JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

# BIOBLEACHING OF ETHANOL-SODA PULP OF *EULALIOPSIS BINATA* BY XYLANASES FROM *ASPERGILLUS FLAVUS* ARC-12 AND *SCHIZOPHYLLUM COMMUNE* ARC-11

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**doi: 10.15414/jmbfs.2021.10.4.669-673**

ARTICLE INFO

Received 1. 11. 2019
Revised 29. 10. 2020
Accepted 9. 11. 2020
Published 1. 2. 2021

Regular article

OPEN ACCESS

**ABSTRACT**

Environmental pollution can be minimized by using xylanase pretreatment of pulp before chemical bleaching. *A. flavus* ARC-12 and *S. commune* ARC-11 produced 234.26 and 1147.11 IU/ml of xylanase under solid-state fermentation that was used for biobleaching of ethanol-soda pulp of *Eulaliopsis binata*. The brightness of bleached ethanol-soda pulp of *E. binata* increased by 3.2 and 1.9% (ISO) with *A. flavus* ARC-12 and *S. commune* ARC-11 xylanase respectively compared chemical bleaching at the same chlorine dioxide charge. While the consumption of chlorine dioxide were mitigated by 2.98 and 3.82% with *A. flavus* ARC-12 and *S. commune* ARC-11 xylanase pretreatment respectively. Moreover, *A. flavus* ARC-12 and *S. commune* ARC-11 xylanase pretreatment reduced AOX generation by 23.80 and 19.04% respectively compared to chemical bleaching.

**Keywords:** ECF bleaching, xylanases, *Aspergillus flavus*, *Schizophyllum commune*, chlorine dioxide

## INTRODUCTION

Pulp and paper industry is 6th largest contaminating industry in world that produces precarious solid, liquid and gaseous wastes **(Sharma *et al.*, 2020)**. During paper manufacturing bulk of lignin is removed by pulping and the remaining lignin in pulp i.e. residual lignin is removed by multistage bleaching process using chlorine compounds including elemental chlorine, chlorine dioxide, and sodium hypochlorite. Chlorine dioxide is widely used to replace elemental chlorine in bleaching section. Chlorine dioxide reduces the generation of chlorinated organic compounds in effluent compared to chlorine bleaching. It shows 2.5 times oxidizing capacity compared to chlorine and attacks lignin more selectively to preserve cellulose **(Raghuveer, 2002; Kumar *et al.*, 2017; Raj *et al.*, 2018)**. During chlorine-based bleaching, raw material components such as lignin, phenols, and resin acids get chlorinated and transformed into highly toxic compounds such as adsorbable organic halides (AOX), dioxins, furans, chlorophenols and volatile organic compounds (VOCs). Some of them are mutagenic, persistent and bioaccumulating due to their lipophilic nature **(Pokhrel & Viraraghavan 2004; Nie *et al.*, 2015; Gautam *et al.*, 2017)**. Therefore, some alternative bleaching process is required to reduce the consumption of chlorine compound. Microbial enzymes are green alternatives for several processes in the pulp and paper industry **(Kumar *et al.*, 2020)**. Biobleaching with microbial enzymes such as xylanases, lignin peroxidases, manganese peroxidases, laccases, and versatile peroxidase has been proved an effective alternative to minimize the consumption of chlorine compounds during bleaching **(Nie *et al.*, 2015; Gautam *et al.*, 2017; Raj *et al.*, 2018; Singh & Arya, 2019; Chaurasia & Bhardwaj, 2019)**. Among them, xylanases are dominantly used for biobleaching of various types of pulps. Filamentous fungi are well known for the production of xylanases in the large amount. The xylanases from different species related to the genus *Aspergillus, Trichoderma, Penicillium, Coprinellus, Humicola, Thermomyces* have been used for biobleaching of pulp **(Bissoon *et al.*, 2002, Lal *et al.*, 2011, Kumar V *et al.*, 2016, Gautam *et al.*, 2017, Campioni *et al.* 2019, Chaurasia & Bhardwaj, 2019)**. Several species of the genus *Aspergillus* such as *A. niger, A. flavus, A. oryzae, A. fumigatus, A. terreus, A. awamori, A. nidulans* have been extensively studied for xylanase production **(Polizeli *et al.*, 2005, Gautam *et al.*, 2015)**.

