# MITIGATION OF ADSORBABLE ORGANIC HALIDES IN COMBINED EFFLUENTS OF WHEAT STRAW SODA-AQ PULP BLEACHED WITH CELLULASE-POOR CRUDE XYLANASES OF *COPRINELLUS DISSEMINATUS* IN ELEMENTAL CHLORINE FREE BLEACHING

SHALINI SINGH and DHARM DUTT

*Department of Paper Technology, Indian Institute of Technology Roorkee, Saharanpur Campus, Saharanpur 247001, India*

*Received* November 15, 2012

Novel cellulase-poor crude xylanases from strains SH-1 NTCC-1163 (enzyme-A), and SH-2 NTCC-1164 (enzyme-B) of *Coprinellus disseminatus*, mitigated kappa number (56.27 and 58.41%) of wheat straw soda-AQ pulp after $OX^AE$ and $OX^BE$ bleaching stages, respectively. Significant reductions in AOX (25.00 and 22.05%), with a slight decrease in some strength properties, owing to removal of hemicelluloses, was observed. The removal of hemicelluloses was further validated by an increase in pulp viscosity (6.82 and 4.93%), COD (43.15 and 43.15%) and colour (51.99 and 62.99%) for $OX^AE_1D_1E_2D_2$ and $OX^BE_1D_1E_2D_2$ sequences, respectively, compared to their respective controls. Compared to controls, AOX reduction was higher in $OX^AE_1DE_2P$ (38.75%) and $OX^BE_1DE_2P$ (36.25%) than $OX^AE_1D_1E_2D_2$ (25.00%) and $OX^BE_1D_1E_2D_2$ (22.05%) bleached pulps, but at the cost of brightness. AOX, COD and colour were significantly reduced on the 6$^{th}$ day of incubation of combined bleaching effluents.

***Keywords***: *Coprinellus disseminates*, biobleaching, wheat straw pulp, AOX

## INTRODUCTION

The pulp and paper industry is customarily associated with large consumption of energy, chemicals and generation of effluents with high concentrations of suspended solids, organic load and toxicity.[1] Pulp bleaching is especially problematic due to the presence of AOX, TOCl, and wood extractives in the effluents.[2] The deliberate discharge and accidental release of harmful chemical compounds into the environment can disrupt natural ecosystems. The use of such chemically treated papers[3-5] is of major concern, as it is associated with chlorinated compounds including animal carcinogens, dioxins and furans, known for causing many health problems.[6,7]

Various up-coming technologies, like oxygen delignification, extended modified continuous cooking, modified conventional batch cooking, isothermal cooking and modified conventional batch cooking, rapid displacement heating to reduce kappa factor before pulp bleaching,[8-11] use of cooking aids,[12] and substitution of chlorine with chlorine dioxide are available. Nowadays, enzymatic prebleaching is a cheaper alternative for a cleaner pulp production.[13] Xylanase treatment improves subsequent bleaching stages.[14] Only few xylanases are reported to be cellulase-free, and alkali-thermo-tolerant.[15] In view of their potential role in pulp bleaching, cost-effective production of enzymes is crucial.

Singh *et al*. observed that, under optimum SSF conditions, strain SH-1 NTCC-1163 of *Coprinellus disseminatus* showed xylanase, CMCase, laccase and lignin peroxidase activities of 727.78 IU/mL, 0.925 IU/mL, 0.640 IU/mL and 0.27 IU/mL, respectively, while the same activities for strain SH-2 NTCC-1164 were of 227.99 IU/mL, 0.660 IU/mL, 0.742 IU/mL and 0.32 IU/mL, respectively.[16] The potential of the same enzyme preparations in conventional and TCF bleaching of wheat straw soda-AQ pulp has been proved.[17] In the present study, the enzyme preparations were evaluated for biobleaching of wheat straw soda-AQ, in an ECF bleaching system. To validate the laboratory findings, a plant trial was also conducted. The pulp produced after brown stock washing had a high temperature (about 70 ºC) and was alkaline in nature (pH about 7.8). Crude cellulase-poor xylanases obtained from *Coprinellus disseminatus* SH-1 NTCC-1163 and SH-2 NTCC-1164 showed maximum activities at 55 ºC and pH 6.4 and retained 32.64 (SH-1) and 35.03% (SH-2) of its activity at pH 8, and 43.01 (SH-1) and 25.00% (SH-2) of its activity at 65 ºC.[9]

## EXPERIMENTAL

### Crude xylanase

Cellulase-poor crude xylanases were obtained from *C. disseminatus* SH-1 NTCC-1163 and SH-2 NTCC-1164 under optimum conditions of SSF.[16] Xylanase activity was determined by measuring the release of reducing sugars using birchwood xylan as a substrate, as per the DNS method.[18] CMCase activity was determined using CMC as a substrate[19] and reducing sugars were measured by the DNS method.[18] Laccase activity in the enzyme samples was determined by continuous spectrophotometric rate determination method given by Ride.[20] Lignin peroxidase activity was measured, as described by Mercer *et al.*, using 2,4-di-chlorophenol as a substrate at 510 nm.[21]

### Pulp sample

Wheat straw was digested in a WEVERK electrically heated rotary digester of 0.02 $m^3$ capacity by the soda-AQ pulping process, which produced a screened pulp yield of 45.05%, of kappa number 18.25 at a dose of 12% (as $Na_2O$) active alkali, 0.1% AQ dose, maximum cooking temperature and time of 150 ºC and 60 min, respectively, with a liquor to raw material ratio of 4:1. The cooked pulp was washed on a laboratory flat stationary screen having a 300 mesh wire bottom, disintegrated, screened through a WEVERK vibratory flat screen with a slot size of 0.15 mm.[22]

