Xylanase-assisted TCF bleaching of *Eulaliopsis binata* pulp

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Abstract
The chlorine-containing bleaching chemicals are completely replaced with other oxidative chemicals in total chlorine-free (TCF) bleaching. Moreover, the xylanase pretreatment of pulp improves the efficiency of oxidative chemicals during TCF bleaching. Brightness of E. binata ethanol-soda pulp increased by 2.5 and 1.4% (ISO) with A. flavus ARC-12 and S. commune ARC-11 xylanases pretreatment of pulp respectively compared to control (without xylanase treatment).

The xylanases from both fungal strains improved the pulp viscosity significantly compared to control. Tear index of xylanase treated pulp increased by 3.67 and 3.25% by A. flavus ARC-12 and S. commune ARC-11 xylanases respectively compared to control while burst index remained almost same. There is no generation of AOX and polychlorinated dioxins as well as furans during TCF bleaching while optical and strength properties of pulp were comparable to ECF bleaching process.

Keywords: TCF bleaching, cellulase-free xylanases, *Eulaliopsis binata, Aspergillus flavus.*

Introduction

The residual lignin in pulp imparts dark colour and it is removed by several stages of bleaching. In the conventional methods of bleaching, chlorine is used which generates AOX which makes effluent discharge as highly toxic. Researchers has focussed on the alternatives of conventional bleaching technologies that can reduce the adsorbable organic hallides (AOX) and total organic chlorides in effluent discharge. Elemental chlorine free (ECF) bleaching uses the chlorine dioxide in place of chlorine, resulting lower AOX in effluent discharge with acceptable quality of pulp brightness[12]. The presence of polychlorinated dioxins and furans in bleach effluents and end-products, compelled for replacement of all chlorine compounds in bleaching process.

Bleaching without elemental chlorine or chlorine containing compounds is termed as totally chlorine free (TCF) bleaching process. These bleach techniques exploit the oxidative agents such as oxygen, ozone and peroxide etc. which degrade lignin by oxidation, thereby decreasing the molecular size and increasing its water and alkali solubility [3,12]. In oxygen delignification, molecular oxygen reacts with pulp in alkaline conditions and allows extended delignification of chemical pulp with positive environmental impacts in presence of Epsom salt ($MgSO_4$), which is a carbohydrate stabilizer[11].

Hydrogen peroxide has a major role in TCF bleaching approaches, it is being used both as an alkaline bleaching agent and as reinforcement agent for alkaline extraction. H_2O_2 acts as a true bleaching agent. Compared with oxygen delignification, H_2O_2 delignification appears to provide better color reduction because of its specific action on chromophores[3,13]. TCF bleaching with oxygen and hydrogen peroxide is superior in terms of environmental impact but their selectivity is not considered as good as chlorine-based bleaching process[9].

The incorporation of xylanases in bleaching process reduces consumption of bleaching chemicals and improves pulp quality. The chemical treatment is not able to remove the lignin completely from fiber, some fraction of residual lignin reprecipitates on surfaces of pulp fibers that causes browning of resultant pulp. Xylanase treatment hydrolyzes relocated hemicelluloses on pulp fibers and attacks lignin-carbohydrates complex that opens up more space for bleaching chemicals. The improved accessibility for bleaching chemicals increases lignin extraction during subsequent bleaching stages[5,13,17]. Xylanase bleaching can significantly improve the final pulp brightness of bleached pulp along with reduction in bleaching cost, when it is used with ozone and hydrogen peroxide[4].

Material and Methods

Enzyme and pulp sources: Xylanases for this study were produced by *Aspergillus flavus* ARC-12 and *Schizophyllum commune* ARC-11 under solid-state fermentation. Xylanase and cellulase assays were performed using birch wood xylan and caboxymethy cellulose respectively as substrate as described by Gautam et al[7]. The pulping of the grass, *Eulaliopsis binata* was performed in WEVERK rotary digester of 0.02 m^3 capacity. The optimization of cooking conditions and pulping chemicals was carried out to produce maximum yield of pulp. Ethanol-soda pulping of chopped *E. binata* biomass was found most suitable that resulted in 47.47% of pulp yield with Kappa number 16.1±0.3[6].

