid treatment (A stage) followed by conventional ECF, with and without the laccase mediator system under different conditions, is in progress.

Table 6. Bleached Pulp Properties

Bleaching sequence	Final brightness (%ISO)	Final viscosity (cP)	CIE whiteness (%ISO)	P.C. number
$D_OEp^*D_1D_2$	87.9	7.2	80.0	0.28
$LEp^{**}D_O$ $Ep^{***}D_1$	88.0	7.1	81.1	0.27
$LE_P{}^{**}A$ $D_OEp^{***}D_1$	88.2	6.9	81.2	0.26
$Sm.XLEp^{**}D_OEp^{***}D_1$	88.3	7.0	82.0	0.25
$Sm.XLEp^{**}AD_OEp^{***}D_1$	88.0	6.8	82.0	0.26
$Sq.XLEp^{**}$ $D_OEp^{***}D_1$	88.0	7.0	82.2	0.25
$Sq.XLEp^{**}AD_OEp^{***}D_1$	88.0	6.8	82.3	0.25

Treatment conditions:
X stage conditions: Xylanase dose 0.075%, pH 8.0, temp. 50 °C, retention time 2h and consistency 10%
L stage conditions: Laccase dose 60 U/g pulp, HBT dose 3%, pH 4.0, temp. 45 °C, consistency 15% and retention time 5 h
A stage conditions: pH 2, temp. 90 °C, retention time 2 h, consistency 10%
Ep* stage conditions: NaOH dose 0.85%, H_2O_2 dose 0.5%, temp. 70 °C, consistency 10% and retention time 2 h
Ep** stage conditions: NaOH dose 1.5%, H_2O_2 dose 0.5%, temp. 70 °C, consistency 10% and retention time 2 h
Ep*** stage conditions: NaOH dose 0.8%, H_2O_2 dose 0.5%, temp. 70 °C, consistency 10% and retention time 2 h
D_O stage conditions: pH 3.5, temp. 55 °C, retention time 30 min, consistency 10%
D_1 stage conditions: ClO_2 dose in 1st, 2nd, 3rd sequence 0.8% and in others 1.1%, pH 3.5, temp. 75 °C, retention time 3 h, consistency 10%
D_2 stage conditions: ClO_2 dose 0.4%, pH 3.5, temp. 75 °C, retention time 3 h, consistency 10%

CONCLUSIONS

Based on the bleaching experiments performed in this study, it is concluded that the enzymes have an encouraging role in reducing the chemical consumption during elemental-chlorine-free bleaching. Upon implementation, the applications from the present findings can be expected to help reduce the amount of pollutants that are produced during future manufacture of bleached kraft pulp.

ACKNOWLEDGMENTS

The authors thank Indo-French Centre for the Promotion of Advanced Research (IFCPAR) for providing financial support.

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Article submitted: March 27, 2006; Accepted: May 8, 2006; Published: June 24, 2006