The difficulty for lignin removal is that xylan forms a complex with lignin, known as lignin-carbohydrate complex that creates a physical barrier for bleaching chemicals action on lignin. The depolymerization of hemicellulose is easier than lignin; therefore biobleaching appears to be more effective with xylanases compared to lignin degrading enzymes. Xylanase breaks the xylan network and open up the polymer to facilitate the removal of residual lignin by mild oxidant. This increases the permeability of pulp fiber surfaces and improves the penetration of bleaching chemicals into the pulp. So, xylanase action assists the removal of trapped lignin from pulp fiber rather than attacking lignin directly **(Motta *et al.*, 2013; Campioni *et al*,. 2019)**. Xylanase pulp bleaching is recognized as an economically viable process that decreases the environmental pollution and disposal of effluents composed of chlorinated organic compounds **(Fernanda *et al.*, 2016)**. The pretreatment of pulp with xylanase minimizes the consumption of chlorine compounds during bleaching. Moreover, the xylanase treatment also decreases the resistance of fiber wall to the outward movement of degraded lignin fragments and improves the extractability of solubilized lignin. Xylanase pretreatment improves the brightness and reduces the kappa number of pulp **(Torres *et al.*, 2000; Gangwar *et al.*, 2014; Gautam *et al.*, 2017)**. Cellulase-free xylanase enzyme preparations are desirable for their applicability in bleaching of pulp. Cellulase can hydrolyze the cellulose and affects the viscosity and physical strength properties adversely **(Subramaniyan & Prema, 2000; Gautam *et al.*, 2017)**. The bleaching efficiency of xylanases is dependent upon several factors such as reaction temperature, pH, enzyme dose, treatment time, pulp consistency, the type of raw material, and the type of pulping and bleaching process **(Bajpai, 1999; Sharma *et al.*, 2020)**. Bokhari et al. (2010) studied the production of xylanase by *Thermomyces lanuginosus* and evaluated its efficacy in ECF bleaching of unbleached wheat straw pulp. During biobleaching of pulp kappa number was decreased by 18.6% while brightness was improved 2.63%. Moreover, xylanase pretreatment resulted in a maximum reduction in chlorine demand by 27.3%. The xylanases produced by *A. niger* and *A. flavus* were utilized for the prebleaching of *Eucalyptus grandis* pulp at consistency of 10%. The xylanase pretreatment was performed at 55 °C for 2 h with enzyme dose of 10 IU/g of pulp. Kappa number was decreased by 25.93% and 36.32% by *A. niger* and *A. flavus* xylanase pretreatments respectively **(de Alencar Guimaraes *et al.*, 2013)**.

## MATERIAL AND METHODS

### Microorganism and xylanase production

The bleaching studies of ethanol-soda pulp of *E. binata* were carried out with crude xylanase from *A. flavus* ARC-12 and *S. commune* ARC-11, previously isolated and identified. Both of the fungal strains, *A. flavus* ARC-12 and *S. commune* ARC-11 were deposited at the National Fungal Culture Collection of India, Agharkar Research Institute, Pune with accession numbers NFCCI 3028

and NFCCI 3029 respectively. The fungal isolate was maintained over potato dextrose agar slants at 4 °C. Xylanase production by *A. flavus* ARC-12 and *S. commune* ARC-11 was carried out under solid-state fermentation using pearl millet stover and wheat bran as substrate **(Gautam *et al*., 2017 & 2018)**.

