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

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ABSTRACT

Environmental pollution can be minimized by using xylanase pretreatment of pulp before chemical bleaching. \textit{A. flavus} ARC-12 and \textit{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 \textit{Eulaliopsis binata}. The brightness of bleached ethanol-soda pulp of \textit{E. binata} increased by 3.2 and 1.9% (ISO) with \textit{A. flavus} ARC-12 and \textit{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 \textit{A. flavus} ARC-12 and \textit{S. commune} ARC-11 xylanase pretreatment respectively. Moreover, \textit{A. flavus} ARC-12 and \textit{S. commune} ARC-11 xylanase pretreatment reduced AOX generation by 23.80 and 19.04% respectively compared to chemical bleaching.

Keywords: ECF bleaching, xylanases, \textit{Aspergillus flavus}, \textit{Schizophyllum commune}, chlorine dioxide

INTRODUCTION

Pulp and paper industry is 6\textsuperscript{th} largest contaminating industry in world that produces precipitous 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 solution. 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 \textit{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 \textit{Aspergillus} such as \textit{A. niger}, \textit{A. flavus}, \textit{A. oryzae}, \textit{A. fumigatus}, \textit{A. terreus}, \textit{A. awamori}, \textit{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 \textit{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 \textit{A. niger} and \textit{A. flavus} were utilized for the prebleaching of \textit{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 \textit{A. niger} and \textit{A. flavus} xylanase pretreatments respectively (de Alencar Guimarães et al., 2013).

MATERIAL AND METHODS

Microorganism and xylanase production

The bleaching studies of ethanol-soda pulp of \textit{E. binata} were carried out with crude xylanase from \textit{A. flavus} ARC-12 and \textit{S. commune} ARC-11, previously isolated and identified. Both of the fungal strains, \textit{A. flavus} ARC-12 and \textit{S. commune} ARC-11 were deposited at the National Fungal Culture Collection of India, Agharkar Research Institute, Pune with accession numbers NFCCI 3028.
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 liberates 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 m³ 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,DEDP, and X,DEDP, bleaching sequences where stands ’X’, represented xylanase from A. flavus ARC-12 and X1, represented xylanase from S. commune ARC-11, ’D’, and ’D’, stood for chloride dioxide 1st and 2nd stages respectively, E’ for alkaline extraction stage, P’ for hydrogen peroxide stage. The xylanase-pretreatment stage was carried out under optimized conditions. The xylanase treatments (X1, and X2) 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 X1 and X2 respectively. After E-stage, samples were treated with 2% chlorine dioxide in ’D1’, and ’D2’ stages (o.d. pulp basis) (1.34% in ’D1’, and 0.66% in ’D2’ 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% H₂O₂, 0.1% MgSO₄ (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 H₂O₂ 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/m² 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 Dextrac 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 as substrate (Gautam et al., 2017 & 2018).
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    | X₁DEDP  | X₂DEDP  |
| 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 (D₁)             |         |         |         |
| ClO₂ applied as available Cl₂, % (o.d. pulp basis) | 1.34    | 1.34    | 1.34    |
| ClO₂ consumed as available Cl₂, % (o.d. pulp basis) | 1.26    | 1.22    | 1.21    |
| ClO₂ consumed on Cl₂ 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 (D₂)             |         |         |         |
| ClO₂ applied as available Cl₂, % (o.d. pulp basis) | 0.660   | 0.660   | 0.660   |
| ClO₂ consumed as available Cl₂, % (o.d. pulp basis) | 0.602   | 0.598   | 0.596   |
| ClO₂ consumed, %                        | 91.21   | 90.60   | 90.3    |
| Final pH                                | 4.1     | 4.1     | 4.1     |
| Peroxide stage (P)                      |         |         |         |
| H₂O₂ applied, % (o.d. pulp basis)       | 0.5     | 0.5     | 0.5     |
| EDTA applied, % (o.d. pulp basis)       | 0.5     | 0.5     | 0.5     |
| MgSO₄ applied, % (o.d. pulp basis)       | 0.1     | 0.1     | 0.1     |
| Final pH                                | 10.4    | 10.9    | 10.8    |
| Total ClO₂ applied, % (o.d. pulp basis) | 2.0     | 2.0     | 2.0     |
| Total ClO₂ consumed, % (o.d. pulp basis) | 1.862   | 1.818   | 1.806   |
| Total ClO₂ consumed on Cl₂ 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                    | X₁    | X₂    | D₁    | E    | D₂    | P    |
|-----------------------------------------|-------|-------|-------|------|-------|------|
| Consistency, %                          | 10    | 10    | 10    | 10   | 10    | 10   |
| Temperature, °C                         | 50±2  | 55±2  | 70±2  | 60±2 | 70±2  | 90±2 |
| Time, min                               | 120   | 120   | 180   | 80   | 140   | 160  |

* ± refers standard deviation

-X represents xylanase from A. flavus ARC-12 and X₂ represents xylanase from S. commune ARC-11

![Figure 1](image1.png)

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 X₁DEDP, and X₂DEDP 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).

![Figure 2](image2.png)

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

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

| Sl. No. | Particulars | DEDP | X₁DEDP | X₂DEDP |
|---------|-------------|------|--------|--------|
|         | Beating level, °SR | 35±1 | 35±1  | 35±1  |
| 1       | Copper number | 0.15±0.003 | 0.11±0.002 | 0.11±0.004 |
| 2       | AOX, kg/t     | 0.42±0.008 | 0.32±0.007 | 0.34±0.007 |

* ± refers standard deviation

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-DEDP and X-DEPD bleaching sequences as compared to DEDP bleaching sequence.

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