An Eco-Friendly Approach: Incorporating a Xylanase Stage at Various Places in ECF and Chlorine-based Bleaching of Eucalyptus Pulp

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A potentially more environmentally compatible approach was evaluated, involving the use of an enzyme (X) stage optimally inserted into various bleaching sequences for *Eucalyptus* kraft pulps. The efficacy of the X stage was evaluated in terms of final brightness, CIE whiteness, post-color number (brightness reversion), effluent characteristics, *etc*. The results showed considerable benefits with an enzymatic pre-treatment bleaching sequence for improved final pulp brightness (1.6 units higher) and reduced adsorbable organic halogens (AOX) (32% lower), in addition to improved biological oxygen demand (BOD) to chemical oxygen demand (COD) ratio, when using 0.5 kg/t pulp dosage of xylanase; enzymatic post-treatment bleaching sequences were observed to boost final CIE whiteness up to 3.4 units and to reduce post color number by 48% at 0.5 kg/t pulp dosage of xylanase. In addition, approximately 32% reductions in AOX released, as well as appreciable improvement in BOD-to-COD ratio, were observed in the bleach effluents. An improved ratio of BOD-to-COD facilitates possible enhancement in the bio-degradability of discharge effluents in a secondary treatment stage. Nine different bleaching sequences were compared. Three sequences for each category (pre-treatment, intermediate, and post-treatment bleaching sequences) were performed to provide an overview of the influence of xylanase treatment on various pulp properties and environmental sum parameters of the ensuing effluents.

Keywords: Adsorbable organic halides (AOX); Bleaching sequences; Biological oxygen demand (BOD); Chemical oxygen demand (COD); Optical properties; Xylanase

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INTRODUCTION

Environmental concerns have influenced the pulp and paper industry to alter the traditional approaches to more environmentally compatible and sustainable processes. Pulp bleaching is one of those processes, in which elemental chlorine is effective at a very low capital cost but is a major environmental threat because of the release of toxic chlorinated compounds and other dioxins that are generated in mill effluents. Elemental chlorine-free (ECF) and total chlorine-free (TCF) bleaching process have been introduced to overcome these environmental issues to some extent. However, the optimum use of an enzyme stage incorporated into a bleach sequence is still a point of research interest for reducing the cost of bleaching (Gangwar *et al*. 2016).

Environmental issues in the pulp bleaching process comprise generation of absorbable organic halogens (AOX), including chlorinated dioxins in mill effluents.

Chlorine-based bleaching sequences in the pulp and paper industry have negative environmental impacts, such as the production of toxic and mutagenic chloroorganic compounds (Comlekcioglu *et al*. 2014). To improve the delignification of pulp with lower bleach chemical usage and costs, alternative methods to traditional bleaching process are being developed. One such method is enzymatic bleaching. Enzymes have been shown to bleach pulp effectively with reduced costs and minimized environmental impacts (Moore 1999; Hart and Rudie 2012). In recent years, there has been increasing interest in the use of xylanases, particularly in the bleaching process of the pulp and paper industry (Sandrim *et al*. 2005). Xylanase enhances the effects of bleaching agents rather than removing lignin directly (Gangwar *et al*. 2015).

In general, enzymes are being used before the bleaching sequences to improve the pulp brightness, while enzymes are also being used after the bleaching sequences to improve the whiteness of the pulp. In this study, we present observations on various bleaching sequences in which the enzyme treatment (X) stage has been incorporated into the bleaching sequence. We examine the impact of the X stage on AOX, BOD, and COD in the ensuing bleaching effluents, which affects the environmental impact of the bleaching process. This comparative approach is very beneficial to find out the best enzymatic bleaching sequences for hardwood kraft pulps in the industrial processing.

EXPERIMENTAL

Eucalyptus (*Eucalyptus globulus*) wood chips, obtained from Ballarpur Industries Ltd, India, were cooked (AA as Na_2O- 19.0%; sulphidity- 24.21%; cooking temperature- 160 ^oC; cooking time- 180 min.; bath ratio- 1:3) in stationary laboratory digester to produce an unbleached kraft pulp with a kappa number of approximately 20 to 21. Cellulase-free commercial xylanase (Optimase CX 72L) was procured from DuPont Genencor Sciences, USA, for this study. The activities of xylanase and cellulase were determined according to the procedure of Bailey *et al*. (1992) and Ghose (1987), respectively.