**Enzyme assays**

Xylanase activity was determined by using 1% (w/v) of birch wood xylan (Sigma Chemical Co. St Louis, MO, USA) in 50 mM citrate buffer at pH 5.5 according to Bailey method (Bailey et al. 1992). One unit of xylanase activity is defined as the amount of enzyme that librates 1 µmole of xylose per min per ml under assay conditions. Cellulase activity was determined as described by **(Kumar *et al*., 2016)**.

**Ethanol-soda pulping of *E. binata***

Fresh *E. binata* grass was collected from Behat, Saharanpur district, India at the end of rainy season and chopped into small pieces. The cooking of chopped *E. binata* was performed in an electronically heated WEVERK rotary digester of 0.02 $m^3$ capacity. The digester contained four bombs of one-liter capacity each. Maximum pulp yield of 47.47% with kappa number, 16.13 was obtained by optimized ethanol-soda pulping. The brightness and viscosity of unbleached pulp were 43.9±0.2 % ISO and 28.2±0.14 cps respectively **(Gautam *et al*., 2016)**.

**Elemental chlorine-free (ECF) bleaching**

Unbleached ethanol-soda pulp of *E. binata* was bleached by DEDP, $X_1$DEDP, and $X_2$DEDP bleaching sequences where stands '$X_1$' represented xylanase from *A. flavus* ARC-12 and $X_2$ represented xylanase from *S. commune* ARC-11, '$D_1$' and '$D_2$' stood for chlorine dioxide 1st and 2nd stages respectively, 'E' for alkaline extraction stage, 'P' for hydrogen peroxide stage. The xylanase pre-treatment stage was carried out under optimized conditions. The xylanase treatments ($X_1$ & $X_2$) of ethanol-soda pulp of *E. binata* were performed at enzyme dosages of 10 IU/g of o.d. pulp for 120 min. During xylanase treatment, the temperature was maintained at 50 and 55 °C for $X_1$and $X_2$ respectively. After E-stage, samples were treated with 2% chlorine dioxide in '$D_1$' and '$D_2$' stages (o.d. pulp basis) (1.34% in '$D_1$' and 0.66% in '$D_2$' stage) at a consistency of 10% at 70 °C for 180 min and pH 4.2. In E-stage NaOH (as such) was conducted at 10% consistency, 60 °C for 60 min, and pH 11.7. In DEDP bleaching sequence, the final stage i.e. peroxide (P) stage was carried out at 10% consistency, temperature 90 °C, pH 10.3 and reaction time 60 min in polythene bag with 0.5% $H_2O_2$, 0.1% $MgSO_4$ (as a carbohydrate stabilizer) and 0.5% EDTA (to mask the activities of d-block elements/transition metals). All the chemicals were added on o.d. pulp basis. The strength of $H_2O_2$ was determined by the method of Vogel's (2002).

**Preparation of laboratory handsheets and evaluation of paper properties**

The ethanol-soda bleached pulp samples of *E. binata* were evaluated for bleaching losses, viscosity (TAPPI T 230 om-08), and copper number (TAPPI T 430 om-88) as per TAPPI Standard Test Methods. The pulp pads were prepared on Büchner funnel (TAPPI T 218 sp-11) and tested for brightness (TAPPI T 452 om-08). Laboratory handsheets of 60 g/$m^2$ were prepared (TAPPI T 205 sp-02) and conditioned at a temperature of 27±2 °C and relative humidity of 65±2%. These laboratory handsheets were tested for various physical strength properties such as tear index (TAPPI T 414 om-98), tensile index (TAPPI T 494 om-01), burst index (TAPPI T 403 om-97), and double fold numbers (TAPPI T 423 cm-98) **(TAPPI, 2007)**.

**Analysis of bleach effluent**

The effluent generated after each stage of bleaching sequence was collected and mixed in equal amounts and were analyzed for COD (closed reflux titrimetric method using Thermoreactor CR2010) (1985), colour (Test method No-204A) as per standard methods for the examination of water and wastewater, American Public Health Association, 1985 (Greenberg et al. 1992) and AOX by column method (2006) with AOX Analyzer Dextar ECS 1200.

