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A GREEN APPROACH FOR REDUCTION IN BLEACH CHEMICALS CONSUMPTION BY REMOVING HEXENURONIC ACID USING XYLANASE ENZYMES IN PULP BLEACHING PROCESS



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Abstract:

Research was carried out to study the trend of Hexenuronic acid (Hex-A) removal for reduction in bleach chemicals consumption and brightness improvement in final paper using xylanase enzyme. This article has shown well improved reduction of hazardous chemicals thus providing a cost-effective solution by following green manufacturing practices in papermaking process. In this manuscript, four different xylanases were studied and compared using eucalyptus (E. globulus) kraft pulps at equivalent and reduced Kappa factor by following elemental chlorine-free (ECF) bleaching sequence; $XD_oE_PD_1D_2$. Enzyme stage (X) reduced Hex-A up to 16.0%, in addition to 2.6 units of brightness gain and 16.9% reduction in Kappa number as compared to control. Whereas, last dioxide stage (D_2) itself was responsible for maximum reduction of Hex-A by 17.5% with 1.5 units of brightness gain over control in final bleached pulp. Due to reduction in the Hex-A components from bleached pulp, brightness reversion (post color number) was also reduced up to 35.0% with enzymatic treatment. Moreover, in comparison to control, maximum reduction of BOD and COD up to 23.8% and 27.1%, respectively were also seen in bleach effluents. Enzymatic treatment also leads to bleach chemicals savings of 14.8% and 20.0% for chlorine dioxide and sodium hydroxide respectively.

Keywords: Bleach effluent, ECF bleaching, Hexenuronic acid, Optical properties, Xylanases

1. Introduction:

During kraft pulping of wood (Eucalyptus and Acacia), Hexenuronic acid (Hex-A) is produced, which gives false information about the kappa number of the pulp usually at higher side. Hex-A also consumes, additional bleach chemicals during bleaching process. Therefore, its removal before bleaching will help in reducing the bleach chemical requirements, in addition to several other direct and indirect benefits. Reduction in bleach chemicals will result in the saving of energy equivalent to that required for producing these chemicals and also reduction in Greenhouse Gas (GHG) emissions related to the energy saved. This manuscript deals with the use of environment friendly xylanase enzymes to remove the Hex-A from hardwood kraft pulps.

Formation of hexenuronic acid during kraft cooking is influenced by the major variables viz; alkali charge, cooking temperature, time [1] and enhanced availability of sites for lignin-carbohydrate linkages. Hex-A is widely distributed among heparin, chondroitin, and lepidimoide (natural polysaccharides). About 75 to 90% of

4-O-methylglucuronic acid side groups (MeGlcA) linked to heteroxylan are removed during the alkaline pulping process and the residual MeGlcA almost completely (83 to 88%) converts to unsaturated hexenuronic acid (4-deoxy-l-threo-hex-4-enopyranosyluronic acid) via the intermediate product, 4-O-methyliduronic acid [2].

Hex-A plays an important role in pulp bleaching because of its undesired neutralization of electrophilic bleaching chemicals such as chlorine dioxide, ozone and peracids, resulting in increased consumption of bleaching chemicals. Hex-A contribute 33 to 67% of the Kappa number in hardwood kraft pulps, whereas 5 to 12% in softwood kraft pulps [1]. Hex-A also consumes potassium permanganate while determining the Kappa number, which results in inaccurate estimates of the quantity of lignin in pulps [3]. Enzymatic pre-treatment of pulp with xylanases (endo-1,4-xylanase activity, EC 3.2.1.8.) before chemical bleaching is an alternative and cost-effective process to reduce consumption of bleach chemicals and to minimize the generation of toxic chlorinated organic substances in effluents of bleach mills [4, 5, 6]. Xylanase is also responsible for reducing the pollution load in bleach effluent by reducing Chemical oxygen demand (COD) and Biochemical oxygen demand (BOD). A ratio of BOD to COD in bleach effluent can be improved with xylanase treatment, which indicates that the effluent becomes more degradable in secondary treatment [7].

To determine the precise profile of the Hex-A decrease at each step of elemental chlorine-free (ECF) bleaching and in terms of change in optical characteristics of pulps, the fate of the xylanase bleach enhancing impact on eucalyptus (E. globulus) was explored. Effluent from each bleaching stage was collected and characterized to examine their remedial efficacy in terms of BOD and COD. Reduction in bleach chemicals were also studied with four different xylanase enzymes. These enzymes were created with a focus on improving the bleach-boosting impact and altering xylanase activity while having little to no cellulase activity. In the current study, the research’s findings are reported and discussed.

