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# Reduction in Pollution Load in Terms of AOX by using Xylanase Enzyme in Bio-Bleaching Studies of *C. equisetifolia*

**Poonam Maan, Dharm Dutt**

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**Abstract** In this study, an attempt has been made to establish the applicability of crude xylanase enzyme produced from the fungus *C. cinerea* RM-1 NFCCI-3086 for the perspective of *C. equisetifolia* kraft pulp bio-bleaching. The xylanase pretreatment enhances the brightness and reduces the kappa number, total chlorine demand and the AOX formation in bleach effluents. COD and color values of bleach effluents are increased in all bleaching sequences by xylanase pretreatment. This indicates the solubilization of residual lignin carbohydrate complexes. This study evaluates an eco-friendly technology of reducing the chemical consumption and pollution load during bleaching of *C. equisetifolia* kraft pulps.

**Keywords** Xylanase, Bio-bleaching, *C. equisetifolia,* Total chlorine demand, AOX.

P. Maan*, D. Dutt
Biotechnology Laboratory, Department of Paper Technology, Indian Institute of Technology, Roorkee, Saharanpur 247001, India
e-mail: prm.iitr@gmail.com
*Correspondence

## Introduction

The rising environmental concerns and legislative pressures have forced the industries to adopt the bio-based cleaner technologies. Like other long-standing industries, the pulp and paper industry contributes a significant role in socio-economic development of a country. In view of environmental impacts, this industry is forced to modify its current traditional bleaching technologies. The use of chlorine in the bleaching process generates various toxic chlorinated organic pollutants such as chlorobenzene, 1, 1, 1-trichloroethane, trichlorophenol, 1, 1-dichloroethane, 1,1,2,2-tetrachloroethane, chloroform, methylene chloride, terachloroethylene and 2,3,7,8-dibenzofurans. The effluents generated during chlorination stage generally contain 2,3,7,8-TCDD and 2,3,7,8- TCDF, while the effluents generated in extraction (E) stage contain the highest concentrations of dioxins. The dioxins are carcinogenic and cause liver damage, lung lesions and tumors and alter the blood chemistry. Chlorinated organic compounds induce genetic defects and cancer in animals and create hazardous environmental impacts [1]. The quantitative measurement of organo- chlorine compounds generated in the bleaching process is done by the AOX (adsorable organic halides) measurement which is closely related to TOCL. The central pollution control board has forced the Indian pulp and paper industry for reducing the AOX level in a fixed time frame. The range of AOX in final discharge for wood based mills is 0.60-9 mg/L.

The current focus is on the alternative bleaching technologies such as bio-bleaching which can reduce the AOX and TOCL in bleach plant effluents. Bio-bleaching process is carried out in presence of micro-organisms or enzymes and is a good alternative for the production of cleaner pulp [2, 3]. Most of the enzymes used in bio-bleaching process are crude enzymes which contain xylanase as main activity and enriched with other enzymes like lipase, mannanase, α–galactosidage, laccase and cellulase [4]. The presence of these enzymes in the crude enzyme mixture increases the bleaching effect with improving drainability and refinability. The xylanase enzyme removes the bond present between residual lignin and hemicellulose (primarily xylan), makes the lignin more accessible for lignolytic enzymes. Xylanase acts as bleach boosting agent in bleaching process and used in the prebleaching of pulps [5]. To obtain good results in bio-bleaching, the enzyme dose, pH, reaction time and pulp consistency must be optimized.

The present study is done to analyze the effect of crude xylanase extracted from *C. cinerea* RM-1 employed in different bleaching sequences and its effect on pollution load (AOX) generated during bleaching of kraft pulo of *C. equisetifolia.*

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## Materials and Methods

Crude xylanase was produced by white rot fungal strain *C. cinerea* RM-1 NFCCI-3086 isolated in our laboratory and unbleached kraft pulp was obtained by pulping of *C. equisetifolia.* The reaction condition i.e. enzyme dose, reaction time and pulp consistency were optimized during enzymatic prebleaching (data not shown here).

### Effect of xylanase pretreatment on conventional bleaching process

The effect of xylanase treatment on kraft pulp was studied under multistage conventional (CEHH and CEHHP) bleaching sequences. For each bleaching sequence, 100 g (oven dry) disintegrated and unbleached pulp was taken. The prebleaching stage (X) was performed at an enzyme dose of 10 IU/g, pH 8.3, reaction time 120 min, temperature 55± 2 $^0$C and pulp consistency 8%. The enzyme treated pulps were extracted ($E_1$ stage). After prebleaching, the pulps were washed and bleached by CEHH bleaching sequence using different chlorine charges. The total chlorine demand (TCD) was calculated as under.

$$\text{TCD \%} = 0.25 \times \text{kappa number}$$

Fifty percent of TCD was charged in C stage and the remaining 50% was charged in hypochlorite 1$^{st}$ ($H_1$) and 2$^{nd}$ ($H_2$) stages in a ratio of 70:30 respectively. The pulps were filtered through four-layered cheese cloth and the collected filtrates were analyzed for residual chlorine while the remaining filtrates were kept at 4 $^0$C for further investigation. The different stages of a sequence were performed according to the conditions given in Table 1.