### Bleaching of enzyme treated pulp using elemental chlorine free bleaching

The effect of enzyme treatment on wheat straw soda-AQ pulp was observed in $OD_1ED_2$ and ODEP bleaching sequences. Wheat straw soda-AQ pulp was delignified with $O_2$ (operating conditions reported in Table 1), and evaluated for kappa number[23] after washing. Enzyme treatment was given after oxygen delignification[24] under optimized conditions of enzyme dose (10 IU/g), reaction time (3 h), and pulp consistency (10% for enzyme-A and 5% for enzyme-B) at the temperature of 55 ºC and pH 6.4.[17] The pulps were washed after enzyme treatment for proper impregnation of subsequent bleaching chemicals.[25] Subsequently, the enzyme treated and control pulps were bleached using $OD_1ED_2$ and ODEP sequences. The pulps obtained after each bleaching stage were filtered through a cheese cloth, and the filtrates were analyzed for residual chlorine, except the alkali extraction stage. The rest of the filtrates were preserved at 4 ºC for further analysis, and the pulps were washed with 2 L of tap water, squeezed, and crumbled.

### Physical and chemical characterization of pulps

Thick pulp pads were prepared (T218 sp-02), and evaluated for kappa number (T236 cm-85), brightness (ISO) (T452 om-02), and CED viscosity (T230 om-04). Pulps were beaten at a beating level of 40 $^o$SR in a PFI mill (T248 sp-00). Laboratory handsheets (60 $g/m^2$) were prepared on a British sheet former (T205 sp-02), conditioned at a relative humidity of 65%±2, and temperature of 27±1 ºC, and evaluated for burst index (T403 om-02), tensile index (T494 om-01), double folds (T423 cm-98), and tear index (T414 om-04), as per Tappi standard test methods.[23]

### Characterization of bleaching effluents

The filtrates from different stages of each bleaching sequence were separately mixed in equal amounts, and the combined effluents, thus obtained, were analyzed for COD, colour and AOX. COD was determined by closed reflux titrimetric method using a Thermo reactor CR2010, and colour by the cobaltiplatinate method at 465 nm. One unit equals the absorbance produced by 1 mg/mL of platinum present in the form of cobaltiplatinate ion at 465 nm. AOX was measured by an AOX Analyzer Dextar ECS 1200. In order to see the effect of residual enzymes on COD, colour and AOX, the combined effluent was analyzed as to the same parameters on 0, 2$^{nd}$, 4$^{th}$ and 6$^{th}$ day of storage at room temperature (18 to 25 ºC).

### Scanning electron microscopy

The fibres of control and enzyme treated pulps were suspended in water, placed on a cover glass and allowed to dry. Then, they were examined under SEM.[26] Electron photomicrographs were taken at 15 kV, using an SE1 detector at desired magnifications.

### Plant trial

A pilot plant trial was conducted in a pulp and paper mill, which had adopted the soda-AQ pulping process, using wheat straw as a cellulosic raw material and pulp bleached by the $OD_1ED_2$ sequence.

### Statistical analysis

Six experimental values for brightness, and three experimental values for kappa number and viscosity, in each case, were taken. The results represent mean values ± their standard deviation (SD).

Table 1
Optimization of enzyme dose, reaction time and pulp consistency during prebleaching of soda-AQ pulp of wheat straw

| Particulars | | Reduction in kappa number,* % | Change in viscosity,* cps | Brightness, % (ISO) | Reducing sugars released, mg/g | Chromophores released, optical density | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | 237 nm | 280 nm | 465 nm |
| Enzyme dose, IU/g | | Optimization of enzyme dose | | | | | | |
| | Control | 17.98±0.12 | 25.65±0.12 | 27.64±0.21 | 0.20 | 0.040 | 0.050 | 0.020 |
| $X^A$ | 5 | 17.30±0.10 | 25.30±0.13 | 38.76±0.34 | 4.05 | 0.309 | 0.310 | 0.242 |
| | 10 | 15.85±0.11 | 24.71±0.11 | 43.97±0.19 | 6.30 | 0.458 | 0.497 | 0.451 |
| | 15 | 16.45±0.12 | 24.33±0.10 | 42.33±0.15 | 10.70 | 0.419 | 0.452 | 0.405 |
| | 20 | 16.68±0.14 | 24.00±0.14 | 41.57±0.11 | 14.00 | 0.390 | 0.429 | 0.360 |
| | 25 | 17.00±0.10 | 23.51±0.13 | 41.24±0.13 | 14.79 | 0.369 | 0.410 | 0.332 |
| | 30 | 17.20±0.08 | 22.98±0.15 | 40.64±0.17 | 15.20 | 0.352 | 0.349 | 0.302 |
| $X^B$ | 5 | 15.20±0.10 | 25.51±0.13 | 44.44±0.29 | 6.14 | 0.318 | 0.321 | 0.294 |
| | 10 | 14.00±0.15 | 24.89±0.12 | 45.58±0.30 | 7.35 | 0.464 | 0.514 | 0.489 |
| | 15 | 14.98±0.20 | 24.60±0.11 | 44.46±0.18 | 12.85 | 0.421 | 0.480 | 0.421 |
| | 20 | 15.34±0.19 | 24.24±0.12 | 43.98±0.20 | 14.89 | 0.410 | 0.454 | 0.380 |
| | 25 | 16.47±0.15 | 23.78±0.14 | 43.45±0.17 | 15.40 | 0.389 | 0.431 | 0.348 |
| | 30 | 16.70±0.21 | 23.10±0.10 | 42.98±0.19 | 16.59 | 0.367 | 0.398 | 0.276 |
| Reaction time, h | | Optimization of reaction time | | | | | | |
| $X^A$ | 1 | 16.39±0.19 | 24.81±0.20 | 39.79±0.31 | 0.151 | 0.242 | 0.265 | 0.151 |
| | 2 | 15.24±0.21 | 24.57±0.23 | 42.14±0.26 | 0.329 | 0.459 | 0.510 | 0.329 |
| | 3 | 14.20±0.14 | 23.69±0.18 | 43.58±0.19 | 0.522 | 0.612 | 0.725 | 0.522 |
| | 4 | 15.16±0.11 | 23.12±0.13 | 42.50±0.24 | 0.411 | 0.565 | 0.698 | 0.411 |
| | 5 | 15.67±0.23 | 22.54±0.20 | 42.12±0.25 | 0.402 | 0.560 | 0.624 | 0.402 |
| $X^B$ | 1 | 15.18±0.14 | 25.60±0.22 | 41.81±0.22 | 5.80 | 0.268 | 0.310 | 0.280 |
| | 2 | 14.80±0.13 | 25.04±0.20 | 43.10±0.25 | 9.20 | 0.478 | 0.520 | 0.460 |
| | 3 | 14.00±0.12 | 24.67±0.15 | 44.00±0.10 | 14.89 | 0.629 | 0.776 | 0.720 |
| | 4 | 13.88±0.10 | 23.95±0.11 | 42.77±0.18 | 17.50 | 0.610 | 0.721 | 0.642 |
| | 5 | 13.08±0.10 | 23.21±0.17 | 41.98±0.12 | 21.02 | 0.610 | 0.700 | 0.600 |
| Consistency, % | | Optimization of pulp consistency | | | | | | |
| $X^A$ | 2 | 15.63±0.17 | 24.85±0.12 | 40.53±0.31 | 5.50 | 0.347 | 0.321 | 0.430 |
| | 5 | 14.18±0.11 | 24.48±0.16 | 43.58±0.27 | 11.78 | 0.458 | 0.642 | 0.540 |
| | 10 | 13.20±0.13 | 23.54±0.20 | 44.68±0.24 | 17.21 | 0.620 | 0.786 | 0.797 |
| | 15 | 13.54±0.15 | 23.00±0.18 | 43.54±0.17 | 17.58 | 0.602 | 0.722 | 0.712 |
| | 20 | 13.92±0.21 | 22.16±0.15 | 42.77±0.22 | 18.14 | 0.576 | 0.700 | 0.764 |
| $X^B$ | 2 | 15.00±0.15 | 24.78±0.24 | 42.72±0.19 | 7.71 | 0.500 | 0.564 | 0.448 |
| | 5 | 13.33±0.11 | 24.41±0.27 | 43.89±0.17 | 15.50 | 0.631 | 0.821 | 0.801 |
| | 10 | 13.89±0.19 | 23.97±0.31 | 43.31±0.22 | 16.40 | 0.600 | 0.789 | 0.756 |
| | 15 | 13.02±0.12 | 23.34±0.29 | 42.77±0.20 | 16.81 | 0.576 | 0.758 | 0.743 |
| | 20 | 12.79±0.11 | 22.89±0.21 | 40.33±0.21 | 17.91 | 0.543 | 0.543 | 0.701 |