Materials and Methods

Raw material

Table 1 describes the initial pulp properties of unbleached hardwood kraft pulps of eucalyptus (E. globulus) were used in this study to check the reduction trend of Hex-A and improvement of bleached pulp properties in ECF bleaching

Commercial Enzymes

Four different cellulase free xylanase enzymes of NOWALZYME series from the Nnoweta Performance Chemicals Pvt. Ltd. were used and studied. These enzymes were categorized based on their xylanase activities and coded as EnzyA, EnzyB, EnzyC and EnzyD respectively for this study. The activity of xylanase was estimated as per the Bailey method [8]. Activity of xylanase at pH 8.0 and temperature 55ºC for all enzymes were 19192+553 (EnzyA), 14123+350 (EnzyB), 46876+226 (EnzyC) and 25315+259 (EnzyD).

Enzyme treatment and bleaching sequence

Kneading mechanism was followed for proper dilutions and mixing of enzyme to be used in experiments. Control pulp was also prepared following similar process, wherein enzyme was replaced with water. Enzyme treatment was incorporated prior to bleaching process. After enzyme treatment, treated and untreated pulps were washed and carried out for ECF bleaching sequence ($D_oE_PD_1D_2$). Table 2 provides specific application conditions for the bleaching steps

Experiments were designed with equivalent and reduced chlorine dioxide (ClO_2) dose in D_o and D_1 stages. Kappa factor (amount of elemental chlorine calculated on the basis of lignin amount present in the pulp) was reduced by 15% in Do stage; i.e., from 0.22 (for higher brightness target level) to 0.187 (for same brightness target level). Polybag method was used to carry out the bleaching process. The enzyme dose of 0.05% was used for all sets except in control. The effectiveness of all four enzymes on eucalyptus pulps were compared for Hex-A removal, post color (PC) number and bleach chemicals reduction and corresponding improvement in optical pulp properties against control. Due to consistency and amount of effluent released are different at each stage, mixed effluent of all bleaching stages ($D_oE_PD_1D_2$) was collected in the ratio of 2.0:1.0:0.5:0.5 and checked for BOD and COD.

Analytical methods

HUT method was followed for measuring Hex-A (mmol/kg), the method is based on the selective conversion of Hex-A to formic acid and furan derivatives (2-furoic acid and 5-carboxy-2-furaldehyde), which were than quantified using UV-visible spectrometer [9]. In the UV spectra, two absorption maxima appeared at 245 and 285 nm, corresponding to 2-furoic acid and 5-carboxy-2-furaldehyde, respectively [3]. Table 3 shows the testing methods followed in the study.

Results and discussion

Table 2 summarizes the conditions of enzyme treatment of pulp after the cooking process. Table 4, presents the variations in Kappa number, brightness and Hex-A obtained with the different xylanases treatment compared with

Initial pulp properties	Mean ± S.D
Kappa number	18.5 ± 0.21
Brightness, %ISO	31.7 ± 0.28
Viscosity, cp	12.8 ± 0.10
Hex-A, mmol/kg	21.3 ± 0.31

Table 1: Properties of unbleached hardwood kraft pulps

Parameter	X stage	D_0 stage	E_P stage	D_1 stage	D_2 stage
Consistency, %	10	5	10	10	10
Treatment time, min.	90	45	120	180	180
Treatment Temp., °C	55	60	80	75	75
pH	8.0	1.8-2.0	10.5-11.0	3.0-4.0	3.0-4.0
Where; X- Enzyme treatment stage, E_P- Extraction stage, D_0, D_1 and D_2- Chlorine dioxide stages					

Table 2: Application conditions used during enzymatic bleaching of eucalyptus pulp

Parameters	Analytical Methods
Kappa number	TAPPI test method T 236 om-99
Brightness, %ISO	TAPPI test method T 452 om-02
CIE Whiteness	TAPPI test method T 560 pm-96
Post Color (PC) No.	TAPPI test method T 260 om-85
Pulp Viscosity, cp	TAPPI test method T 230 om-99
BOD, kgtp^{-1}	APHA 5210
COD, kgtp^{-1}	APHA 5220