TCF bleaching: The sequences QOPP, X_1QOPP and X_2QOPP were used for bleaching of *E. binate* pulp where 'X_1' stood for xylanase from *A. flavus* ARC-12 and X_2 represented xylanase from *S. commune* ARC-11, 'Q' stood for chelating stage, 'O' stood for oxygen delignification and

'P' stood for hydrogen peroxide stage. Ethanol-soda pulp samples were treated with 0.2% DTPA at a pulp consistency of 3% and pH 4.5 for 30 min at ambient temperature[8]. After xylanase and DTPA treatments, the pulp was subjected to O_2 delignification in a WEVERK rotary digester.

The pulp samples were mixed with 0.1% $MgSO_4$, 0.2% EDTA, 2% NaOH and 10% consistency and placed in a vessel at following conditions: oxygen pressure 5.0 kg/cm^2, temperature 90 °C, reaction time 45 min and pH 12.0. In peroxide stage, 1.5% peroxide charge was given with 2% NaOH and 0.1% $MgSO_4$ at consistency 10%, temperature 90 °C for 60 min and pH 11.8. In final stage, 1.5% H_2O_2 was applied on o.d. pulp basis at conditions described in table 1.

Hand sheets preparation and analysis: The pulp obtained after ethanol-soda pulping was bleached with TCF sequences and was analyzed for bleaching losses, viscosity and copper number as per TAPPI Standard Test Methods. The pulp pads were prepared for brightness determination and hand sheets of 60 g/m^2 for physical strength properties were prepared according to TAPPI test methods. Tear index, tensile index, burst index and double fold number were also analyzed as per standard methods of TAPPI[18]. The pulp beating level of 35 °SR was used for determination of physical pulp and paper properties.

Analysis of bleach effluent: After bleaching process, the effluents were collected from all stages and were mixed in equal proportions for analysis.

Table 1
TCF bleaching *E. binate* pulp

Particulars	**Bleaching sequence**		
	QOPP	**X_1QOPP**	**X_2QOPP**
Xylanase treatment stage (X)			
Xylanase, IU/g	–	10	10
pH	–	6.0	5.0
Chelating stage (Q)			
DTPA applied, %	0.2	0.2	0.2
Final pH	4.6	4.6	4.6
Oxygen stage (O)			
O_2 pressure, kg/cm^2	5.0	5.0	5.0
$MgSO_4$ applied, % (o	0.1	0.1	0.1
EDTA applied, %	0.2	0.2	0.2
NaOH applied, %	2.0	2.0	2.0
Final pH	12.0	12.0	12.0
Peroxide stage (P_1)			
H_2O_2 applied, %	1.5	1.5	1.5
H_2O_2 consumed, %	1.47	1.45	1.46
DTPA applied, %	0.5	0.5	0.5
$MgSO_4$ applied, %	0.1	0.1	0.1
Final pH	11.8	11.8	11.7
Peroxide stage (P_2)			
H_2O_2 applied, %	1.5	1.5	1.5
H_2O_2 consumed, %	1.43	1.26	1.30
DTPA applied, %	0.5	0.5	0.5
$MgSO_4$ applied, %	0.1	0.1	0.1
Final pH	11.8	11.8	11.7
Total H_2O_2 applied, %	3.0	3.0	3.0
Total H_2O_2 consumed, %	2.90	2.71	2.76
Bleached pulp yield, %	43.52±0.39	42.94 ±0.48	42.65±0.55
Pulp brightness ,% (ISO)	82.1±0.3	84.6±0.4	83.5±0.3
Pulp viscosity, cps	9.0±0.011	9.3±0.023	9.5±0.014

± refers standard deviation
* Xylanase and all bleaching chemicals were used as oven dried pulp basis

Bleaching conditions:
1. **Consistency**- X1 and X2, O & P stages (10%) while Q (3%)
2. **Temperature-** X1 (50±2 °C), X2 (55 ±2 °C), Q (25±2 °C), O and P (90±2 °C)
3. **Time-** X1 and X2 (120 min), Q (30 min), O (45 min), P (60 min)

Table 2
Physical strength properties of TCF bleached pulp and effluent analysis

S.N.	Particulars	QOPP	X_1QOPP	X_2QOPP
1	Copper number	0.09±0.002	0.07±0.001	0.06±0.002
2	Beating level, °SR	35±1	35±1	35±1
3	Tear index, mNm^2/g	11.98±0.21	12.42±0.25	12.37±0.16
4	Burst index, $kPam^2/g$	7.70±0.19	7.45±0.17	7.38±0.15
5	Tensile index, Nm/g	82.94±1.72	81.15±2.40	80.34±1.84
6	Double fold, number	309±4	294±3	290±5
7	COD, mg/L	1348±28	1562±31	1475±27
8	Color, PTU	1862±30	2065±36	2087±31

± refers standard deviation

The COD was determined by using Thermorecator VR2010 through closed reflux titrimetric process[1]. The colour of the combined effluent was estimated as per standard protocol (Test method no. 204A)[10].

