STUDIES ON XYLANASE AND LACCASE ENZYMATIC PREBLEACHING TO REDUCE CHLORINE-BASED CHEMICALS DURING CEH AND ECF BLEACHING

Vasanta V. Thakur, Rakesh Kumar Jain,* and Rajeev Mohan Mathur

The biobleaching efficiency of xylanase and laccase enzymes was studied on kraft pulps from wood and nonwood based raw materials employed in the Indian paper industry. Treatment of these pulps with xylanase enzyme could result in improved properties, showing 2.0% ISO gain in pulp brightness and/or reducing the demand of chlorine-based bleach chemicals by up to 15% with simultaneous reduction of 20 to 25% in AOX generation in bleach effluents. Further, mill-scale trial results revealed that enzymatic prebleaching can be successfully employed with xylanases to reach the same bleach boosting efficacy. Laccase bleaching was also studied on hardwood pulp at a pH around 8.0, where most of the pulp mills in India are operating, in contrast to earlier studies on laccase enzyme bleaching, which were conducted at acidic pHs, *i.e*. 4.0 to 5.0. In case of laccase bleaching, interesting results were found wherein a bleach-boosting effect was observed even at pH 8.0. Further studies carried out with HOBT as mediator in comparison to the commonly used and expensive ABTS laccase mediator system (LMS) resulted in improvement of the bleaching efficiency with reduction in demand of chlorine dioxide by more than 35%. Potential for further reduction was indicated by the brightness gain, when compared with a control using the DE(p)D bleach sequence.

Keywords: Enzymes; Prebleaching; Xylanase; Laccase; Laccase mediators; Chlorine dioxide; ECF bleaching, AOX reduction

Contact information: a: Central Pulp & Paper Research Institute, Saharanpur, 247 001, U.P, India
**Corresponding author: rkjain_knhpi@yahoo.co.in*

INTRODUCTION

As global trends and customer preferences are switching towards cleaner and greener products, a key challenge faced by the pulp and paper industry nowadays is the adoption of elemental chlorine-free (ECF) and totally chlorine free (TCF) bleaching technologies, which reduce the toxic chemical usage and the pollution load with potential for better product quality. The main aim of these technologies is to reduce or to phase out the use of elemental chlorine and chlorine-based chemicals from the bleaching sequences. Paper mills are moving in this direction by adopting eco-friendly technologies such as oxygen delignification and enzymatic prebleaching. The enzymatic prebleaching technology is a cleaner, greener option for the pulp and paper industry.

Biobleaching, employing enzymatic bleaching techniques, is now considered to be one of the preferred routes, primarily because of number of advantages offered over conventional chemical bleaching. The major advantage of the process is a reduction in adsorbable organic halide (AOX) levels in the discharge effluents by reducing the requirement of chlorine during bleaching and improved pulp quality gain in brightness and the reduction in Post Colour Number (PC number).

Xylanases are the enzymes that have mainly bean used for prebleaching of pulps. Xylanases that are thermostable and cellulase-free are preferred for biobleaching of paper pulp (Soltanali and Shirkolaee 2007). Application of xylanases in the pulp and paper industry has increased significantly during the past few years (Rajasekar 2007). Xylanases have saved chemical costs for the industry without interfering with the existing processes. This technology has increased the bleaching speed in both TCF and ECF processes and, in the case of chlorine dioxide bleaching, has actually increased the throughput of the plant due to debottlenecking at the chlorine dioxide generator (Paice *et al*. 1995).

Other enzymes used for biobleaching include peroxidases, which use hydrogen peroxide, and laccases, which use oxygen and eventually react with the lignin-derived moieties. Most of the direct bleaching with enzymes is done using oxidative enzymes that directly attack the color-producing compounds in the lignin. The enzymes that attack the lignin components of the fiber are oxidative (Arias *et al*. 2003). Laccases are considered to be some of the most promising enzymes for such applications (Reid 1998). Treating pulp with fungal enzymes was found to partially enhance the brightness, but it did not brighten the pulp to the same extent as fungal growth on the pulp (Kenealy and Jeffries 2003). However, the use of oxidative enzymes rather than whole organisms does avoid the problem of cellulose hydrolysis. Laccases from fungi have been found to reduce the kappa number and enhance the bleaching of kraft pulp when they are used in the presence of chemical mediators such as 2, 2’-azinobis-(3-ethylbenzthiazoline-6-sulfonate (ABTS) (Wong *et al.* 1999). The more common laccase mediators, 1-hydroxybenzotriazole (1-HBT) and ABTS, have been used by many researchers comparing effects with various laccases. Different laccases have also been shown to react differently with mediators (Arias *et al*. 2003). 1-HBT and ABTS were better mediators for delignification than other mediators tested. However, both are expensive (Bourbonnais *et al*. 1997). The need for an inexpensive, effective, nontoxic mediator for laccase treatment is a critical problem. Use of laccase for bleaching has been limited by the availability of the enzyme and the cost of the mediators needed for lignin destruction (Arias *et al*. 2003). Hence, there is an immediate need for a laccase mediator that is cheap and easily available to the paper industry.