Table 3: Analytical methods used in the study

control. All enzymes tested viz; EnzyA, EnzyB, EnzyC and EnzyD reduced the Kappa number by 9.3% (EnzyB) to 16.9% (EnzyC) after the X stage. Brightness gain was in the range of 1.4-2.6 units, with maximum for EnzyC (2.6 units) and minimum with EnzyB (1.4 units). The extent of Hex-A removal in the treated pulps was in the range of 9.3-16.0% as compared to control. Previous studies had reported 13.0%-15.0% reduction in Hex-A amount after the enzyme treatment [10]. In the present study, EnzyC showed relatively higher reduction in Hex-A content, when compared to other enzymes. By reducing Hex-A, xylanase also facilitates increase in the pulp brightness (Table 4) and reduce brightness reversion as well (as chromophores are removed by xylanases treatment) as shown in Table 5. EnzyC showed the best results in reduction of post color number of the final bleached pulps in comparison to other enzymes. Table 5 shows the post color number as 0.60, 0.44, 0.45, 0.39 and 0.41 units with control and enzymes (EnzyA, EnzyB, EnzyC and EnzyD) respectively at equivalent kappa factor (KF).

After bleaching process, Hex-A participates in discoloration reactions, which leads to increase in brightness reversion of bleached pulps. In eucalyptus hardwood pulp, brightness reversion or post color number is influenced by various factors and is directly related to acid groups and its chemical composition, such as Hex-A [1]. Xylanase treatment assist in removing hexenuronic acid or xylan derived chromophores with dissolved xylooligosaccharide fractions and hence contribute in improving brightness and brightness stability of final bleached pulp.

Parameter	Control	EnzyA	EnzyB	EnzyC	EnzyD
End Kappa No.	18.3 ± 0.10	16.4 ± 0.17	16.6 ± 0.20	15.2 ± 0.10	15.7 ± 0.20
Brightness, %ISO	31.9 ± 0.10	33.5 ± 0.17	33.3 ± 0.35	34.5 ± 0.20	33.9 ± 0.10
Hexenuronic acid, mmol/kg	20.90 ± 0.69	18.47 ± 0.46	18.95 ± 0.59	17.56 ± 0.37	18.08 ± 0.44
Viscosity, cp	12.1 ± 0.10	12.4 ± 0.10	12.2 ± 0.0	12.8 ± 0.20	12.6 ± 0.10

Table 4: Effect of EnzyA, EnzyB, EnzyC and EnzyD on pulp properties after Enzyme (X) stage

Further, xylanase treatment also facilitates improvement in brightness of pulp due to removal of residual lignin bleach chemicals [11]. After the bleaching process, whiteness gain at equivalent kappa factor was recoded as 2.21, 1.68, 2.87 and 2.23 units, while at reduced KF it was 0.94, 0.63, 1.65 and 1.14 units in bleached pulp with enzymes EnzyA, EnzyB, EnzyC and EnzyD, respectively as compared to control (Table 5). This increase in viscosity is probably due to the enzyme induced dissolution of low molecular weight xylan components (oligosaccharides). However, at the end of bleaching process, drop in viscosity in enzyme treated pulps was noted over the control (Table 5) due to the enhanced delignification of enzyme treated pulps during bleaching process.

Parameter	KF	Control	EnzyA	EnzyB	EnzyC	EnzyD
CIE Whiteness	0.22	76.11 ± 0.23	78.32 ± 0.28	77.79 ± 0.22	78.98 ± 0.52	78.34 ± 0.23
	0.187	---	77.05 ± 0.38	76.74 ± 0.34	77.76 ± 0.32	77.25 ± 0.26
Post Color (PC) No.	0.22	0.60 ± 0.03	0.43 ± 0.03	0.45 ± 0.04	0.39 ± 0.01	0.41 ± 0.04
	0.187	---	0.50 ± 0.04	0.53 ± 0.04	0.44 ± 0.03	0.46 ± 0.02
Viscosity, cp	0.22	10.6 ± 0.58	10.4 ± 0.31	10.5 ± 0.20	10.2 ± 0.10	10.3 ± 0.35
	0.187	---	10.5 ± 0.30	10.6 ± 0.15	10.4 ± 0.12	10.4 ± 0.15
Shrinkage, %	0.22	4.6 ± 0.20	5.9 ± 0.31	6.1 ± 0.45	5.4 ± 0.20	5.8 ± 0.25
	0.187	---	5.4 ± 0.26	5.6 ± 0.30	5.1 ± 0.15	5.2 ± 0.23
BOD, kgtp^{-1}	0.22	45.4 ± 0.87	38.1 ± 0.60	40.5 ± 0.70	34.6 ± 0.42	36.2 ± 0.35
	0.187	---	41.8 ± 0.45	43.5 ± 0.50	38.7 ± 0.45	39.9 ± 0.50
COD, kgtp^{-1}	0.22	75.6 ± 0.26	66.2 ± 0.59	68.2 ± 0.46	55.1 ± 0.40	65.4 ± 0.60
	0.187	---	70.1 ± 0.70	71.8 ± 0.64	63.2 ± 0.40	68.8 ± 0.38