$X^A$ = Enzyme-A, $X^B$ = Enzyme-B, Crude xylanases from *C. disseminatus* SH-1 (enzyme-A preparation) exhibited 727.78 IU/mL xylanase, 0.925 IU/mL CMCase, 0.640 U/mL laccase and 0.270 U/mL lignin peroxidase activities, while *C. disseminatus* SH-2 designated enzyme-B presented 227.99 IU/mL xylanase, 0.660 IU/mL CMCase, 0.742 U/mL laccase and 0.320 U/mL lignin peroxidase activities. Kappa number = 18.25, unbleached pulp viscosity, cp = 26.04; * Values after alkali extraction with 2% NaOH at 70 ºC for 90 min and 10% pulp consistency
Pulp treatment conditions: Enzymes A and B = pH 6.4, temperature – 55 ºC
Optimization of enzyme dose: Reaction time – 2 h and pulp consistency – 5%
Optimization of reaction time: Enzyme dose – 10 IU/g (oven dry pulp basis) and pulp consistency – 5%
Optimization of consistency: Reaction time – 3 h and enzyme dose – 10 $IUg^{-1}$ (oven dry pulp basis)
± refers to standard deviation; experiments were conducted in triplicates

## RESULTS AND DISCUSSION

### Effect of enzyme treatment on pulp, paper and effluent properties

Oxygen treatment reduced pulp kappa number by 45.20% (Table 1). Enzymes A and B reduced kappa number of oxygen delignified pulp by 20.20 and 24.10%, respectively (Tables 2-3). The attack of xylanase on LCC or xylan associated with lignin may have led to pulp delignification.[27] The brightness of $OX^AE_1D_1E_2D_2$ and $OX^BE_1D_1E_2D_2$ bleached pulps improved by 7.58 and 10.06%, respectively, compared to $OD_1ED_2$ pulp (Tables 2 and 3). In ODEP, enzymes A and

B improved pulp brightness by 6.29 and 10.78%, respectively, as compared to the control.

The viscosity of the $OX^AE_1D_1E_2D_2$ and $OX^BE_1D_1E_2D_2$ pulps improved by 6.82 and 4.93%, respectively. In the case of ODEP bleached pulps, the enzyme treatment enhanced pulp viscosity by 6.33, and 4.22%, compared to the control. The increase in viscosity might be due to the selective removal of lower degree of polymerization (DP) xylan and enrichment of high molecular weight polysaccharides.[28] There was no adverse effect of the small amount of CMCases present in the crude enzyme, as viscosity drops when cellulases cleave cellulose chains, lowering the degree of cellulose polymerization and destroying fibre integrity.[29] In fact, a mild cellulase activity might improve pulp fibrillation and induce better fibre bonding.[30]

The tensile index, burst index and double fold of $OX^AE_1D_1E_2D_2$ decreased by 16.12, 10.47 and 10.60%, respectively, compared to the control. This decrease for the same parameters was lesser in the $OX^BE_1D_1E_2D_2$ pulp. The tear index improved almost to the same extent for both enzyme treated pulps. The tensile and burst indexes for $OX^AE_1DE_2P$ pulp decreased by 16.30 and 9.46%, respectively, as compared to the control. This decrease was slightly lower for the $OX^BE_1DE_2P$ pulp. On the other hand, double folds and tear index for $OX^AE_1DE_2P$ were higher by 5.97 and 2.61% than those of the control.