### Analysis of combined bleach effluent

The effluents collected after each bleaching stage were mixed in equal amounts at the end of each bleaching sequence and were analyzed for various effluent characteristics. The COD load was determined by closed reflux titrimetric method (using thermoreactor CR 2010) as adopted by Kumar and co-workers [6]. The color was determined by cobaltiplatinate method by measuring the wavelength at 465 nm. AOX was measured by column method [7].

### Statistical analysis

All experiments were carried out independently in triplicate and the results were the mean ± standard deviation of three replicate experiments.

## Results and Discussion

### Effect of xylanase pretreatment on kappa number and brightness

Table 1 illustrates the effect of crude xylanase pretreatment on kappa number, brightness, TCD and viscosity during CEHH and CEHHP bleaching sequences. Crude xylanase pretreatment followed by

**Table 1.** Effect of xylanase pretreatment on conventional bleaching sequences. Unbleached pulp kappa number=17.46 ±0.26, unbleached pulp brightness = 41.49 ± 1.0% and unbleached pulp viscosity = 995 ± 18.5 $cm^3/g$.

| Particular | Bleaching sequence CEHH | XECEHH | CEHHP | XECEHHP |
|---|---|---|---|---|
| Xylanae (X) stage | | | | |
| #Enzyme dose (IU/g) | – | 10 | – | 10 |
| Extraction ($E_1$) stage | | | | |
| #NaOH applied (%) | – | 2 | – | 2 |
| Kappa number of xylanase treated pulp | – | 14.47 ± 0.06 | – | 14.47 ± 0.06 |
| Chlorination (C) stage | | | | |
| #$Cl_2$ applied as available $Cl_2$ (%) | 2.19 | 1.81 | 2.19 | 1.81 |
| #$Cl_2$ consumed as available $Cl_2$ (%) | 2.17 | 1.80 | 2.15 | 1.79 |
| Amount of $Cl_2$ consumed (%) | 99.1 | 99.5 | 98.2 | 98.9 |
| Extraction ($E_2$) stage | | | | |
| #NaOH applied (%) | 2 | 1 | 2 | 1 |
| Hypochlorite ($H_1$) stage | | | | |
| #$Ca(OC_l)_2$ applied as available $Cl_2$ (%) | 1.53 | 1.27 | 1.53 | 1.27 |
| #$Ca(OCl)_2$ consumed as available $Cl_2$ (%) | 1.52 | 1.23 | 1.51 | 1.21 |
| Amount of Ca $(OCl)_2$ consumed (%) | 99.3 | 96.9 | 98.7 | 95.3 |
| Hypochlorite ($H_2$) stage | | | | |
| #$Ca(OCl)_2$ applied as available $Cl_2$ (%) | 0.66 | 0.54 | 0.66 | 0.54 |
| #$Ca(OCl)_2$ consumed as available $Cl_2$ (%) | 0.62 | 0.51 | 0.64 | 0.50 |
| Amount of Ca $(OCl)_2$ consumed (%) | 93.9 | 94.4 | 96.9 | 92.6 |
| Peroxide (P) stage | | | | |
| #$H_2O_2$ applied (%) | | | 2 | 2 |
| #$H_2O_2$ consumed (%) | – | – | 1.98 | 1.96 |
| #$MgSO_4$ applied (%) | | | 0.1 | 0.1 |
| #EDTA applied (%) | | | 0.5 | 0.5 |
| Total $Cl_2$ applied (%) | 4.37 | 3.62 | 4.37 | 3.62 |
| Total $Cl_2$ consumed (%) | 4.31 | 3.54 | 4.30 | 3.50 |
| Amount of total $Cl_2$ consumed (%) | 98.6 | 97.8 | 98.4 | 96.7 |
| Total residual $Cl_2$ (%) | 1.4 | 2.2 | 1.6 | 3.3 |
| Bleaching losses (%) | 8.2 | 7.0 | 8.8 | 7.8 |
| Bleached pulp yield (%) | 43.1 | 44.3 | 42.5 | 43.5 |
| Pulp brightness (%) ISD | 75.2 ± 0.80 | 76.2 ± 1.0 | 79.0 ± 0.78 | 81.1 ± 0.83 |
| Pulp viscosity ($cm^3/g$) | 510.5 ± 7.6 | 525.0 ± 8.9 | 485.0 ± 7.2 | 506.4 ± 11.4 |