Biobleaching technology is in the developmental stage as far as the Indian paper industry is concerned. A number of xylanase enzymes are available in the market, but these enzyme preparations are highly sensitive to pH and temperature, which vary considerably from mill to mill depending upon the kind of fibrous raw materials employed and also the process conditions. Under the conditions prevalent in the Indian industry it is required to identify tailor-made enzymes that could tolerate the extreme conditions, particularly pH and temperature, existing in the mills.

Central Pulp & Paper Research Institute (CPPRI) has been engaged in the area of enzymatic prebleaching of pulps for the last decade. Effects of both imported and indigenously developed xylanases on pulps produced from various raw materials, i.e. eucalyptus, bamboo, and bagasse, etc., used in the Indian paper industry were studied in a detailed and systematic manner. The objective was to develop and/or identify suitable enzymes effective for bleaching of pulps resulting from wood-based (eucalyptus) and nonwood (bagasse and bamboo) with a goal to reduce the requirement of chlorine-based chemicals and AOX levels in bleach effluents while achieving improved brightness and other optical properties of the pulp.

The present paper discusses the results of the enzymatic prebleaching of wood and nonwood pulps using xylanase and laccase enzymes and also the results of the implementation of the xylanase bleaching in an Indian Paper mill based on woody raw materials to produce writing and printing grades of paper.

EXPERIMENTAL

Materials

Pulps used in the study

Eucalyptus kraft pulp and kraft bagasse pulps were collected from an integrated paper mill. Unbleached screened kraft pulps were collected from the high density tower of the respective pulp and paper mills before going into the bleaching section. The kappa number of the pulps used in the present study were 17.0 (hardwood pulp) and 10.0 (bagasse pulp), respectively.

Enzymes

The xylanase (Pulpzyme HC) enzyme used in the study was procured from a commercial enzyme supplier (Novozymes). (Xylanase activity, 5000 IU/mL)

The laccase enzyme used in the study was a crude enzyme produced from an isolated white rot fungus in the laboratory. (Laccase enzyme activity, 350 U/mL)

Microorgansim

White rot fungus was isolated from soil collected from pulp and paper mill. The fungus was purified, grown, and maintained on malt extract agar.

Laccase enzyme production

Laccase production was carried out in flask level with 100 mL malt extract broth inoculated with 10% of 5 day old fungal discs and incubated at optimum temperature (30°C) and pH (5.0). Crude laccase enzyme filtered after 7 days was used in present study.

Methods

Enzymes and activity measurements

Xylanase activity was determined according to the Bailey method (Bailey *et al.* 1992). The activity was determined by measuring the release of reducing sugars from oat spelt xylan. Appropriately diluted enzyme solution (0.2 mL) and 1.8 mL 1% oat spelt xylan solution in 0.05 M sodium phosphate buffer (pH 7.0) were incubated for 30 min. at 50°C. After incubation, the reaction was stopped by adding 3 mL DNS (Dinitro salicylic acid) reagent and the solution was boiled for 5 minutes, and the absorbance at 540 nm was measured. One International unit of enzyme was defined as the amount of enzyme releasing 1 μmole of xylose sugars per minute per mL of enzyme.

Laccase enzyme activity was analysed following the procedure described in Arora and Sindhu (1985). Enzyme activity is assayed at 30°C by using 10 mM guaiacol with 100 mM sodium citrate buffer. The change in absorbance of the reaction mixture containing guaiacol was monitored at 470 nm for 30 minutes of incubation. One unit of activity was defined as the change in absorbance at 470 nm of 0.001 per minute per mL of enzyme.

Xylanase enzyme treatment of the pulp

Xylanase enzyme treatment of the hardwood and bagasse pulps was carried out using the pretreatment conditions given in the Table 1. Pulps used in the study were procured from integrated mills. Xylanase enzyme dose was optimized on both pulps based on the yield loss, kappa number reduction, and brightness improvement of the pulp and also lignin and color of the pulp filtrates after enzyme treatment (Data not given). Optimised dose of the identified enzyme was added to the pulps after sufficient dilution and mixed properly by kneading mechanism. Control pulps were prepared identically to the enzyme treated pulp with enzyme replaced with water. After enzyme treatment, pulps were washed and subjected to characterization.