**Statistical analysis**

All the experiments were carried out in triplicate and experimental results were represented as the mean ± standard deviation of three experimental values.

**RESULTS AND DISCUSSION**

**Xylanase production**

Xylanase production was carried out under solid-state fermentation conditions by *A. flavus* ARC-12 and *S. commune* ARC-11 using millet stover and wheat bran respectively as the carbon source under SSF conditions. *A. flavus* ARC-12 and *S. commune* ARC-11 produced 234.26 and 1147.11 IU/ml of xylanase respectively at optimum cultural conditions. Cellulase activity was not detected in crude xylanase from *A. flavus* ARC-12 while *S. commune* ARC-11 produced 1.47 IU/ml of cellulase. However, cellulase activity was very low in crude xylanase from *S. commune* ARC-11, the ratio between xylanase and cellulase activity was 780 to 1 respectively. **Campioni *et al.* (2019)** also used *T. reesei* QM9414 enzyme having xylanase to cellulase ratio 200 to 1 respectively for the biobleaching of kraft pulp.

**Effect of xylanase pre-treatment on ECF bleaching**

**Table 1** showed the results and bleaching conditions of DEDP, $X_1$DEDP, and $X_2$DEDP sequences of ethanol-soda pulp of *E. binata* and its effect on brightness, viscosity, and bleached pulp yield. The brightness of ethanol-soda pulp by DEDP, $X_1$DEDP, and $X_2$DEDP bleaching sequences was 82.6, 85.8, and 84.5% respectively. Bleached pulp yield was improved up to 45.24 and 45.57% in bleaching sequences $X_1$DEDP, and $X_2$DEDP as compared to DEDP bleaching sequence (44.30%). The brightness of $X_1$DEDP, and $X_2$DEDP bleached ethanol-soda pulp of *E. binata* increased by 3.2 and 1.9% (ISO) respectively compared DEDP bleaching sequences at the same chlorine dioxide charge. Xylanase treatment enhances the porosity of pulp fibres and which subsequently improves the accessibility of bleaching chemicals into the pulp compared untreated pulp. It allows the lignin fragments to remove from the pulp. Therefore, higher brightness of pulp can be obtained by xylanase treatment at the same bleaching dosage **(Torres *et al*., 2000; Johannes & Majcherczyk, 2000; Lin *et al*., 2013; Raj *et al*., 2018)**. Pre-treatment of bagasse pulp with *T. lanuginosus* SSBP xylanase (DED bleaching sequence) increased the pulp brightness by 4.5% compared to control **(Bissoon *et al*., 2002)**. Pretreatment with a commercial enzyme (Xylanase-P) increased the brightness of bagasse pulp by 3.1% and softwood kraft pulp by 5.1% compared to control after ECF bleaching **(Madlala *et al*., 2001)**. **Campioni *et al.* (2019)** performed the pretreatment of *Eucalyptus* kraft pulp with *T. reesei* QM9414 xylanase and observed a 10% reduction in chlorine dioxide consumption, maintaining the same brightness as in control.