Table 5: Effect of EnzyA, EnzyB, EnzyC and EnzyD on final pulp and bleach effluent properties

After X stage, both treated and untreated pulp samples were subjected to $D_OE_PD_1D_2$ bleaching sequence and characterized for Hex-A contents and brightness improvement at each stage viz; XD_O, XD_oE_P, $XD_OE_PD_1$ and $XD_OE_PD_1D_2$. Table 6 shows the effect of xylanases during bleaching process is often evaluated through analyzing of optical properties of the final bleached pulps at equivalent kappa factor. On the basis of residual chlorine dioxide amount in equivalent kappa factor experiments at stages (D_O, D_1 and D_2), we also examined optical properties and Hex-A reduction at reduced kappa factor (Table 6). The target for equivalent

Parameter	KF	Control	EnzyA	EnzyB	EnzyC	EnzyD
D_0 stage						
Brightness, %ISO	0.22	54.7 ± 0.55	56.8 ± 0.67	56.5 ± 0.65	57.8 ± 0.50	57.1 ± 0.40
	0.187	---	56.1 ± 0.47	56.0 ± 0.45	57.2 ± 0.25	56.4 ± 0.36
Hexenuronic acid, mmol/kg	0.22	13.10 ± 0.40	9.21 ± 0.71	9.37 ± 0.64	8.87 ± 0.33	9.14 ± 0.49
	0.187	---	9.81 ± 0.76	10.21 ± 0.45	9.26 ± 0.23	9.57 ± 0.56
E_P stage						
Brightness, %ISO	0.22	75.6 ± 0.61	77.4 ± 0.57	77.1 ± 0.40	77.9 ± 0.47	77.5 ± 0.60
	0.187	---	76.6 ± 0.57	76.5 ± 0.61	77.2 ± 0.46	76.9 ± 0.40
Hexenuronic acid, mmol/kg	0.22	4.10 ± 0.19	2.83 ± 0.17	3.01 ± 0.34	2.43 ± 0.30	2.66 ± 0.35
	0.187	---	3.23 ± 0.39	3.44 ± 0.46	2.81 ± 0.52	2.97 ± 0.42
D_1 stage						
Brightness, %ISO	0.22	85.3 ± 0.42	86.3 ± 0.47	86.1 ± 0.35	86.9 ± 0.36	86.4 ± 0.29
	0.187	---	85.7 ± 0.78	85.5 ± 0.61	86.1 ± 0.50	85.8 ± 0.60
Hexenuronic acid, mmol/kg	0.22	2.70 ± 0.24	2.20 ± 0.35	2.26 ± 0.33	2.00 ± 0.26	2.13 ± 0.12
	0.187	---	2.41 ± 0.46	2.53 ± 0.55	2.24 ± 0.37	2.36 ± 0.16
D_2 stage						
Brightness, %ISO	0.22	87.3 ± 0.62	88.2 ± 0.67	88.1 ± 0.82	88.8 ± 0.68	88.5 ± 0.57
	0.187	---	87.2 ± 0.62	87.1 ± 0.35	87.9 ± 0.36	87.6 ± 0.72
Hexenuronic acid, mmol/kg	0.22	2.40 ± 0.28	2.09 ± 0.09	2.19 ± 0.11	1.98 ± 0.31	2.06 ± 0.26
	0.187	---	2.31 ± 0.18	2.39 ± 0.39	2.10 ± 0.20	2.22 ± 0.33