Table 1. Enzymatic Pretreatment Conditions

Particulars	Kraft hardwood pulp		Kraft bagasse pulp	
	Control	Xylanase Treated	Control	Xylanase Treated
Enzyme dose, kg/t pulp	-	0.5	-	0.5
Treatment Time, (min)	150	150	120	120
Temperature, ^{o}C	50	50	50	50
Consistency, %	10.0	10.0	10.0	10.0
pH	8.5-8.7	8.5-8.7	8.8	8.8

Characterisation of pulp and effluents after enzyme treatment

After enzyme treatment, enzyme treated and untreated pulps were analysed for yield loss, kappa number, and brightness and pulp effluents squeezed after enzyme treatment were subjected to chemical analysis like colour and lignin.

Studies on ECF Bleaching of enzyme treated pulps

Enzyme-treated and untreated wood and bagasse pulps were bleached with an ECF sequence (D_0E(p)DD) to explore the potential of savings of chlorine dioxide, AOX reduction in effluents, and to assess the gain in final brightness of the pulp. Process conditions used in bleaching studies are given in Table 2.

Characterisation of pulp and effluents after bleaching

After bleaching, both enzyme-treated and untreated pulps were anlysed for brightness and micro kappa number (during mill scale studies). Handsheets were prepared at neutral pH following TAPPI Test method 205 and analysed for strength and optical properties such as burst index, tensile and tear index values, and PC number. The final bleach pulp effluents were analysed for AOX, chemical oxygen demand (COD), biological oxygen demand (BOD), colour and lignin.

Table 2. Process Conditions Used During ECF Bleaching (D_0E (p) DD) of Pulps

Particulars	ClO_2 (D_O) stage	Alkali Extraction (Peroxide) stage	D_1 stage	D_2 stage
Temperature, °C	55	70	80	80
Consistency, %	3.5	10.0	10.0	10.0
Treatment Time (min.)	45	60	180	180
pH	2.0-3.0	11.0	3.0-4.0	3.0-4.0
D_0E (p) D1D2 – Bleaching sequence. D_0 – chlorine dioxide 1st stage , E(p) Extraction stage with hydrogen peroxide, D_1 - chlorine dioxide second stage , D_2 - chlorine dioxide third stage				

Adsorbable Organic halogens (AOX)

AOX of the effluents was measured using the procedure standardized in Germany in 1985 and known as the DIN 38 409 Tail 14 AOX procedure. This is an instrumental method, and the values obtained in AOX determination include most chlorinated organics; results are reported as originally bound chlorine. However, some highly volatile compounds including chlorinated methane are excluded. The test involves the adsorption of chlorinated organics onto activated carbon and then combustion at higher temperature in presence of oxygen followed by micro-coulometric titration of produced halides. The test has been adopted as a legally enforceable measure for control of chlorinated organic compounds in effluent.

Lignin

Lignin in the effluents was measured taking the absorbance of the samples at 280 nm. Dilutions of the samples were made when the absorbance values were out of the range 0.2 to 0.8. Lignin was calculated using the formula, Lignin, mg/mL = $Ab_{280} \times$ dilution factor/absorptivity of lignin, where the value 21 is used for hardwood and 20 for agro-based lignin. Other parameters were analysed using the methods listed in Table 3.

Table 3. Standard Methods Used in the Experimental Work

Parameter	**Method**
Characterization of Pulp	
Kappa number	TAPPI –T236 om-99
Micro kappa number	TAPPI – T206-208
Brightness	ISO 2470 1999 (E)
CED Viscosity	TAPPI – T230 om-89
Tensile Index	ISO 1924 -2:1994(E)
Tear Index	ISO 1974 1990
Burst Index	ISO 2758: 2001(E)
PC Number	TAPPI-260 om-85
Characterisation of Effluent	
COD	APHA 5220
BOD	APHA 5210
Colour	APHA (Platinum-cobalt method)

RESULTS AND DISCUSSION

Xylanase Pretreatment Followed by ECF Bleaching of Pulps from Wood and Nonwood Pulps

Characterisation of the unbleached pulp after enzyme treatment

Unbleached pulp samples of both enzyme-treated and untreated conditions were characterized for kappa number, brightness (% ISO), and other parameters of interest. Results are shown in Table 4. Enzyme-treated pulps showed slight reduction in kappa number and improvement in brightness when compared with the control. Several reports described the reduction in kappa number by different xylanases. The xylanase from *Streptomyces chartreuses* L1105 xylanase showed *ca.* 12.2% reduction in kappa number of wheat straw pulp (Li *et al*. 2011), and *Bacillus pumilus* xylanase showed a 14% reduction in kappa number of kraft pulp (Bim and Franco 2000). Colour ($A_{465\text{nm}}$) and lignin ($A_{280\text{nm}}$) levels of all the xylanase enzyme treated pulp filtrates showed remarkable increase when compared to the untreated pulps. The release of chromophores and hydrophobic compounds ($A_{280\text{nm}}$ and $A_{465\text{nm}}$) and the reduction in kappa number coupled to the release of reducing sugars suggested the dissociation of LCC from the pulp fibres (Beg *et al.* 2000), and the release of these compounds was maximal after 2h of reaction time (Li *et al.* 2011). Not much pulp yield loss was observed with optimized and enzyme dose used in the present study, but pulp loss was observed with high doses of enzyme during optimization studies (Data not given).