Table 2
Effect of enzyme treatment on ODED bleaching of soda-AQ pulp of wheat straw

| Particulars | Bleaching sequences | | |
|---|---|---|---|
| | $OD_1ED_2$ | $OX^AE_1D_1E_2D_2$ | $OX^BE_1D_1E_2D_2$ |
| Oxygen (O) stage*,** | | | |
| Kappa number after oxygen delignification | 10.00 ± 0.14 | 10.00 ± 0.14 | 10.00 ± 0.14 |
| Enzyme dose, $X^A$, IU/g* | — | 10 | — |
| Enzyme dose, $X^B$, IU/g* | — | — | 10 |
| Extraction ($E_1$), NaOH applied,* % | — | 2 | 2 |
| Kappa number | — | 7.98± 0.13 | 7.59 ± 0.11 |
| $D_1$ stage*♦ | | | |
| % $ClO_2$ consumed as available $Cl_2$ | 0.96 | 0.97 | 0.99 |
| $D_2$ stage*♦ | | | |
| % $ClO_2$ consumed as available $Cl_2$ | 0.99 | 0.97 | 0.95 |
| Extraction ($E_2$), NaOH applied,* % | 0.5 | 0.5 | 0.5 |
| Final brightness, % (ISO) | 73.85 | 79.45± (+7.58) | 81.28 ± (+10.06) |
| Final viscosity, cps | 9.53 ± 0.10 | 10.18 ± 0.13 (+6.82) | 10.00 ± 0.11 (+4.93) |
| Mechanical strength properties⌂ | | | |
| Tensile index, Nm/g | 49.60 | 41.60 (—16.12) | 43.60 (—12.09) |
| Burst index, kPa $m^2$/g | 3.34 | 2.99 (—10.47) | 3.01 (—9.88) |
| Double fold, no | 66 | 59 (—10.60) | 60 (—9.09) |
| Tear index, $mNm^2$/g | 5.30 | 5.46 (+3.01) | 5.44 (+2.64) |
| AOX, kg/t (Combined bleaching effluent) | 0.68 | 0.51(—25.00) | 0.53 (—22.05) |
| COD, mg/L (Combined bleaching effluent) | 1365.72 | 1955.16 (+43.15) | 1957.62 (+43.33) |
| Colour, PCU (Combined bleaching effluent) | 149.40 | 227.08 (+51.99) | 243.52 (+62.99) |

| Bleaching conditions | $X^A$ | $X^B$ | $E_1$ | O | $D_1$ | $E_2$ | $D_2$ |
|---|---|---|---|---|---|---|---|
| pH | 6.4 | 6.4 | 11.0 | 11.0 | 2.5 | 11.0 | 2.5 |
| Consistency, % | 10 | 5 | 10 | 15 | 7 | 10 | 7 |
| Retention time, min | 180 | 180 | 90 | 90 | 120 | 90 | 120 |
| Temperature, ºC | 55±2 | 55±2 | 70±2 | 110±2 | 70±2 | 70±2 | 70±2 |

$X^A$ = Crude xylanases from strain SH-1, $X^B$ = Crude xylanases from strain SH-2; * Chemical charge on oven dry pulp basis; ** $O_2$ pressure – 5 kg/$cm^2$, 2% NaOH, 0.1% $MgSO_4$; +/– stand for % difference compared to control pulp; ♦ Total $ClO_2$ applied in $D_1$ and $D_2$ stages – 2% (1% in each stages), ± Standard deviation from the mean; Unbleached pulp kappa number = 18.25; Unbleached pulp brightness, % (ISO) = 27.41; Unbleached pulp viscosity, cps = 26.04, ⌂ 40 Shopper-Riegler degrees ($^0$SR)

Double folds and tear index for $OX^BE_1DE_2P$ improved by 7.46 and 3.17%, respectively, compared to the ODEP pulp. Xylanase treated wheat straw pulps have longer average fibre, and lower fines content than the control (disadvantageous for fibre bonding). Longer average fibre length might increase tear index.[31] The quantity, chemical structure, distribution and DP of hemicelluloses influence paper strength.[32] The removal of hemicelluloses and the concomitant reduction in DP of residual hemicelluloses might reduce tensile and burst indexes. Though excess xylan removal reduced burst and tensile strengths by reducing inter-fibre bonding, yet fibres themselves were not weakened, thus increasing tear index.[6]

Table 3
Effect of enzyme treatment on ODEP bleaching of soda-AQ pulp of wheat straw

| Particulars | Bleaching sequences | | |
|---|---|---|---|
| | ODEP | $OX^AE_1DE_2P$ | $OX^BE_1DE_2P$ |
| Oxygen (O) stage*,** | | | |
| Kappa number after oxygen delignification | 10.00 ± 0.14 | 10.00 ± 0.14 | 10.00 ± 0.14 |
| Enzyme dose, $X^A$, IU/g* | — | 10 | — |
| Enzyme dose, $X^B$, IU/g* | — | — | 10 |
| Extraction ($E_1$), NaOH applied,* % | — | 2 | 2 |
| Kappa number | | 7.98 ± 0.13 | 7.59 ± 0.11 |
| Chlorine dioxide (D) stage*,♦ | | | |
| % $ClO_2$ consumed as available $Cl_2$ | 1.99 | 1.96 | 1.98 |
| Extraction ($E_2$), NaOH applied,* % | 1 | 1 | 1 |
| Peroxide (P) stage*,$^{\Delta}$ | | | |
| $H_2O_2$ consumed, % | 2.99 | 2.96 | 2.96 |
| Final brightness, % (ISO) | 70.50±0.22 | 74.94 ±0.18 (+4.44) | 78.1±0.13 (+7.6) |
| Final viscosity, cps | 9.47± 0.06 | 10.07± 0.03 (+6.33) | 9.87± 0.05 (+4.22) |
| Mechanical strength properties$^{\triangle}$ | | | |
| Tensile index, Nm/g | 49.97 | 41.82 (—16.30) | 42.61(—14.72) |
| Burst index, kPa $m^2$/g | 3.38 | 3.06 (—9.46) | 3.08 (—8.87) |
| Double folds, no | 67 | 71(+5.97) | 72 (+7.46) |
| Tear index, $mNm^2$/g | 5.35 | 5.49 (+2.61) | 5.52 (+3.17) |
| AOX, kg/t (Combined bleaching effluent | 0.80 | 0.49 (—38.75) | 0.51(—36.25) |
| COD, mg/L (Combined bleaching effluent) | 996.00 | 1402.86 (+40.84) | 1474.08 (+48.00) |
| Colour, PCU (Combined bleaching effluent) | 126.20 | 193.79 (+53.55) | 210.62 (+66.89) |