The viscosity of $X_1$DEDP, and $X_2$DEDP bleached *E. binata* ethanol-soda pulp increased by 3.40 and 2.27% respectively compared to DEDP bleaching sequence. It is well established that the crude xylanase hydrolyzes xylan only and not cellulose chains in pulp **(da Silva *et al*., 1994; Vidal *et al*., 1997; Roncero *et al*., 2003)**. The copper number decreased by 28.0 and 25.33% for *E. binata* ethanol-soda pulp after $X_1$DEDP, and $X_2$DEDP bleaching sequences compared to DEDP. Xylanase pre-treatment reduced the degree of damage to cellulose of the ethanol-soda pulp after full bleaching sequences in terms of reduction in copper number. The pulp viscosity of ethanol-soda pulp by DEDP, $X_1$DEDP, and $X_2$DEDP bleaching sequences were 8.8, 9.1, and 9.0% cps respectively. This slight increase indicated that there was no adverse effect on cellulose chain polymer. Tear index improved by 20.29 and 15.53% during $X_1$DEDP and $X_2$DEDP sequences compared to DEDP (**Figure 1**). While improvement in other mechanical strength properties like burst index and double-fold numbers were insignificant except a slight improvement in tensile index during bleaching sequences $X_1$DEDP and $X_2$DEDP compared to DEDP. **Agrawal et al. (2016)** performed bleaching of plywood veneer soda anthraquinone pulp with xylano-pectinolytic enzyme from *Bacillus pumilus* AJK and observed 8.5, 13.4, and 10.8% increase in breaking length, burst factor and tear factor respectively. **Lin et al. (2013)** also reported slight improvements in burst, tear, and tensile index on the treatment of wheat straw soda-AQ pulp with recombinant xylanase from *B. halodurans* during ECF bleaching (Lin et al. 2013). Pretreatment of pulp with xylanase and its subsequent bleaching with sequence $CDED_1D_2$ improved various physical properties of the pulp i.e. viscosity, tensile strength, breaking length, burst factor, and tear factor and by 44%, 32%, 21%, 6%, and, 7% respectively, which greatly improves the quality of the paper **(Battan *et al*., 2007)**.

The consumed $ClO_2$ during $X_1$DEDP and $X_2$DEDP bleaching sequences were mitigated by 2.98 and 3.82% respectively. Xylanase pretreatment reduced AOX generation by 23.80 and 19.04% after $X_1$DEDP, and $X_2$DEDP bleaching sequences respectively compared to DEDP (**Table 2**). It is well proved that 4-O-methylglucuronic acid side chain of hemicelluloses is converted into hexenuronic acid (HexA) during pulp cooking. Several researchers indicated that the formation of AOX during bleaching has close relationship with HexA content of pulp **(Björklund *et al*., 2002, 2004; Nie *et al*., 2015; Dai *et al*., 2016)**. Various studies proposed that HexA consumes chlorine dioxide during bleaching. It is primarily the in-situ generated hypochlorous acid that reacts with HexA to form AOX **(Torngren & Ragnar, 2002; Ventorim *et al*., 2008)**. Therefore, when hemicelluloses and HexA were removed from the fibre due to xylanase action, the lignin could easily react with $ClO_2$ and AOX generation decreased at the same dose of $ClO_2$ **(Nie *et al*., 2015)**. **Nie et al. (2015)** studied the xylanase-aided chlorine dioxide bleaching of bagasse pulp and concluded that lignin and HexA were the main sources of AOX generation and xylanase pretreatment removed HexA, mitigated AOX formation by 21.4–26.6% to achieve same level of brightness. **Dai et al. (2016)** also studied the correlation between HexA content of pulp and AOX formation and found that AOX could be reduced up to 29.8% by xylanases treatment ($XD_0$) compared to chlorine dioxide bleaching stage ($D_0$). Chlorophenol compounds were completely removed after xylanase treatment.