Table 4. Characterization of Enzyme Treated and Untreated Pulps

Particulars	Kraft hardwood pulp		Kraft bagasse pulp	
	Control	Xylanase Treated	Control	Xylanase Treated
Pulp Yield , %	99.6	99.1	99.8	99.2
Kappa Number	16.7	16.0	9.96	8.56
ISO Brightness, %	29.7	30.9	33.43	35.6
Analysis of pulp filtrates of enzyme treated and untreated pulps				
Lignin A_{280}, kg/t pulp	0.5	0.96	0.30	1.10
Colour A_{465}, Kg/t pulp	8.91	12.9	4.7	13.0

Effect of enzymes on brightness of the pulp

Results shown in the Table 5 indicate that the enzyme-treated pulps could be bleached to higher brightness with a gain in brightness level of more than 2.0% ISO. The brightness of the hardwood and bagasse pulp was increased by 2%. Improvement in brightness (0.7 to 1.0) unit over the control pulp was observed with Xyn5B and Xyn11A (Gallardo *et al*. 2010).

Effect of enzyme treatment on bleach chemical requirement

The potential benefit of the xylanase treatment lies in reduction of bleaching chemicals. Enzyme-treated pulps differ in response to bleach chemical when compared to untreated pulps. Results shown in Table 5 showed that the chlorine dioxide dose of the two enzyme treated pulps was reduced by 15% in comparison to that of untreated pulps.

Table 5. ECF Bleaching of Enzyme-Treated and Untreated Pulps

Particulars	Kraft hardwood pulp			Kraft bagasse pulp		
	Control	Xylanase-Treated		Control	Xylanase-Treated	
Applied ClO_2, kg/t pulp (D_0 stage)	35.0	35.0	29.5	28.0	28.0	23.8
ClO_2 Savings, %	-	-	15.6	-	-	15
ISO Brightness, %	82.0	84.0	82.9	82.9	85.0	83.0
Brightness improvement, unit	-	2.0	0.9	-	2.1	-

For Hardwood kraft pulp - E(p) – NaOH – 20 kg/t pulp D_1 - ClO_2 – 18 kg/t pulp
H_2O_2 - 10 kg/t pulp D_2 - ClO_2 – 5 kg/t pulp

For Bagasse kraft pulp - E(p) – NaOH – 20 kg/t pulp D_1 - ClO_2 – 13 kg/t pulp
H_2O_2 - 10 kg/t pulp D_2 - ClO_2 – 5 kg/t pulp

Bleaching of the control and enzyme treated pulps showed remarkable reduction in chlorine dioxide, which was reduced from 35.0 kg/ton to 30.0 kg/ton in the case of enzyme treated hardwood pulps, and from 28.0 kg/ton to 23.8 kg/ton for bagasse pulps. Further, xylanase-treated hardwood pulp also showed a 0.9 unit brightness improvement after savings of chlorine dioxide 15%, which was an additional benefit of the enzymatic treatment. The results of xylanase pretreatment of wheat straw pulp demonstrated that the additive effects of the action of xylanase rendered the fibers more accessible to chemical bleaching agents, assisting in lowering the concentration of the latter in the subsequent bleaching process (Li *et al*. 2011). Other reports have indicated a substantial saving of chlorine dioxide (15 to 35%) in an ECF sequence (Allison *et al*. 1996). The hardwood pulp shows a xylanase (luminase) enzyme response at a dosage of 10 g/t pulp reduces 20% of ClO_2 and NaOH with 1 to 2% higher pulp brightness (Yin *et al*. 2011).