| Bleaching conditions | $X^A$ | $X^B$ | $E_1$ | O | D | $E_2$ | P |
|---|---|---|---|---|---|---|---|
| pH | 6.4 | 6.4 | 11.0 | 11.0 | 2.5 | 11.0 | 11.8 |
| Consistency, % | 10 | 5 | 10 | 15 | 7 | 10 | 10 |
| Retention time, min | 180 | 180 | 90 | 90 | 120 | 90 | 120 |
| Temperature, ºC | 55±2 | 55±2 | 70±2 | 110±2 | 70±2 | 70±2 | 90±2 |

$X^A$ = Crude xylanases from strain SH-1, $X^B$ = Crude xylanases from strain SH-2; * Chemical charge on oven dry pulp basis; ** $O_2$ pressure – 5 kg/cm$^2$, 2% NaOH, 0.1% $MgSO_4$; +/– stand for % difference compared to control pulp; ♦ Total $ClO_2$ applied in D-stage 2%, $^{\Delta}$ $H_2O_2$ applied in P-stage – 3%, 2% NaOH, 5% sodium silicate, 0.05% $MgSO_4$; ± Standard deviation from the mean; Unbleached pulp kappa number = 18.25; Unbleached pulp brightness, % (ISO) = 27.41; Unbleached pulp viscosity, cps = 26.04; $^{\triangle}$ 40 $^0$SR

For the combined bleaching effluent, AOX decreased by 25.00 and 22.05%, COD increased by 43.15 and 43.33%, and colour increased by 51.99 and 62.99%, respectively, for $OX^AE_1D_1E_2D_2$ and $OX^BE_1D_1E_2D_2$ pulps, as compared to the control pulps (Table 3). For $OX^AE_1DE_2P$ and $OX^BE_1DE_2P$ bleached pulps (Table 3), AOX decreased by 38.75 and 36.25%, COD increased by 40.84 and 48.00% and colour increased by 53.55 and 66.89%, respectively. The hydrolytic action of the enzyme weakens pulp carbohydrate bonds and causes dissolution. This increases the concentration of lignin, and hydrolyzed xylan in the effluent, leading to increased COD, and colour.[33] AOX reduction was remarkable, as a 20-45% reduction in the AOX of xylanase treated pulp effluents has been reported earlier.[34-37]

As Table 4 shows, AOX decreased progressively up to the 6$^{th}$ day of incubation. On the 6$^{th}$ day of incubation, residual enzymes A and B, in the combined effluent, reduced AOX by 35.29 and 33.82% for $OX^AE_1D_1E_2D_2$ and $OX^BE_1D_1E_2D_2$ bleached pulps, respectively, as compared to the $OD_1ED_2$ bleached pulp. Similarly, on the 6$^{th}$ day of incubation, COD was reduced by 30.82 and 13.75% and colour by 49.50 and 38.78% for the combined effluent of $OX^AE_1D_1E_2D_2$ and $OX^BE_1D_1E_2D_2$ bleaching sequences, respectively, compared to the control.

In $OX^AE_1DE_2P$ and $OX^BE_1DE_2P$ bleaching sequences, AOX reduced by 46.25 and 42.50%, COD by 11.91 and 5.93% and colour by 23.75

and 16.52%, respectively, on the 6th day of incubation. Enzyme-A was observed to be more effective in reducing AOX, COD and colour, as compared to enzyme-B. The accessory enzymes (CMCase, in negligible amount, laccase and lignin peroxidise) present in the crude enzymes A and B might also have played a significant role in the reduction of AOX, COD and colour in the combined bleaching effluent.

## Scanning electron microscopy

The enzyme treated pulps (Plate 1B, C, E and F) underwent a peeling process, giving rise to flakes and filaments of material detached from the fibre surface, they were rougher, more heterogeneous and showed cracking and delamination of the cell wall. On the other hand, the control pulp (Plate 1A and D) was smoother, cleaner, did not present fibrillation, but exhibited fibres of different thickness. The enzymes produced changes in the surface of the fibre, owing to the hydrolysis they cause.[38] Xylanase treatment seems to facilitate diffusion of larger lignin macromolecules out through the fibre cell wall[28] and thus improved the accessibility of pulps for bleaching chemicals, thereby decreasing kappa number and increasing brightness of the enzyme treated pulps.

## Plant trials

Table 5 reports the pilot plant trial findings with enzymes A and B during pulp bleaching, using $OX^AE_1D_1E_2D_2$ and $OX^BE_1D_1E_2D_2$ bleaching sequences. Enzyme-A mitigated AOX, COD and colour by 24.18, 44.61 and 52.35%, with an increase in suspended solids (12%), in the $OX^AE_1D_1E_2D_2$ bleaching sequence, compared to the control. Similarly, enzyme-B mitigated AOX, COD and colour by 21.97, 45.33 and 62.85%, respectively, in the $OX^BE_1D_1E_2D_2$ bleaching sequence, compared to the control. However, the results for mechanical strength properties are comparatively lower than those obtained at a laboratory scale. Plant trial results indicated that enzyme-A was more effective in mitigating AOX, compared to enzyme-B, which validated the laboratory results.