| Bleaching conditions | X | $E_1$ | C | $E_2$ | $H_1$ | $H_2$ | P |
|---|---|---|---|---|---|---|---|
| Consistency (%) | 8 | 10 | 3 | 10 | 10 | 10 | 10 |
| pH | 8.3 | 11.5 | 2 | 11.5 | 11 | 11 | 11.5 |
| Temperature ($^0C$) | 55±2 | 70±2 | Ambient | 70±2 | 70±2 | 70±2 | 90±2 |
| Reaction time (min) | 120 | 90 | 30 | 90 | 60 | 60 | 120 |

alkali extraction mitigates kappa numbers of unbleached pulp 2.99 units (17.12%). While, the brightness after XECEHH and XECEHHP bleaching sequences improves by 1.0 and 2.1% respectively, as to pulp brightness of CEHH and CEHHP bleaching sequences. This improvement in brightness and reduction in kappa number after xylanase treatment, possibly results from the partly degradation of xylan on the fiber surface and destruction of LCC; thus improving the accessibility of bleaching chemicals with facilitating the removal of dissolved lignin during washing and bleaching steps [5]. Xylanase treatment increased the penetration of bleaching chemicals into the pulp. The TCD of kraft pulp bleached by XECEHH and XECEHHP reduces by 17.16% in comparison with TCD of both CEHH and CEHHP bleaching sequences. Enzymatic prebleaching of hardwood and softwood pulps showed about 35-41% and 10-20% reduction respectively, in the active chlorine at the chlorination stage [5].

The viscosity after XECEHH and XECEHHP bleaching sequences improves by 2.76 and 4.23% re-

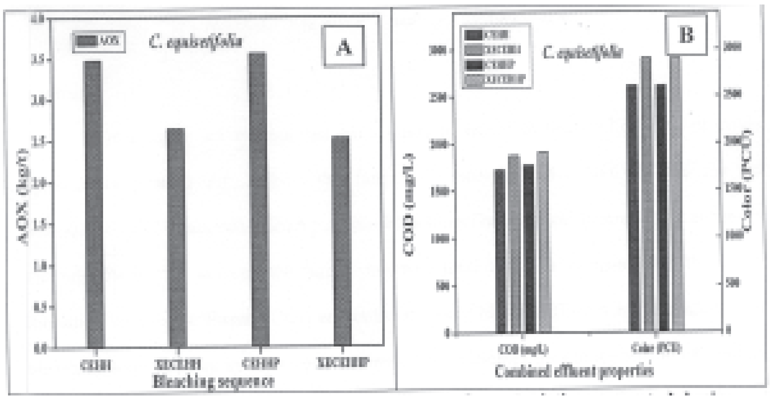


**Fig. 1.** Comparison of combined bleach effluent characteristics generated during CEHH, XECEHH, CEHHP and XECHHP bleaching sequences.

spectively, over viscosity of CEHH and CEHHP bleached pulp. The improvement in viscosity of xylanase pretreated pulps reflects the selective hydrolysis of low DP xylan and enrichment of high molecular weight carbohydrates. Similar results were observed by several workers who found the slight increment in viscosity of soda-AQ pulps of lemon and sofia grasses after crude xylanase pretreatment [8—10].

### Effect of xylanase pretreatment on combined bleach effluent characteristics

The AOX level is reduced by 23.85 and 28.93% after XECEHH and XECEHHP bleaching sequences respectively, as compared to their respective controls (CEHH and CEHHP) (Fig. 1A). The COD load and color values of combined effluent generated during XECEHH bleaching sequence are increased by 8.0 and 10.31% respectively, as compared to CEHH bleaching sequence whereas the improvement in COD load and color values are 7.31 and 9.93% respectively, during XECEHHP bleaching sequence as compared to CEHHP bleaching sequence (Fig 1B).

The chlorine reacts with residual lignin present in fibers and produces the organochlorine compounds during bleaching process [8]. The xylanase pretreatment reduces the TCD in bleaching sequences, this results into the low toxicity of the spent bleach liquor [9]. Hence, the bio-bleaching using xylanase enzyme reduces the amount of chlorophenols and AOX in the bleaching effluent. Enzymes dissolve the carbohydrate bonds present in pulp; this leads to an increase in the concentration of hydrolyzed xylan and residual lignin carbohydrate complexes in the effluent which ultimately results into an increase in COD and color. Many researchers observed the similar results [10, 11].

## Conclusion

The crude xylanase enzyme produced by *C. cinerea* RM-1 has been used successfully for its application in bio-bleaching of *C. equisetifolia* kraft pulps and reduction of toxicity in effluents generated, in terms of AOX. The total chlorine demand is reduced by 17.16% with significant reduction in AOX level after enzymatic treatment. This study explores the future prospects for pulp bleaching using a cleaner technology which results in the lower chemical consumption and pollution load.

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