The optimum temperature, pH, and activity of Optimase CX 72L were 55 °C, 8.0, and 46,876 IU/mL, respectively. A control pulp was also treated in the same way as the xylanase-treated pulp in each and every experiment, where the enzyme was replaced with water. Three different types of bleaching sequences were examined with and without xylanase: (CD)(EP)D_1D_2, D_0(EP)D_1D_2, and $D_0(EP)_1D_1(EP)_2$ (where (CD) represents a chlorination stage with partial chlorine dioxide substitution, D_0 represents a chlorine dioxide delignification stage, (EP) indicates an extraction stage with caustic and hydrogen peroxide reinforcement, and D_1 and D_2 represent the first and second chlorine dioxide brightening stages after the (EP) stage). Experimental conditions for each bleaching sequences are shown in Table 1.

Different bleaching sequences were designed for this study, which included the direct action of chlorine at the first stage and substitution of a chorine stage with chlorine dioxide (ECF) in another sequence. In another design for a sequence, the last chlorine dioxide (D_2) stage was replaced with extraction stage to study the effect of alkali and peroxide on pulp properties. In total, nine different bleaching sequences were studied for each category (pre, post, and intermediate) to determine the best enzymatic beaching sequence in terms of brightness, CIE whiteness, and PC number of bleached pulps. These nine sequences are given in Table 2.

Table 1. Experimental Conditions and Dosages Used for Bleaching

Parameter	X stage	CD stage	D_0 stage	EP stage	D_1 stage	D_2 stage
Temperature, °C	55	Ambient	60	80	75	75
pH	8.0	1.8-2.0	1.8-2.0	10.5	2.8-3.0	2.8-3.0
Time, min.	90	45	45	120	180	180
Consistency, %	10	5	5	10	10	10
Dosage: *Kappa factor- 0.22; Active chlorine- based on kappa number; Chlorine dioxide- based on active chlorine in D_0 stage, 0.8% in D_1 and 0.3% in D_2 stage; Caustic- half of active chlorine dose; Hydrogen peroxide- 0.5%*						

Table 2. Incorporation of the Enzymatic Stage Before, Within, and After the Bleach Sequence

Sequence Type	Enzymatic Bleaching Sequence		
Pre-Treatment	$X(CD)(EP)D_1D_2$	$XD_0(EP)D_1D_2$	$XD_0(EP)_1D_1(EP)_2$
Post-Treatment	$(CD)(EP)D_1D_2X$	$D_0(EP)D_1D_2X$	$D_0(EP)_1D_1(EP)_2X$
Intermediate	$D_0X(EP)D_1D_2$	$D_0(EP)D_1XD_2$	$D_0(EP)_1XD_1(EP)_2$

After bleaching, the effluents from enzyme-treated and untreated bleaching sequences (*i.e.*, controls) were characterized to determine the contents of adsorbable organic halogens (AOX), chemical oxygen demand (COD), and biological oxygen demand (BOD). Inter-stage washing was followed for the collection of mixed bleach effluent for all stages (CD/D_0, EP, D_1, D_2) in the ratio 2:1:0.5:0.5 as per the consistency maintained in the particular bleaching stage.

Kappa number, a measure of residual lignin in the pulp, was determined using TAPPI Test Method T236 om-99 (1999). Handsheets were prepared with a smooth and reproducible surface for reflectance measurements in accordance with TAPPI Test Method T205 sp-02 (2002). The brightness (% ISO) and CIE whiteness of pulp were measured using a Konica Minolta (Model No.-CM 3600A) in accordance to TAPPI Test Methods T452 om-02 (2002) and T560 om-96 (1996), respectively. The brightness reversion of the pulp was estimated in terms of the post color (PC) number in accordance with TAPPI Test Method T260 om-85 (1985). COD (kg/t pulp) and BOD (kg/t pulp) were characterized using test methods APHA 5220:B (1999) and APHA:B 5210 (1999), respectively. AOX of the bleaching effluents was measured using a ThermoFisher Scientific instrument (Model No. ECS 1200) in accordance with ISO-9562 test procedure; the values obtained by the AOX determination included most chlorinated organics and are reported as organically bound chlorine (ppm).