Effect of enzyme treatment on Strength & Optical properties of the pulp

Enzyme-treated pulps and untreated pulps were refined in a PFI mill up to 250 to 350 mL CSF. Handsheets of the final pulp were made and evaluated for desired strength and optical properties. Results are shown in Table 6. The pulp quality in terms of burst, tensile, and tear indexes of the hardwood and bagasse enzyme treated pulps was at par with the untreated pulps, showing that enzyme treatment did not show any negative effect on strength of the pulp. Effect of xylanase on strength properties was similar to that reported by Shakhes and Marandi (2011), *i.e.* no great modifications of mechanical properties were observed after a xylanase treatment. Atik *et al*. (2006) reported that a significant difference due to the xylanase enzyme treatment of pulp was not found in tensile or bursting strength properties of aspen wood pulps. Unbleached softwood kraft pulp treated with Pulpzyme HC had a decreased freeness with minimal changes in tear and tensile index at a given apparent density; *i.e*. the tear/tensile relationship remained essentially unchanged after Escher-Wyss refining (Dickson *et al*. 2000). The treatment of pulp with xylanase only decreases the tear index, which may be explained by action of xylanases that reduce the intrinsic fibrillar resistance due to removal of superficial hemicelluloses (Batalha *et al.* 2011). The increase in xylanase dose decreased the strength properties and bulk of wheat straw soda-AQ pulp. The decreased strength may have been due to the removal of hemicellulose, therefore decreasing the fiber bonding capacity (Ates *et al.* 2009). Xylanase treatment of eucalyptus kraft pulp improved the pulp properties such as tensile strength and burst factor by up to 63% and 8%, respectively (Beg *et al.* 2000).

Table 6. Strength Properties and Optical Properties of Enzyme-Treated and Untreated Pulps

Particulars	Hardwood kraft pulp		Bagasse kraft pulp	
	Control	Xylanase Treated	Control	Xylanase Treated
Strength Properties				
PFI Revolutions	4000	4000	1500	1500
Freeness, CSF	250	300	260	330
Burst Index, KPa.m^2/g	6.2	6.0	4.6	4.5
Tensile Index, Nm/g	81.0	80.5	68.5	67.5
Tear Index, mn.m^2/g	7.2	7.4	5.6	5.6
Optical properties				
Yellowness, %	9.2	8.7	6.12	5.42
PC Number	3.1	1.9	2.8	1.12
Reduction of PC Number, %	-	38	-	40

Results of optical property testing shown in Table 6 indicate that yellowness and post colour number of enzyme treated pulps (Fig. 1) were significantly reduced compared to the unbleached pulps without enzyme treatment. There was considerable decrease in post colour number of enzyme treated pulps to the tune of 40% compared to the untreated pulp. The PC number of the fully bleached pulps both with and without xylanase treatment in TCF bleaching was 0.14 to 0.24 and 0.38 to 0.81, respectively. The carboxylic acids and their counter ions which resulted in color reversion were removed by xylanase treatment. In addition, it would appear that the partial removal of xylan in pulps by xylanase treatment also yielded brightness stability because of the decreased chance of colour reversion (Kim and Paik 2000).

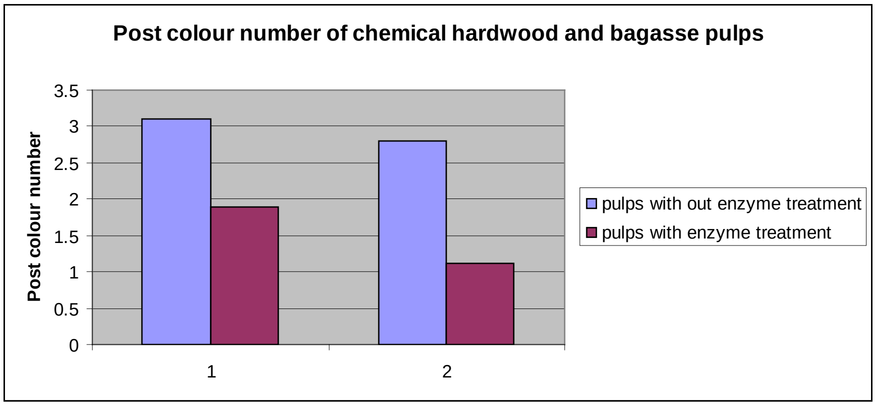

Fig. 1. Post colour number of bleach pulps with and without enzyme treatment

Effect of enzymatic treatment on the environment

The main attractive benefit of the eco-friendly enzyme prebleaching technology is the potential for AOX reduction in bleach effluents. To determine the effect of enzyme treatment on the environment, the pollution load of the enzyme-treated and untreated pulp bleach effluents was analysed in terms of COD, BOD, and AOX.

Table 7. Characterization of Bleach Effluents of Enzyme-Treated and Untreated Pulps

Particulars	Kraft hardwood pulp		Kraft bagasse pulp	
	Control	Xylanase Treated	Control	Xylanase Treated
AOX, kg/t pulp	0.9	0.67	0.5	0.36
AOX Reduction, %	-	25.6	-	28
COD, kg/t pulp	62.48	62.12	39.56	31.01
BOD, kg/t pulp	39.56	31.01	24.06	20.57

Reduction in total chlorine dioxide demand of around 15% during D_0E (p) D1D2 bleach sequence resulted in lowering the toxicity of the bleach plant effluent in enzyme treated pulps remarkably. From the results shown in Table 7 it is evident that the AOX level in the bleach effluent generated from pulps treated with enzyme was reduced to the tune of 25 to 28%, *i.e.* 25.6% in hardwood pulps and 28% in bagasse, respectively, compared to the untreated pulps.