Statistical analysis: The experiments were carried out in triplicate and results were shown as mean value ± standard deviation of vales of three experiments.

Results and Discussion

Xylanase production: Previously isolated and identified fungal strains i.e. *Aspergillus flavus* ARC-12 and *Schizophyllum commune* ARC-11 were employed for xylanases production and 234 and 1147 IU/ml of xylanases activities were observed using millet stover and wheat bran as substrate respectively.

TCF bleaching: ISO brightness, viscosity and Kappa number were found to be 43.9±0.2 % (ISO), 28.2±0.14 cps and 16.1±0.3 respectively for unbleached pulp. Table 1 depicted the bleaching of ethanol soda pulp of *E. binata* for QOPP, X_1QOPP and X_2QOPP bleaching sequences. The brightness of QOPP, X_1QOPP and X_2QOPP bleached ethanol-soda pulp was 82.1, 84.6 and 83.5% respectively and their respective viscosity values were 9.0, 9.3 and 9.5 cps. The xylanases from *A. flavus*-ARC12 and *S. commune*-ARC-11 treatment resulted in improvement in pulp viscosity by 3.33 and 5.55 % respectively. The xylanases treatment causes the removal of comparatively short xylan chains that increase average degree of polymerization of polysaccharides in the pulp and increases the intrinsic viscosity of pulp. The slight improvement in pulp viscosity by xylanases treatment by was reported several researchers[5,17].

Brightness of *E. binata* ethanol-soda pulp increased by 2.5 and 1.4% (ISO) after X_1QOPP and X_2QOPP bleaching sequences respectively compared to QOPP bleaching sequence (Table 2). Xylanase treatment results in better penetration of bleaching chemicals into the pulp due to enhancement in accessibility of pulp, which in turn enhances the brightness of the pulp after subsequent oxygen and hydrogen peroxide bleaching stages[2,14]. Matos et al[16,17] studied the implementation of xylanases prior to oxygen delignification of kraft pulp (eucalyptus) and reported brightness increase by 1.1% (ISO) compared to control (without xylanases treatment) at pH 8.0. Bleached pulp yields were 42.94 and 42.65% after X_1QOPP and X_2QOPP bleaching sequences compared to QOPP (43.52%) bleaching sequence.

Table 2 showed comparisons of mechanical strength properties and combined effluent characteristics of QOPP, X_1QOPP and X_2QOPP bleaching sequences. Copper number of ethanol-soda pulp of *E. binata* decreased by 22.22 and 33.33% in X_1QOPP and X_2QOPP bleached pulps respectively compared to QOPP bleached pulp. Tear index increased by 3.67 and 3.25% after X_1QOPP and X_2QOPP bleaching sequences compared to QOPP bleaching sequence. Conversely, burst index remained almost constant. In the similar way, tensile index and double fold numbers were also decreased slightly after X_1QOPP and X_2QOPP bleaching sequences compared to QOPP bleaching sequence.

Xylanase pretreatment of ethanol-soda pulp of *E. binata* during X_1QOPP and X_2QOPP bleaching sequences increased the COD values by 15.87 and 9.42% respectively compared to QOPP bleaching sequence. The colour of combined bleach effluent generated during X_1QOPP and X_2QOPP bleaching sequences increased by 10.90 and 12.08% respectively compared to control. The increase in COD and colour of bleach effluent is due to the xylanase pre-treatment, since the hydrolytic action of xylanase leads to weakening of the carbohydrate bonds in the pulp and dissolution of lignin and hydrolyzed xylan into the media[15,19].

Gautam et al[5-7] studied the ECF bleaching of ethanol soda pulp of *E. binate* and reported 0.42 kg/t of AOX generation with DEDP bleaching sequence of ECF while the xylanase pretreatment reduced the AOX generation up to 23.8%. Therefore, TCF bleaching is considered environmentally friendly process, as it is not generating AOX during bleaching.

Moreover, the optical and physical strength properties of pulp bleached by TCF process were comparable to ECF bleaching.

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Conclusion

Pulp bleaching is one of the most significant stages in terms of pollutants generation. TCF bleaching is considered an alternate for replacement of chlorine compounds in bleaching process and minimizing the pollutants generation. The xylanase from *A. flavus* ARC-12 and *S. commune* ARC-11 was employed for pretreatment of pulp that improved the efficiency of non-chlorine oxidative chemicals for the bleaching of pulp. The ISO brightness of pulp was improved significantly while the physical strength properties were also maintained.