Table 4
Effect of residual enzyme on combined bleaching effluent COD, AOX and color

| Parameters | Incubation periods, days | AOX, kg/T | COD, mg/L | Colour, PCU |
|---|---|---|---|---|
| $OD_1ED_2$ | 0 | 0.68 | 1365.72 | 149.40 |
| $OX^AE_1DE_2P$ | 0 | 0.51 (–25.00) | 1955.16 (+43.15) | 227.08 (+51.99) |
| | 2 | 0.49 (–27.94) | 1544.58 (+13.10) | 134.08 (–10.25) |
| | 4 | 0.46 (–32.35) | 1402.64 (+2.70) | 118.56 (–20.64) |
| | 6 | 0.44 (–35.29) | 944.73 (–30.82) | 75.45 (–49.50) |
| $OX^BE_1DE_2P$ | 0 | 0.53 (–22.05) | 1957.62 (+43.33) | 243.52 (+62.99) |
| | 2 | 0.51 (–25.00) | 1585.95 (+16.12) | 134.46 (–10.00) |
| | 4 | 0.48 (–29.41) | 1468.26 (+7.51) | 119.83 (–19.79) |
| | 6 | 0.45 (–33.82) | 1177.98 (–13.75) | 91.46 (–38.78) |
| ODEP | 0 | 0.80 | 996.00 | 126.20 |
| $OX^AE_1DE_2P$ | 0 | 0.49 (–36.25) | 1402.86 (+40.84) | 193.79 (+53.55) |
| | 2 | 0.46 (–42.5) | 1112.06 (+11.65) | 159.59 (+26.46) |
| | 4 | 0.45 (–43.75) | 1042.38 (+4.66) | 151.85 (+20.32) |
| | 6 | 0.43 (–46.25) | 877.35 (–11.91) | 96.22 (–23.75) |
| $OX^BE_1DE_2P$ | 0 | 0.51 (–36.25) | 1474.08 (+48.00) | 210.62 (+66.89) |
| | 2 | 0.50 (–37.50) | 1173.07 (+17.78) | 188.18 (+49.11) |
| | 4 | 0.48 (–40.00) | 1123.99 (+12.85) | 168.74 (+33.71) |
| | 6 | 0.46 (–42.50) | 936.92 (–5.93) | 105.34 (–16.52) |

+/ – stand for % change compared to their respective controls

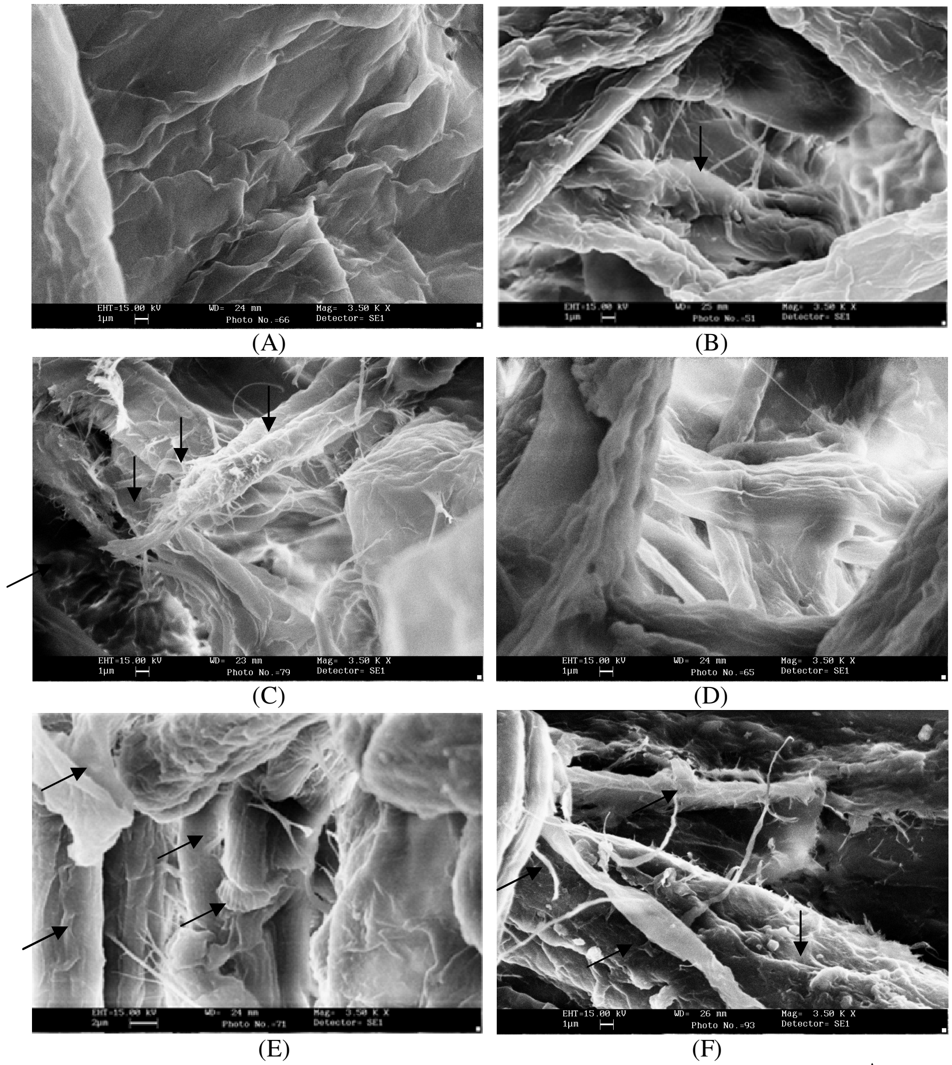


Plate 1: Scanning electron micrograph showing (A) ODED bleached pulp fibers (3.5 kx); (B) $OX^{A}EDED$ bleached pulp fibers (3.5 kx); (C) $OX^{B}EDED$ pulp fibers (3.5 kx); (D) ODEP bleached pulp fibers (3.5 kx); (E) $OX^{A}EDEP$ bleached pulp fibers (3.5 kx); (F) $OX^{B}EDEP$ pulp fibers (3.5 kx)