Further, both COD and BOD of the effluents of the enzyme treated pulps were significantly reduced when compared to the untreated pulps. Overall, enzyme treatment showed promise in reducing the pollution load of the bleach effluents of chemical pulps. Xylanase bleaching led to a reduction in effluent AOX, and the BOD to COD ratio of the bleach effluent was increased, indicating that the effluent became more amenable to biological degradation in secondary treatment (Bajpai 2010). Yin *et al*. (2011) observed different COD levels (COD increase and decrease) in various mills with the same xylanase enzyme. Further, they stated that the effective low dosage xylanase may be used to achieve improved bleaching cost reduction without a pulp yield loss and filtrate COD increase. Kim and Paik (2000) reported that the environmental end-load decreased with xylanase treatment and also when compared to CEDED, the OC/DED and TCF bleaching with and without xylanase treatment resulted in the reduction in COD and BOD.

The overall impact of xylanase enzyme on hardwood and bagasse pulp ECF bleaching was encouraging. The major benefits of this technology were 15% chlorine dioxide reduction, 25 to 28% AOX reduction in bleach effluents, and 30 to 40% PC number reduction.

Commercial Mill Scale Trial of Xylanase Prebleaching in an Integrated Hardwood Based Indian Paper Mill

Having realized the need and specific requirements of the Indian paper industry and to create confidence among Indian paper mills, the Institute has carried out commercial scale mill trials to demonstrate the enzymatic prebleaching process on a mill scale with an aim for improving the quality (optical) properties of pulp and to reduce environment impact, and in particular to reduce the AOX level in bleach effluent.

The mill considered uses wood, bagasse, waste paper, and purchased pulp for making of various grades of specialty papers, writing and printing grades, packing grades, *etc*. A considerable amount of pulp is made from wood, *i.e.* *Eucalyptus* and *Casuarina*. Brown stock washer (BSW) pulp is washed through vacuum washers and screened prior to thickening for high density storage and bleaching. The bleach sequence used was CE(p)1E(p)2H, *i.e.* chlorination (Cl_2 stage), alkali extraction stage with hydrogen peroxide, 1 & 2 stages (E(p)1& E(p)2), and a calcium hypochlorite stage (Hypo stage). The total chlorine used for a given quantity of wood was high, and the mill management wanted to reduce the amount of total chlorine through an enzymatic bleaching route; therefore, enzymatic prebleaching technology was implemented at the site.

After commissioning of the additional equipment required for enzymatic prebleaching of pulp, *i.e.* a medium consistency pump, standpipe, dosing pump, *etc*., the mill trials were conducted employing xylanase. Before starting the trial, the pulping and bleaching conditions prevailing in the mill were constantly monitored for a period of 10 days with respect of temperature, pH, kappa number, carryover of the unbleached pulp, and bleach chemical requirements, as well as the residual chlorine and other parameters in the filtrates and pulp mats at various washers.

Effect of enzyme on requirement of chlorine chemicals

Enzymatic treatment was carried out at pH 9 to 10 and a temperature of 50 to 60 °C. The effects of the enzyme treatment on mill scale are recorded in Table 8. A reduction in kappa number of the unbleached pulp was observed, with a drop from an average of 23.0-25.0 to 21.0-22.0. There was a drop in the kappa number at all the stages during bleaching, *i.e.* at the C stage it dropped from 7.0 to 6.0, at the EP1 stage from 5.0 to 4.0, and at the EP2 stage from 4.0 to 3.0 with gain in brightness *i.e.* around 2 to 3 units in each stage, respectively. Having observed the drop in kappa number and the potential to reduce chlorine consumption, the chlorine charge at ‘C’ stage was reduced to 4.0 kg/t pulp from an average mill required dose of 5.0 kg/t pulp. Further, the hypochlorite flow rate was reduced from 75% to 60-65% (15 m^3/h to 13 m^3/h), and the hypochlorite consumption was reduced from 45.0 kg/t pulp to 38.0-40.0 kg/t pulp, indicating a saving of 10 to 12% of hypo while maintaining the targeted ISO brightness of 82-83%. Finally, it was observed that around 15% savings of the total chlorine both ‘C’ and ‘H’ stages could be possible in bleaching of hardwood pulp using xylanase enzymes on mill scale.

Further the PC Number of final bleached pulp after enzyme treatment was reduced from an average value of 4.0 to less than 2.5.