**Table 1** Effect of *A. flavus* ARC-12 and *S. commune* ARC-11 xylanases pretreatment on ECF bleaching of *E. binata*

| Particulars | Bleaching sequence | | |
|---|---|---|---|
| | **DEDP** | **$X_1$DEDP** | **$X_2$DEDP** |
| Unbleached pulp kappa number | 16.1±0.3 | 16.1±0.3 | 16.1±0.3 |
| Unbleached pulp brightness, % (ISO) | 43.9±0.2 | 43.9±0.2 | 43.9±0.2 |
| Unbleached pulp viscosity, cps | 28.2±0.14 | 28.2±0.14 | 28.2±0.14 |
| **Xylanase stage (X)** | | | |
| Amount of xylanase added (on o.d. pulp basis), IU/g | – | 10 | 10 |
| pH | – | 6.0 | 5.0 |
| **Chlorine dioxide stage ($D_1$)** | | | |
| $ClO_2$ applied as available $Cl_2$, % o.d. pulp basis) | 1.34 | 1.34 | 1.34 |
| $ClO_2$ consumed as available $Cl_2$, % (o.d. pulp basis) | 1.26 | 1.22 | 1.21 |
| $ClO_2$ consumed on $Cl_2$ basis, % | 94.02 | 91.04 | 90.2 |
| Final pH | 4.1 | 4.0 | 4.0 |
| **Alkali extraction stage (E)** | | | |
| NaOH applied, % (o.d. pulp basis) | 1.2 | 1.2 | 1.2 |
| Initial pH | 11.1 | 10.8 | 10.7 |
| Final pH | 11.3 | 11.2 | 11.2 |
| **Chlorine dioxide stage ($D_2$)** | | | |
| $ClO_2$ applied as available $Cl_2$, % (o.d. pulp basis) | 0.660 | 0.660 | 0.660 |
| $ClO_2$ consumed as available $Cl_2$, % (o.d. pulp basis) | 0.602 | 0.598 | 0.596 |
| $ClO_2$ consumed, % | 91.21 | 90.60 | 90.3 |
| Final pH | 4.1 | 4.1 | 4.1 |
| **Peroxide stage (P)** | | | |
| $H_2O_2$ applied, % (o.d. pulp basis) | 0.5 | 0.5 | 0.5 |
| EDTA applied, % (o.d. pulp basis) | 0.5 | 0.5 | 0.5 |
| $MgSO_4$ applied, % (o.d. pulp basis) | 0.1 | 0.1 | 0.1 |
| Final pH | 10.4 | 10.9 | 10.8 |
| Total $ClO_2$ applied, % (o.d. pulp basis) | 2.0 | 2.0 | 2.0 |
| Total $ClO_2$ consumed, % (o.d. pulp basis) | 1.862 | 1.818 | 1.806 |
| Total $ClO_2$ consumed on $Cl_2$ basis, % | 93.1 | 90.9 | 90.3 |
| Bleached pulp yield, % | 44.30±1.4 | 45.24±1.5 | 45.57±1.1 |
| Pulp brightness, % (ISO) | 82.6±0.2 | 85.8±0.3 | 84.5±0.2 |
| Pulp viscosity, cps | 8.8±0.008 | 9.1±0.016 | 9.0±0.012 |

| **Bleaching conditions** | $X_1$ | $X_2$ | $D_1$ | E | $D_2$ | P |
|---|---|---|---|---|---|---|
| Consistency, % | 10 | 10 | 10 | 10 | 10 | 10 |
| Temperature, $^0$C | 50±2 | 55±2 | 70±2 | 60±2 | 70±2 | 90±2 |
| Time, min | 120 | 120 | 180 | 60 | 180 | 60 |

± refers standard deviation

-$X_1$ represents xylanase from *A. flavus* ARC-12 and $X_2$ represented xylanase from *S. commune* ARC-11