RESULTS AND DISCUSSION

Nine bleaching sequences were studied, in which a xylanase (X) stage was included at various sequence positions, as shown in Table 2. The optical properties and pulp shrinkages of these various bleaching sequences of Table 2 are shown in Table 3. In xylanase pre-treatment bleaching sequences, the final brightness increased by 0.9, 1.3, and 1.6 units and the CIE whiteness increased by 1.1, 2.2, and 2.8 units when xylanase was applied at dosages of 0.01%, 0.03%, and 0.05% on pulp, respectively, for X(CD)(EP)D_1D_2 sequence. When the chlorination stage (*i.e.*, (CD)) was replaced with a chlorine dioxide delignification stage (D_0), the final brightness increased by 0.7, 1.1, and 1.5 units and the CIE whiteness increased by 1.4, 2.0, and 2.5 units when xylanase was applied at dosages of 0.01%, 0.03%, and 0.05% on pulp, respectively, *versus* the control. When the ECF sequence was changed by replacing the second chlorine dioxide (D_2) stage with a second extraction stage (*i.e.*, $(EP)_2$), the final brightness increased by 0.6, 1.0, and 1.3 units and the CIE whiteness increased by 0.9, 1.6, and 2.0 units when xylanase was applied at dosages of 0.01%, 0.03%, and 0.05% on pulp, respectively.

During the xylanase post-treatment bleaching sequences, in the case of (CD)(EP)D_1D_2X, the final brightness increased by 0.5, 1.0, and 1.2 units, whereas the CIE whiteness increased by 1.6, 2.7, and 3.4 units *versus* the control. When the ECF bleaching sequence, D_0(EP)D_1D_2X, was used, the final brightness and CIE whiteness values were augmented by 0.5, 0.8, and 1.1 units and 1.5, 2.5, and 3.2 units, respectively, as compared with the control (Table 3). When the other ECF sequence, $D_0(EP)_1D_1(EP)_2$X, was used, the final brightness went up by 0.4, 0.7, and 1.0 units, whereas the CIE whiteness went up by 1.3, 2.4, and 2.8 units when compared with the control. Post-treatment results (Table 3) showed that the enzymatic (X) stage after the bleaching sequence was more beneficial for improved final CIE whiteness of the bleached pulp than X stage pre-treatment in the bleach sequences.

Intermediate application of the X stage in the various bleaching sequences was examined to see if xylanase could further improve the optical properties of the eucalypt pulps *versus* the other X stage bleach sequence placements (Table 3). Kappa number and brightness (% ISO) of the pulp were determined prior to bleaching. In the X stage, enzyme dosages of 0.01%, 0.03%, and 0.05% on pulp were applied, as has been reported earlier. After the completion of the bleach sequence, the bleached pulps were analyzed for the brightness, CIE whiteness, and PC number. In Table 3, the final brightness and CIE whiteness gains versus the control were 0.4, 0.7, and 1.0 units and 0.7, 1.0, and 1.2 units, respectively, for the D_0X(EP)D_1D_2 bleaching sequence. When the X stage was incorporated within the ECF bleaching sequence (*i.e.*, D_0(EP)D_1XD_2), the final brightness and CIE whiteness gains over the control were 0.4, 0.7, and 1.1 units and 0.75, 1.1, and 1.4 units, respectively. While integrating the X stage between $(EP)_1$ and D_1 stages (*i.e.*, $D_0(EP)_1$X$D_1(EP)_2$), the final brightness increased by 0.9, 1.2, and 1.4 units, whereas the CIE whiteness increased by 1.3, 1.8, and 2.3 units as compared with the control. The increase in the brightness and whiteness level with xylanase treatment was presumably due to the influence of xylanases through variety of mechanisms such as increased accessibility of bleach chemicals on the pulp fibers, removal of hexenuronic acid components after the chlorine dioxide stages, removal of xylan derived chromophores, and hydrolysis of re-precipitated xylan on the cellulose fibre during the kraft pulping.

Table 3 also indicates that the maximum PC number reduction was 48% for the ECF bleaching sequence, where the X stage was used at the end (*i.e.*, post-treatment sequence D_0(EP)D_1D_2X) and had a xylanase dose of 0.05%. The minimum PC number reduction of 39% was obtained when a pre-treatment X stage sequence was employed (*i.e.*, X$D_0(EP)_1D_1(EP)_2$), where the D_2 stage was replaced with $(EP)_2$ stage while using a xylanase dose of 0.05%. A minimal pulp yield loss (*i.e.*, shrinkage) was observed across all the enzymatic bleaching sequences (pre, post, and intermediate) as compared with the respective controls.