Table 6: Effect of EnzyA, EnzyB, EnzyC and EnzyD on Hex-A and brightness at equivalent and reduced Kappa factor (KF)

and reducing KF was to get increased and same brightness levels respectively as compared to control. Although all enzymes have shown significant improvement in the brightness and whiteness of the bleached pulp, EnzyC was observed superior when compared to other enzymes. As shown in Table 6, after the XD_O stage, brightness gain of 2.1, 1.8, 3.1 and 2.4 units was obtained for enzyme EnzyA, EnzyB, EnzyC and EnzyD respectively as compared to control at equivalent kappa factor. The decreasing trend of brightness gain was observed as we carried out for further stages and it was 1.8, 1.5, 2.3 and 1.9 units after XD_OE_P; 1.0, 0.8, 1.6 and 1.1 units after $XD_OE_PD_1$ bleaching sequences. The final gain in brightness of 0.9, 0.8, 1.5 and 1.2 units were observed after ending of bleaching ($XD_OE_PD_1D_2$) process. At reduced kappa factor by 15% (Table 6), brightness gain was observed only for EnzyC and Enzy D and maximum of 0.6 unit was obtained with EnzyC with the reduction of 14.8 and 20.0% of chlorine dioxide (ClO_2) and sodium hydroxide (NaOH) chemicals (Table 7). It clearly shows better candidature of EnzyC among these four different xylanases.

The current observations highlights that the bleaching effect differs in each bleaching stage and varies significantly within the bleaching sequence with continued decrease in Hex-A content resulting in reduced bleach boosting effects towards the end of the sequence. Table 6 also presents the profile of Hex-A contents at equivalent and reduced KF across the bleaching stage with treatment of EnzyA, EnzyB, EnzyC and EnzyD as compared to control. Although all enzymes notably reduced the Hex-A contents, the extent of Hex-A removal with EnzyC treatment was better throughout the bleaching sequence (Figure 1).

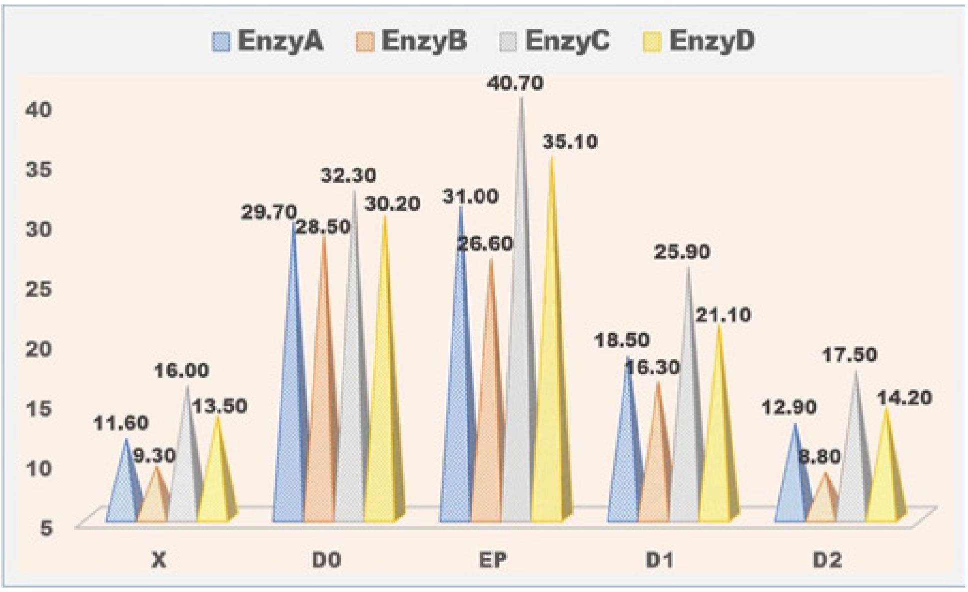

Figure 1: Trend of Hex-A reduction (%) with enzymes (EnzyA, EnzyB, EnzyC and EnzyD) at different stages of ECF bleaching sequences