References

1. Chemical oxygen demand method No-508 B, In Standard methods for the examination of water and wastewater 532, APHA, AWWA, WPCF, Washington **(1985)**

2. Daneault C., Leduc C. and Valade J., The use of xylanases in kraft pulp bleaching: a review, *TAPPI Journal*, **77**, 125–131 **(1994)**

3. Dence C. and Reeve D.W., The technology of chemical pulp bleaching. in Pulp bleaching, principles and practice, ed., Cw D., TAPPI press, Atlanta, USA, 213–443 **(1996)**

4. Gangwar A.K., Prakash N.T. and Prakash R., Applicability of microbial xylanases in paper pulp: A review, *BioResource* **9(2)**, 3733–3754 **(2014)**

5. Gautam A., Kumar A. and Dutt D., Biobleaching of ethanol-soda pulp of *Eulaliopsis binata* by xylanases from *Aspergillus flavus* ARC-12 and *Schizophyllum commune* ARC-11, *Journal of Microbiology, Biotechnology and Food Science*, **10(4)**, 669-673 **(2021)**

6. Gautam A., Kumar A. and Dutt D., Effects of ethanol addition and biological pretreatment on soda pulping of *Eulaliopsis binata*, *Journal of Biomaterials and Nanobiotechnology*, **7(2)**, 78–90 **(2016)**

7. Gautam A., Kumar A., Bharti A.K. and Dutt D., Rice straw fermentation by *Schizophyllum commune* ARC-11 to produce high level of xylanase for its application in pre-bleaching, *Journal of Genetic Engineering and Biotechnology*, **16**, 693–701 **(2018)**

8. Ghasemi S., Behrooz R., Fatehi P. and Ni Y., Impact of acid washing and chelation on $Mg(OH)_2$-based hydrogen peroxide bleaching of mixed hardwoods CMP at a high consistency, *Bioresources*, **5**, 2258–2267 **(2010)**

9. Goto T. et al, Evaluating chelating agents and their effects on cellulosic pulps during P-stage bleaching, Part 1: analytical method development, *Cellulose*, **30**, 3887–3900 **(2023)**

10. Greenberg A., Clesceri L. and Eatonne A., Standard methods for the of water examination and wastewater, Washington, DC (USA), American Public Health Association (USA), American Water Works Association (USA), Water Environment Federation (USA) **(1992)**

11. Gullichsen J.F.C., Bleaching applications: oxygen bleaching. in Chemical pulping, Papermaking science and technology, ed., Gullichsen J.F.C., 617–665 **(1999)**

12. Immerzeel P. and Fiskari J., Synergism of enzymes in chemical pulp bleaching from an industrial point of view: A critical review, *Candian Journal of Chemical Engineering*, **101**, 312–321 **(2023)**

13. Kumar A., Biobleaching: An eco-friendly approach to reduce chemical consumption and pollutants generation, *Physical Sciences Reviews*, **6(4)**, 20190044 **(2021)**

14. Lin, X. et al, Bleach boosting effect of xylanase from *Bacillus halodurans* C-125 in ECF bleaching of wheat straw pulp, *Enzyme and Microbial Technology*, **52**, 91–98 **(2013)**

15. Mathur S., Kumar, S. and Rao, N., Application of commercial xylanases in bleaching-A review, *IPPTA Journal*, **13**, 13–24 **(2001)**

16. Matos J.M.S., Evtuguin D.V., de Sousa A.P.M. and Carvalho M.G.V.S., Xylanase treatment of eucalypt kraft pulps: effect of carryover, *Applied Microbiology and Biotechnology*, **108**, 210 **(2024)**

17. Matos J.M.S., Evtuguin D.V., Sousa A.P.M. and Carvalho M.G.V.S., Effect of xylanase-assisted treatment of oxygen-delignified eucalypt kraft pulp on ECF Bleaching, *Forests*, **14(2)**, 896 **(2023)**

18. TAPPI, TAPPI standard test methods **(2007)**

19. Vidal T., Torres A., Colom J. and Siles J., Xylanase bleaching of eucalyptus kraft pulp: an economical ECF process: The use of xylanase in the bleaching of eucalyptus kraft pulp, *APPITA Journal*, **50(2)**, 141-148 **(1997)**.

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