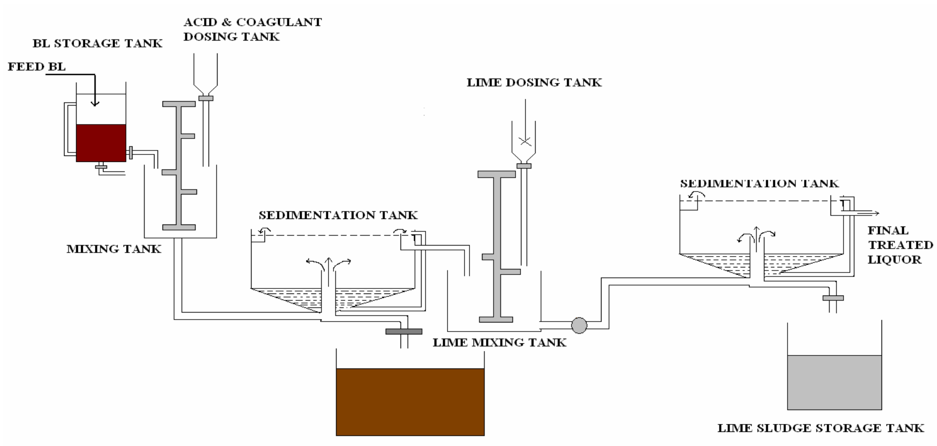


Plate 2: A schematic general flow diagram of a pilot plant for the lignin removal process (LRP)

Table 5
Comparison of pollution parameters of the combined effluent generated during bleaching of wheat straw soda-AQ pulp by ODED, $OX^AEDED$ and $OX^BEDED$ bleaching sequences during plant trials

| Parameters | Bleaching sequences | | |
|---|---|---|---|
| | $OD_1ED_2$ | $OX^AE_1DE_2P$ | $OX^BE_1DE_2P$ |
| Bleached pulp brightness, % (ISO) | 76.2 | 81.3 (+5.1) | 83.5 (+7.3) |
| Tensile index, Nm/g | 45.55 | 38.75 (–14.92) | 40.90 (–10.21) |
| Burst index, kPa $m^2$/g | 3.19 | 2.75 (–13.79) | 2.87 (–10.03) |
| Double folds, no | 61 | 54 (–11.47) | 56 (–8.20) |
| Tear index, $mNm^2$/g | 4.85 | 5.15 (+6.19) | 5.05 (+4.12) |
| Volume of effluent discharged per day, $m^3$ | 3240 | 3385 | 3420 |
| pH | 6.2 | 6.4 | 6.3 |
| Suspended solids, mg/L | 750 | 840 (+12) | 825 (+10) |
| Colour, PCU | 1500 | 2285.0 (+52.35) | 1457.8 (+63.85) |
| COD, kg/T | 1650 | 2386.7 (44.61) | 2398.3 (+45.33) |
| AOX, kg/T | 0.91 | 0.69 (–24.18) | 0.71 (–21.97) |

Note: Conditions of pulp bleaching, chemical and enzyme dosages as per Table 1; the values reported in the table are the average data of 24 h; +/ – stand for % change compared to control; installed capacity of the mill = 66000 T/year, bleached pulp production per day = 216 T; kappa number of unbleached pulp = 19±2

Paper industries manufacturing kraft paper based on agro residues do not have a chemical recovery plant. Black liquor treatment is carried out by the lignin recovery process (LRP) or lignin separation process, which involves precipitation of lignin using mineral acids followed by separation of precipitated mass from the black liquor by the filtration technique, such as dissolved air floatation system, filter bed, filter press, drum filter etc. The diagrammatic sketch of LRP is shown in Plate 1. Black liquor is collected in a separate pool before sending to LRP. Residual enzymes have sufficient time to react with the solid contents of the black liquor.

## CONCLUSION

The results indicate that the novel cellulase-poor crude xylanases of strains SH-1 NTCC1163 and SH-2 NTCC1164 of *Coprinellus disseminatus* may successfully be used for pulp biobleaching. The enzyme treatment effectively mitigates AOX, thus, making the process environment friendly. The reduction in the values of COD and colour (owing to residual enzyme activity in the stored effluents) for the enzyme treated pulps, as compared to the control pulps, after 6 days of incubation of the combined bleaching effluents, is significant.

## ABBREVIATIONS

| | |
|---|---|
| AQ | Anthraquinone |
| AOX | Adsorbable organic halides |
| CMC | Carboxymethylcellulose |
| CMase | Carboxymethylcellulase |
| COD | Chemical oxygen demand |
| D | Chlorine dioxide stage |
| $D_1$ | I$^{st}$ chlorine dioxide stage |
| $D_2$ | II$^{nd}$ chlorine dioxide stage |
| DNS | Di-nitro salicylic acid |
| $E_1$ | I$^{st}$ alkali extraction stage |
| $E_2$ | II$^{nd}$ alkali extraction stage |
| ECF | Elemental chlorine free |
| EMCC | Extended modified continuous cooking |
| ITC | Isothermal cooking |
| MCBC | Modified conventional batch cooking |
| MCC | Modified continuous cooking |
| P | Hydrogen peroxide stage |
| ODL | Oxygen delignification |
| RDH | Rapid displacement heating |
| TCF | Total chlorine free |
| TOCl | Total organic chlorides |
| $X^A$ | Enzyme-A |
| $X^B$ | Enzyme-B |