Moreover, in order to study the effects of xylanase pretreatment, bleach effluents from different bleaching stages were also characterized for BOD and COD. Reduction in BOD and COD clearly indicated the remedial efficiency of treated pulp samples over control. Table 5 demonstrates that the reduction of BOD was in the range of 10.8-23.8% and 4.2-14.8% for kappa factor 0.22 and 0.187, respectively. Same trend was also observed for COD reduction and it was in range 9.8-27.1% and 5.0-16.4% for kappa factor 0.22 and 0.187 respectively. Table 5 indicates maximum reduction in BOD and COD and the ratio of BOD to COD was also increased with EnzyC, which leads to easier treatment of bleach effluent in secondary treatment at effluent treatment plant. Table 5 also specifies more yield loss (pulp shrinkage) in enzymatic process compared to control. It clearly shows that the enzymatic process lead to reduction of organic loads in the pulps as it reduces Hex-A components from the pulps in to the effluents. Sometimes it also destroys part of xylans or hemicellulose in the pulps, which may cause of yield loss or increases pulp shrinkage as compared to control.

As shown in Table 3, reduction in Kappa number is directly proportional to reduction in Hex-A vis-à-vis increase in the brightness of the pulp. As shown in Figure 2, during alkaline cooking process of hardwood pulps, bound Hex-A with the xylan entraps lignin components [11]. This bounded Hex-A cause hindrance in penetration of bleach chemicals onto the fibre surface, giving higher Kappa number and thus indicating higher lignin content than actual lignin amount in the pulps and resulted in higher addition vis-à-vis consumption of bleach chemicals during bleaching process. Xylanase effectively hydrolyzes the re-precipitated xylan and removes the bound Hex-A from the pulp fibre thus releasing the entrapped lignin content [12].

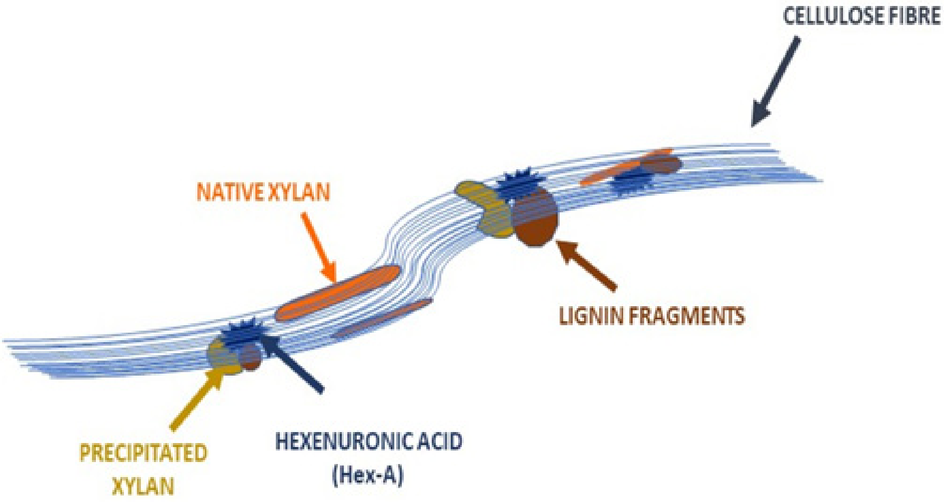

Figure 2: Schematic diagram of bounded Hexenuronic Acid (Hex-A) with Xylan

Therefore, xylanase treatment facilitates measurement of the true Kappa number vis-à-vis the lignin content leading to lesser use and better penetration of bleach chemicals. Amongst the four enzymes, EnzyC shows better reduction in bleach chemicals consumption (Table 7) during bleaching of eucalyptus hardwood pulps and obtained the possible reduction of 14.8 and 20.0%, respectively for ClO_2 and NaOH at reduced kappa factor with improvement in optical properties of the final pulp. This chemical savings leads to its promising behavior and better suitability at industrial level by using xylanase during ECF bleaching of eucalyptus hardwood kraft pulps.

Particulars	ClO_2, kgtp^{-1}	Savings, kgtp^{-1}	NaOH, kgtp^{-1}	Savings, kgtp^{-1}
Control	26.3	---	20.1	---
EnzyA	23.1	3.2	17.1	3.0
EnzyB	23.3	3.0	17.4	2.7
EnzyC	22.4	3.9	16.1	4.0
EnzyD	22.8	3.5	16.7	3.4

Table 7: Reduction in bleach chemicals consumption in ECF bleaching of eucalyptus hardwood pulps

CONCLUSIONS:

This study concludes the role of Hex-A in eucalyptus kraft pulps and its interference on other pulps properties. Results indicate the stage wise reduction behavior of hexenuronic acid during ECF bleaching sequence; $XD_OE_PD_1D_2$. The incorporation of enzyme treatment prior to conventional bleaching sequence and its efficacy in removal of Hex-A in addition to improvement in optical properties of pulps was studied. Amongst the enzymes tested, EnzyC was found to exhibit better performance with reference to removal of Hex-A contents across all the stages (X, XD_O, XD_OE_P, $XD_OE_PD_1$ and $XD_OE_PD_1D_2$) tested respectively over to control. Maximum reduction in kappa number and bleach chemicals consumption with improvement in optical properties was observed with EnzyC over other enzymes (EnzyA, EnzyB and EnzyD) throughout the enzymatic bleaching process. EnzyC is also considered as the best for reducing the pollution load in terms of BOD and COD in bleach effluent at all bleaching stages. These results also demonstrate the clear trend of Hex-A reduction at all bleaching stages for eucalyptus hardwood pulps. This novel approach can be used as bleach boosting process and can potentially be used to develop the environmentally friendly technologies and preparing paper industries towards green manufacturing practices.

ABBREVIATIONS

BOD	: Biochemical Oxygen Demand
COD	: Chemical Oxygen Demand
D_O	: Chlorine Dioxide Stage before Extraction Stage
D_1	: First Chlorine Dioxide Stage after Extraction Stage
D_2	: Second Chlorine Dioxide Stage after Extraction Stage
ECF	: Elemental Chlorine Free
E_P	: Alkali Extraction with Hydrogen Peroxide Stage
Hex-A	: Hexenuronic Acid
KF	: Kappa Factor
PC No.	: Post Color Number
X	: Enzymatic Treatment Stage

REFERENCES

[1] Gangwar AK, Tejo Prakash N, Prakash R (2014) Applicability of microbial xylanases in paper pulp bleaching: A Review. BioResources 9(2):3733-3754.

[2] Chauhan S, Choudhury B, Singh SN, Ghosh P (2006) Application of xylanase enzyme of Bacillus coagulants as a prebleaching agent on non-woody pulps. Process Biochem 41(1):226-231.

[3] Shatalov AA, Pereira H (2009) Impact of hexenuronic acids on xylanase-aided bio-bleaching of chemical pulps. Bioresour Technol 100(12):3069-3075.

[4] Bajpai P, Anand A, Sharma N, Mishra SP, Bajpai PK, Lachenal D (2006) Hexenuronic acids in different pulps and its removal effects on bleaching and pulp properties. BioResources 1(1):34-44.

[5] Gangwar AK, Tejo Prakash N, Prakash R (2015) Amenability of Acacia and Eucalyptus Hardwood Pulps to Elemental Chlorine-Free Bleaching: Application and Efficacy of Microbial Xylanase. BioResources 10(4):8405-8413.

[6] Gupta GK, Kapoor RK, Shukla P (2020) Advanced techniques for enzymatic and chemical bleaching for pulp and paper industries. Microbial Enzymes and Biotechniques. Springer Singapore, pp. 43-56.

[7] Gangwar AK, Tejo Prakash N, Prakash R (2016) An eco-friendly approach: Incorporating a xylanase stage at various places in ECF and chlorine-based bleaching of Eucalyptus pulp. BioResources 11(2):5381-5388.

[8] Bailey MJ, Biely P, Poutanen K (1992) Inter laboratory testing of methods for assay of xylanase activity. J Biotechnol 23(3):257-270.

[9] Tenkanen M, Gellerstedt G, Vuorinen T, Teleman A, Pertulla M, Li J, Buchert J (1999) Determination of hexenuronic acid in softwood kraft pulps by three different methods. J Pulp Pap Sci 25(9):306-311.

[10] Fillat U, Roncero MB, Bassa A, Sacon VM (2012) Effect of commercial xylanases applied at extreme conditions in a eucalyptus pulp mill. TAPPI J 11(10):53-59.

[11] Woolridge EM (2014) Mixed Enzyme Systems for Delignification of Lignocellulosic Biomass. Catalysts 4(1):1-35.

[12] Dukare A, Saxena S, Sharma K, Vigneshwaran, Kautkar S (2021) Microbial xylanase: An eco-friendly tool for bleaching of lignocellulosic paper pulp. Vigyan Varta An International E-Magazine for Science Enthusiasts 2(12):62-65.