Effect of xylanase enzyme on Environment

The main attractive benefit of the eco-friendly enzyme prebleaching technology is the AOX reduction in bleach effluents. Reduction in total chlorine demand of around 15% during CE (p) 1E (p) 2H bleach sequence resulted in lowering the toxicity of the bleach plant effluent in enzyme treated pulps remarkably. From the results in Table 9 it is evident that the AOX level in the bleach effluent generated during enzyme treatment was reduced to the tune of 23% i.e. from 5.25 to 4.01 kg/t pulp. Further, there was not much change in COD and in BOD of the bleached effluents during enzyme treatment.

Table 8. Consolidated Results of Xylanase Prebleaching of Mill Trial

Particulars	Without Enzyme	With Enzyme
BSW pulp kappa No.	23-25	23-25
Screened Pulp Kappa No.	22-24	21-22
Kappa No. Reduction	1.0 –1.5	2.0-3.0
Pulp Brightness, %	27-30	29-32
CED Viscosity, cm^3/g	727-836	779-892
Chlorination Stage		
Appl. Cl_2 Dose, %	5.0	4.8
Appl. Cl_2 Dose , kg/t pulp	50	48
Cl_2 Savings, kg/t pulp	-	2.0
Cl_2 Savings, %	-	4%
Residual Cl_2 in Filtrate ,mg/L	50-135	100-450
Residual Cl_2 in pulp , mg/L	20-36	30-70
Micro Kappa No.	7.0-8.0	6.0-7.0
ISO Brightness , %	36-38	40-43
ISO Brightness Improvement	-	3-4
Ep-1st Stage		
Applied NaOH, %	1.5	1.5
Applied H_2O_2 ,%	0.5	0.5
Micro kappa No.	5.0-5.9	4.0-5.0
ISO Brightness, %	42-47	43-50
ISO Brightness Improvement	-	2-3
Ep - 2nd Stage		
Applied NaOH, %	0.5	0.5
Applied H_2O_2,%	0.5	0.5
Micro kappa No.	3.6-3.8	3.0-3.5
ISO Brightness , %	53-54	54-59
ISO Brightness Improvement	-	3-4
Hypo Stage		
Hypo Flow , m^3/h	75	62-65
Applied Hypo, kg/t pulp	45	38-40
Applied Hypo, %	4.5	3.8-4.0
Hypo Savings, %	-	11-15
Residual Cl_2 in Filtrate, mg/L	460-560	400-1000
Residual Cl_2 in pulp, mg/L	25-85	50-300
ISO Brightness, %	82-83	82-83
PC Number	3.0-4.0	2.0-2.5
CED Viscosity, cm^3/g	239-377	299-401

Table 9. Nature of Bleach Effluents (combined) during Enzyme Treatment

Particulars	Without enzyme	With enzyme
AOX , kg/t pulp	5.25	4.01
AOX Reduction , %	-	23.6
COD , kg/t pulp	700-820	672-800
BOD, kg/t pulp	230-250	230-248

The overall impact of xylanase enzyme on hardwood pulp bleaching on a mill scale is shown in Table 10. The major benefits of this technology were a two-unit reduction in unbleached kappa number, 15% chlorine reduction, 20 to 25% AOX reduction in bleach effluents, and 30 to 40% PC number reduction. The results of the mill trial showed that the technology is technically viable in the Indian paper industry.

Table 10. Overall Impact of Enzyme Prebleaching on Hard Wood Pulp

Parameters	Enzyme effect
Unbleach kappa no. reduction	2 unit
Total Cl_2 reduction , %	15
AOX Reduction, %	20-25
PC number reduction,%	30-40

Implementation of enzyme prebleaching technology in a mill

Before introducing enzyme prebleaching technology in any mill it is important to evaluate a particular enzyme preparation for its response towards pulp for various parameters such as temperature, pH, enzyme activity, and cellulase contamination. Good washing practices in a mill will increase the efficiency of the enzyme and also lower the enzyme dose, thereby reducing the cost of the enzyme technology. Incorporation of enzyme prebleaching technology in ClO_2 using mills can be highly cost effective, since it can decrease the amount of ClO_2 and the cost of generating ClO_2. The use of xylanase in the paper industry will boost enzyme production rates, which may result in lowering of xylanase cost, and enzyme may be available in the market at lower price. Because the pulp and paper sector can be expected to benefit from enzyme prebleaching technology, there is motivation to promote this technology and explore the possibilities of achieving the desired standard norms in respect of the AOX levels to clean the environment.