## REFERENCES

[1] P. R. Berube and K. A. Kahmark, *Water Environ. Res.*, **73**(**5**), 1 (2001).
[2] C. Xavier, S. Chamorro and G. Vidal, *Bull. Environ. Contam. Toxicol.*, **75**, 670 (2005).
[3] D. Dutt, J. S. Upadhyaya and C. H. Tyagi, *Cellulose Chem. Technol.*, **41**(**4-6**), 293 (2007).
[4] D. Dutt, C. H. Tyagi, R. S. Malik and J. S. Upadhyaya, *J. Sci. Ind. Res.*, **63**(**5**), 420 (2004).
[5] D. Dutt, J. S. Upadhyaya, C. H. Tyagi, S. Agnihotri and M. K. Upadhyay, *J. Sci. Ind. Res.*, **64**(**6**), 443 (2005).
[6] K. V. Sarkanen and G. Goyal, in *Procs. Tappi International Pulp Bleaching Conference*, Tappi Press, Atlanta, 1985, pp. 77-84.

[7] A. Poland and A. Kende, in "Inorganics of Human Cancer", edited by H. H. Hiatt, J. D. J. Watson and A. Winston, Cold Spring Harbor, New York, 1977, p. 847.
[8] M. Näsman, S. Backa and M. Ragnar, *Nordic Pulp Pap. Res. J.*, **22**(**1**), 42 (2007).
[9] H. Peter and C. Daniel, *Tappi J.*, **5**(**4**), 23 (2006).
[10] M. Bäckström and A. Jensent, *Appita J.*, **54**(**2**), 203 (2001).
[11] C. A. Bianchini and M. K. Azad, *Ippta J.*, **19**(**1**), 57 (2007).
[12] R. C. Francis, S. J. Shin, S. Omori, T. E. Amidon and T. J. Blain, *J. Wood Chem. Technol.*, **26**(**2**), 141 (2006).
[13] G. Z. Fu, A. W. Chan and D. E. Minns, *Int. J. Life Cycle Ass.*, **10**(**2**), 136 (2005).
[14] C. Valls and M. B. Roncero, *Bioresource Technol.*, **100**, 2032 (2009).
[15] T. Collins, C. Gerday and G. Feller, *FEMS Microbiol. Rev.*, **29**, 3 (2005).
[16] S. Singh, D. Dutt, C. H. Tyagi and J. S. Upadhyaya, *New Biotechnol.*, **26**(**3/4**), 165 (2009).
[17] S. Singh, D. Dutt and J. S. Upadhyaya, *New Biotechnol.*, **28**(**1**), 47 (2010).
[18] G. L. Miller, *Anal. Chem.*, **31**, 238 (1959).
[19] M. Mandels, *Biotech. Bioeng. Symp.*, **5**, 81 (1975).
[20] J. P. Ride, *Physiol. Plant Pathol.*, **16**, 187 (1980).
[21] D. K. Mercer, M. Iqbal, P. G. G. Miller and A. J. McCarthy, *Appl. Environ. Microbiol.*, **62**(**6**), 2186 (1996).
[22] S. Singh, D. Dutt and C. H. Tyagi, *BioResources*, **6**(**4**), 3876 (2011).
[23] TAPPI test methods, standard methods for pulp and paper, Technical Association of Pulp and Paper Ind., TAPPI Press, Atlanta, USA, 2007.
[24] A. Blanco, T. Vidal, J. F. Colom and F. I. J. Pastor, *Appl. Environ. Microbiol.*, **61**(**12**), 4468 (1995).
[25] L. S. Pederson, P. Kihlgren, A. M. Nissen, N. Munk, H. C. Holm and P. P. Choma, in *Procs. Tappi Pulping Conference*, Boston, USA, 1992, vol. 1, p. 31.
[26] B. L. Gabriel, "Biological Scanning Electron Microscopy", Von Nostrand Reinhold Company, New York, Toronto, London, Melbourne, 1982.
[27] Y. L. Yang, K.-E. L. Eriksson, *Holzforschung*, **46**, 481 (1992).
[28] M. G. Paice, L. Jurasek, C. Ho, R. Bourbonnais and F. Archibald, *Tappi J.*, **72**, 217 (1989).
[29] S. Högman, H. Jövesm, E. Rosenberg and Y. Shohem, in "Biotechnology in Pulp and Paper Industry", edited by M. Kuwahara and M. Shimada, Uni Publishers Co Ltd., Tokyo, Japan, 1992, pp. 107-115.
[30] B. Maiti and D. Whitmore, *Chem. Eng. Comm.*, **162**, 169 (1997).
[31] J. Zhao, X. Li, Y. Qu and P. Gao, *Enzyme Microbiol. Technol.*, **30**, 734 (2002).
[32] T. Eremeeva, T. Bykova and A. Treimanis, in *Procs. The 8th International Symposium on Wood Pulping Chemistry*, Helsinki, Finland, 1995, vol. 3, pp. 225-230.
[33] J. C. Roberts, A. J. McCarthy, N. J. Flynn and P. Broda, *Enzyme Microbiol. Technol.*, **12**, 210 (1990).
[34] R. K. Jain, V. V. Thakur, M. Manthan, R. M. Mathur and A. G. Kulkarni, in *Ippta Convention Issue*, 2001, pp. 57-64.
[35] D. K. Mishra, H. C. Joshi, H. S. Bhatia, D. P. Chandarana and R. L. Lakhotia, in *Ippta Convention Issue*, 2001, pp. 5-14.
[36] C. Leduc, C. Daneault, P. Delaunois, C. Jaspers and M. J. Penninckx, *Appita J.*, **48**(**6**), 435 (1995).
[37] D. J. Senior and J. Hamilton, *J. Pulp Pap. Sci.*, **18**(**5**), J165 (1992).
[38] P. L. Pham, I. Alric and M. Delmas, *Appita J.*, **48**(**3**), 213 (1995).