Table 3. Effect of Bleaching Sequences with an Incorporated Xylanase Stage on Eucalyptus Pulp

Bleaching Sequences		Set	Final Brightness (% ISO)	CIE Whiteness	Post Color Number	Pulp Shrinkage (%)
Pre-treatment Bleaching	X(CD)(EP)D_1D_2	Control	87.5±0.67	77.2±0.38	0.58±0.07	5.7±0.49
		0.01%	88.4±0.42	78.3±0.45	0.48±0.06	5.9±0.46
		0.03%	88.8±0.49	79.3±0.31	0.40±0.05	6.1±0.30
		0.05%	89.1±0.36	79.9±0.36	0.34±0.04	6.3±0.45
	XD_0(EP)D_1D_2	Control	87.3±0.46	76.9±0.57	0.62±0.07	5.5±0.46
		0.01%	88.0±0.38	78.3±0.49	0.50±0.04	5.7±0.32
		0.03%	88.4±0.17	78.8±0.34	0.42±0.04	5.9±0.32
		0.05%	88.8±0.36	79.3±0.34	0.35±0.03	6.1±0.26
	X$D_0$$(EP)_1$$D_1$$(EP)_2$	Control	87.1±0.50	76.01±0.32	0.61±0.06	5.4±0.40
		0.01%	87.7±0.38	76.91±0.31	0.51±0.02	5.7±0.26
		0.03%	88.1±0.17	77.61±0.23	0.44±0.04	5.8±0.25
		0.05%	88.4±0.36	78.01±0.38	0.37±0.05	6.0±0.20
Post-treatment Bleaching	(CD)(EP)D_1D_2X	Control	87.3±0.55	76.5±0.49	0.51±0.05	6.0±0.36
		0.01%	87.8±0.50	78.2±0.42	0.40±0.05	6.0±0.35
		0.03%	88.3±0.25	79.3±0.39	0.34±0.06	6.3±0.46
		0.05%	88.5±0.36	79.9±0.41	0.29±0.02	6.6±0.46
	D_0(EP)D_1D_2X	Control	87.1±0.51	76.0±0.58	0.54±0.04	5.7±0.31
		0.01%	87.6±0.59	77.5±0.31	0.40±0.04	5.8±0.21
		0.03%	87.9±0.36	78.5±0.33	0.32±0.06	6.0±0.21
		0.05%	88.2±0.21	79.2±0.27	0.28±0.06	6.2±0.35
	$D_0$$(EP)_1$$D_1$$(EP)_2$X	Control	86.9±0.55	75.6±0.38	0.52±0.05	5.5±0.23
		0.01%	87.3±0.32	76.9±0.23	0.42±0.03	5.6±0.35
		0.03%	87.6±0.35	77.9±0.40	0.36±0.03	5.7±0.35
		0.05%	87.9±0.40	78.4±0.22	0.30±0.02	5.9±0.21
Intermediate Bleaching	D_0X(EP)D_1D_2	Control	85.8±0.38	74.0±0.27	0.58±0.07	5.4±0.42
		0.01%	86.2±0.20	74.6±0.34	0.46±0.01	5.5±0.26
		0.03%	86.5±0.44	75.0±0.53	0.38±0.03	5.7±0.46
		0.05%	86.8±0.40	75.1±0.41	0.32±0.06	5.8±0.15
	D_0(EP)D_1XD_2	Control	87.2±0.60	76.2±0.41	0.63±0.05	5.4±0.49
		0.01%	87.6±0.35	77.0±0.49	0.49±0.05	5.6±0.10
		0.03%	87.9±0.44	77.3±0.50	0.39±0.07	5.7±0.36
		0.05%	88.3±0.50	77.6±0.39	0.34±0.05	5.9±0.44
	$D_0$$(EP)_1XD_1$$(EP)_2$	Control	87.0±0.30	76.0±0.33	0.60±0.04	5.5±0.42
		0.01%	87.9±0.40	77.3±0.34	0.49±0.04	5.6±0.12
		0.03%	88.2±0.31	77.7±0.35	0.43±0.05	5.8±0.29
		0.05%	88.4±0.45	78.2±0.50	0.36±0.04	6.0±0.38

The pollution loads of enzyme-treated and control bleach effluents for various bleach sequences were determined in terms of COD, BOD, and AOX to evaluate the environmental impacts of enzymatic treatment. Composite effluent solutions from each bleach sequence were collected from all respective stages where the xylanase dosage was 0.05%. Similar composite effluent solutions from each bleach sequence were collected for the corresponding control sequences.