Laccase Pretreatment followed by ECF bleaching of pulps from hardwoods

Having vast experience of the xylanase bleaching with CEH bleaching with around 15% chlorine savings in both lab and mill scale, which is not much economical at this stage in the Indian Paper Industry, the Institute had realized the need of more reduction in chlorine demand and extended its Research and Developmental activities in laccase enzymatic ECF bleaching. Most of the laccase bleaching done by other workers has been in the pH range 4.0 to 5.0 (Bajpai *et al*. 2006). In the present study, laccase enzyme treatment was done at a weakly alkaline pH of the pulp (7.5 to 8.0), which is the most prevalent pH in mills currently.

Laccase enzyme treatment and ECF bleaching

Laccase enzyme treatment was carried out on hardwood pulp collected from an integrated paper mill using the conditions shown in Table 11. Laccase treatment was done at pH 8.0 without adjusting the pH of the pulp. To improve the bleaching efficiency of the laccase enzyme, H_OBT and ABTS were added as mediators. Both the enzyme-treated and untreated pulps were subjected to DE_PD bleach sequence.

Table 11. Laccase Enzyme Pretreatment Conditions

Particulars	Control	Enzyme treated pulp		
		Laccase	Laccase+$\mathrm{H_OBT}$	Laccase + ABTS
Enzyme dose, U/g	-	100	100	100
Treatment Time, (min)	240	240	240	240
Temperature, ^{o} C	50	50	50	50
Consistency, %	10.0	10.0	10.0	10.0
pH	7.8	7.8	7.2	7.2
$\mathrm{H_OBT}$,%	-	-	0.5	-
ABTS, %	-	-	-	0.5

Effect of laccase treatment on brightness and chlorine dioxide demand

Results shown in Table 12 indicate that the enzyme treated pulps could be bleached to higher brightness with a gain in brightness level of 3.0 units compared to the control.

Table 12. $D_0E_PD_1$ Bleaching of Hardwood Pulps

Particulars	Control	Enzyme treated pulp							
		Laccase		Laccase+ $\mathrm{H_OBT}$			Laccase+ ABTS		
Applied ClO_2, kg/t pulp	38.0	38.0	28.5	38.0	28.5	24.7	38.0	28.5	24.7
ClO_2 Savings, %	-	-	25	-	25	35	-	25	35
ISO Brightness, %	82.6	83.1	82.3	85.5	84.1	83.0	85.8	83.5	82.7
ISO Brightness improvement, unit	-	0.5	-	2.9	1.5	0.8	3.2	0.9	-
For Hardwood kraft pulp - E(p) – NaOH – 25 kg/t pulp D_1 - ClO_2 – 18 kg/t pulp H_2O_2 - 10 kg/t pulp D_2 - ClO_2 – 5 kg/t pulp									

An appreciable reduction in chlorine dioxide consumption was observed even with the LMS system at a pH of nearly 8.0. The demand was reduced to 35% with a brightness improvement of 1.5 units in comparison to the untreated sample, which indicated that there is a further scope of reduction in chlorine dioxide consumption. Bajpai *et al.* (2006) reported a reduction in chlorine dioxide demand with an LMS system when used at pH 4.0. The results also showed that $\mathrm{H_OBT}$ can be used as a mediator. $\mathrm{H_OBT}$ showed better response when compared with the more expensive mediator ABTS. Further studies on laccase mediator systems are in progress.

CONCLUSIONS

1. Based on studies carried out on enzymatic prebleaching of eucalyptus and bagasse chemical pulps employing xylanase and laccase enzymes, it was concluded that there is considerable potential for its implementation in the Indian Paper Industry.
2. The implementation of enzymatic prebleaching of chemical pulps from the above mentioned commonly used raw materials could play a significant role in reducing

the requirement of chlorine-based chemicals by more than 15%, thereby helping to reduce the AOX level in bleaching effluents by 20 to 25%.

3. An improvement in the optical properties of the pulp, particularly a gain in brightness level by around 2% was noticed, along with reduction in post colour number (PC No.) by more than 30% without loss in strength properties.
4. The studies further confirmed the potential of laccase enzyme for prebleaching of eucalyptus chemical pulps and the results were highly encouraging with respect to reducing the demand for chlorine-based chemicals by up to 35% even at a pH level near to neutral/alkaline (8.0), in contrast to the more commonly studied pH of around 5.0 at which most of the laccase enzymes had been found effective.
5. The effectiveness of the identified laccase enzyme for prebleaching at pH level of 8.0, where most of the wood based pulp and paper mills in India are operating, has given confidence to implement the process on a commercial scale without any requirement of acid addition to bring the pulp pH to around 5.0.
6. Thus, implementation and demonstration of the xylanase enzyme prebleaching in wood (eucalyptus) based paper mills has increased the confidence level in Indian paper industry to promote and to adopt the enzyme bleaching, which should further help in improving the environmental status, in addition to improving pulp quality.

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