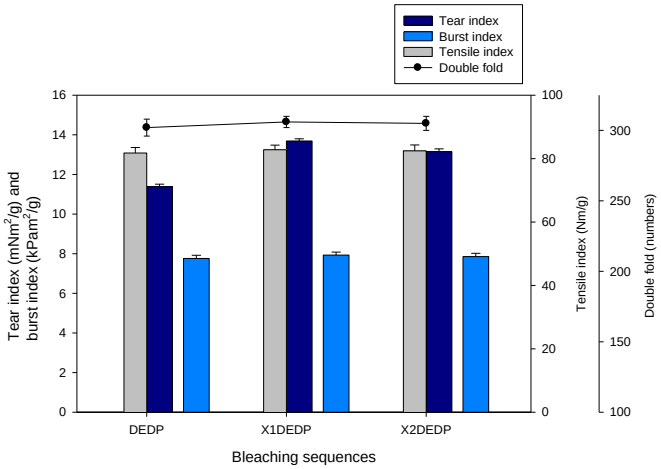


**Figure 1** Comparison of mechanical strength properties during ECF bleaching of pulp of *E. Binata*

On contrary to this, the COD showed an increase of 9.87 and 7.96% respectively in combined bleached effluent obtained from $X_1$DEDP, and $X_2$DEDP bleached pulps compared to DEDP (**Figure 2**). The increase in COD of combined bleach effluent of xylanase prebleaching sequences may be explained due to the dissolution of xylan and lignin fragments with carbohydrates compared to control **(Valls *et al*., 2013; Sharma *et al*., 2014)**. **Sharma et al. (2014)** analyzed biological oxygen demand (BOD) and chemical oxygen demand (COD) of effluent from chlorine dioxide bleaching and enzyme pretreatment with subsequent chlorine dioxide bleaching. BOD and COD were increased by 13.98 and 26.39% respectively in effluents generated from the enzyme (xylanase and laccase) treated pulps **(Sharma *et al*., 2014)**.

**Table 2** Comparison of copper number and AOX during ECF bleaching of *E. binata* pulp

| Sl. No. | Particulars | DEDP | $X_1$DEDP | $X_2$DEDP |
|---|---|---|---|---|
| 1 | Beating level, $^0$SR | 35±1 | 35±1 | 35±1 |
| 2 | Copper number | 0.15±0.003 | 0.11±0.002 | 0.11±0.004 |
| 3 | AOX, kg/t | 0.42±0.008 | 0.32±0.007 | 0.34±0.007 |

± refers standard deviation

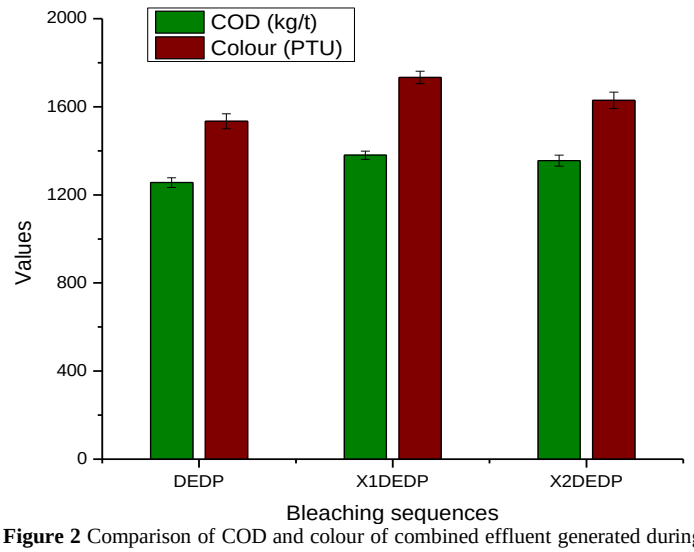


**Figure 2** Comparison of COD and colour of combined effluent generated during ECF bleaching of *E. binata* pulp

## CONCLUSION

Xylanases from two filamentous fungi namely *A. flavus* ARC-12 and *S. commune* ARC-11 were utilized for pretreatment of ethanol-soda pulp of *E. binata* before

chlorine dioxide bleaching. Xylanases from both fungi were found effective for improvement in ISO brightness. During xylanase bleaching, pulp viscosity improved slightly and physical strength properties were maintained except tear index that increased significantly. Xylanase pretreatment reduced both chlorine dioxide consumption and AOX generation. CODs were increased in combined bleached effluent obtained from $X_1$DEDP and $X_2$DEDP bleaching sequences as compared to DEDP bleaching sequence.

**Acknowledgments:** The first author is thankful to the Council of Scientific and Industrial Research, NewDelhi forawarding her Fellowship and for providing partial financial support to carryout this work.

**Conflict of interest:** The authors declare that there are no conflicts of interest regarding the publication of this paper.

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