As shown in Table 4, bleaching sequences with enzyme pre-treatment (*i.e.*, X(CD)(EP)D_1D_2, XD_0(EP)D_1D_2, and X$D_0$$(EP)_1$$D_1$$(EP)_2$) had AOX reductions in the range of 29 to 32% *versus* the controls. In contrast, bleach sequences with X stage post-treatment (*i.e.*, CD)(EP)D_1D_2X, D_0(EP)D_1D_2X and $D_0$$(EP)_1$$D_1$$(EP)_2$X) had reductions in AOX (16% to 22% less) when compared with X stage pre-treatment bleaching sequences. When the X stage was incorporated within the bleaching sequences, the amount of AOX reduction when compared with the controls was on the order of 17% to 22%.

Incorporating an X stage between chlorine dioxide delignification and extraction stages (*i.e.*, $D_0X(EP)D_1D_2$) reduced the AOX by a relatively higher amount than the other intermediate ECF bleaching sequences that incorporated an X stage.

Table 4. Comparison of Bleach Effluent Properties Where Enzyme Treatment is Pre, Post, or Intermediate Sequence Placement at a 0.5 kg/t Pulp Enzyme Dosage

Enzyme treatment	Bleaching sequence	BOD (kg/t pulp)	COD (kg/t pulp)	AOX (kg/t pulp)	AOX (% reduction)
Pre-treatment bleaching sequences	Control	45.2±0.40	75.1±0.66	0.87±0.05	--
	$X(CD)(EP)D_1D_2$	36.1±0.47	74.1±0.46	0.59±0.05	32.2
	Control	41.3±0.51	69.9±0.55	0.78±0.04	---
	$XD_0(EP)D_1D_2$	34.7±0.51	66.7±0.47	0.54±0.03	30.8
	Control	39.3±0.49	67.3±0.42	0.72±0.03	---
	$XD_0(EP)_1D_1(EP)_2$	33.3±0.50	64.4±0.45	0.51±0.04	29.2
Post-treatment bleaching sequences	Control	51.5±0.51	77.9±0.55	0.96±0.05	---
	$(CD)(EP)D_1D_2X$	40.1±0.69	72.1±0.25	0.75±0.05	21.9
	Control	46.3±0.31	73.4±0.45	0.91±0.04	---
	$D_0(EP)D_1D_2X$	38.7±0.40	64.7±0.15	0.76±0.02	16.5
	Control	43.5±0.51	70.3±0.40	0.82±0.05	---
	$D_0(EP)_1D_1(EP)_2X$	36.3±0.40	61.4±0.35	0.69±0.05	15.9
Intermediate bleaching sequences	Control	33.5±0.46	55.6±0.30	0.72±0.05	---
	$D_0X(EP)D_1D_2$	32.6±0.50	51.2±0.46	0.56±0.03	22.2
	Control	38.1±0.53	57.7±0.38	0.75±0.07	---
	$D_0(EP)D_1XD_2$	37.4±0.35	56.7±0.59	0.62±0.04	17.3
	Control	32.1±0.61	53.2±0.36	0.69±0.04	---
	$D_0(EP)_1XD_1(EP)_2$	31.5±0.29	52.1±0.42	0.56±0.07	18.8

COD and BOD levels of the bleach effluents were examined for the various sequences with an X stage at various positions and compared with the controls. Overall, xylanase treatments were promising and showed reduction in COD and BOD levels in combined bleaching effluents for each sequence *versus* the respective controls. Other investigators have also reported that xylanase bleaching led to a reduction in AOX found in bleaching filtrates and to an appreciably higher BOD-to-COD ratio for the xylanase pre-bleaching filtrates (Senior and Hamilton 1991; Bajpai 2010; Thakur *et al*. 2012; Gangwar *et al*. 2014).

CONCLUSIONS

1. The study facilitates a better understanding of the influence of xylanase incorporation at various stages of bleaching sequence.
2. Enzyme pre-treatment bleaching sequences are more effective at boosting final pulp brightness (1.6 units for $X(CD)(EP)D_1D_2$ and 1.5 units for ECF bleaching sequence, $XD_0(EP)D_1D_2$).
3. In addition, a 32% reduction of AOX was observed for enzyme pre-treatment bleaching sequence *versus* enzyme post-treatment and intermediate bleaching sequences.

4. In contrast, enzyme post-treatment was observably a better option *versus* enzyme pre-treatment or intermediate bleaching when the desired target is to increase the CIE final whiteness of the pulp (*e.g.*, 3.4 units higher for (CD)(EP)D_1D_2X and 3.2 units higher for D_0(EP)D_1D_2X).
5. It is also concluded that out of the nine different sequences examined, the maximum reduction in PC number (48%) was obtained with enzyme post-treatment bleaching sequences and the minimum reduction (39%) was obtained with enzyme pre-treatment bleaching sequence.
6. Incorporation of an enzyme stage at any position in a bleaching sequence improved the BOD-to-COD ratio of the resulting effluents, which indicated better degradability of the bleach effluents in secondary treatment when compared to the control.
7. A marginal increase in pulp shrinkage was also observed across all the enzymatic bleaching sequences versus their respective controls.

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REFERENCES CITED

APHA 5210:B (1999). "Standard method for BOD test. B. 5-Day BOD test," American Public Health Association, Washington, DC.

APHA 5520:B (1999). "Standard method for COD test. B. Open reflux method," American Public Health Association, Washington, DC.

Bailey, M. J., Biely, P., and Poutanen, K. (1992). "Inter laboratory testing of methods for assay of xylanase activity," *J. Biotechnol*. 23(3), 257-270. DOI: 10.1016/0168-1656(92)90074-J

Bajpai, P. (2010). "Recent developments in cleaner production," in: *Environmentally Friendly Production of Pulp and Paper*, P. Bajpai (ed.), John Wiley & Sons, Inc., Hoboken, NJ, pp. 264-344. DOI: 10.1002/9780470649657.ch6

Comlekcioglu, U., Tutus, A., Cicekler, M., Gunes, M., and Aygan, A. (2014). "Application of recombinant xylanase from *Orpinomyces* sp. in elemental chlorine-free bleaching of kraft pulps," *Rom. Biotechnol. Lett*. 19(1), 8941-8950.

Gangwar, A. K., Tejo Prakash, N., and Prakash, R. (2014). "Applicability of microbial xylanases in paper pulp bleaching: A review," *BioResources* 9(2), 3733-3754. DOI: 10.15376/biores.9.2.3733-3754

Gangwar, A.K., Tejo Prakash, N., and 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. DOI: 10.15376/biores.10.4.8405-8413

Gangwar, A.K., Tejo Prakash, N., and Prakash, R. (2016). "Questioning conventional wisdom regarding the most suitable sequence of enzyme usage in pulp bleaching: Editorial," *BioResources* 11(1), 6-7. DOI: 10.15376/biores.11.1.6-7

Ghose, T. K. (1987). "Measurement of cellulase activities," *Pure Appl. Chem*. 59(2), 257-268. DOI: 10.1351/pac198759020257

Hart, P. W., and Rudie, A. W. (2012). *The Bleaching of Pulp*, 5th Ed., *TAPPI Press*, Atlanta.

ISO 9562 (2004). "Water quality - Determination of adsorbable organically bound halogens (AOX)," International Organization for Standardization, Geneva, Switzerland.

Moore, B. R. (1999). *The Effect of Using Hemicellulase and Oxidase Successively as Pre-Bleaching Agents for Hardwood Kraft Pulp*, Ph.D. dissertation, Western Michigan University, Kalamazoo, MI.

Sandrim, V. C., Rizzatti, A. C. S., Terenzi, H. F., Jorge, J. A., Milagres, A. M. F., and Polizeli, M. L. T. M. (2005). "Purification and biochemical characterization of two xylanases produced by *Aspergillus caespitosus* and their potential for kraft pulp bleaching," *Process Biochem*. 40(5), 1823-1828. DOI:10.1016/j.procbio.2004.06.061

Senior, D. J., and Hamilton, J. (1991). "Use of xylanases for the reduction of AOX in kraft pulp bleaching," in: *Proceedings of the Environmental Conference 1991 of the Technical Section*, Canadian Pulp and Paper Association, Montréal, PQ, pp. 63-67.

TAPPI T205 sp-02 (2002). "Forming handsheets for physical tests of pulp," TAPPI Press, Atlanta, GA.

TAPPI T236 om-99 (1999). "Kappa number of pulp," TAPPI Press, Atlanta, GA.

TAPPI T260 om-85 (1985). "Test to evaluate the ageing properties of bleached chemical pulps," TAPPI Press, Atlanta, GA.

TAPPI T452 om-02 (2002). "Brightness of pulp, paper, and paperboard (directional reflectance at 457 nm)," TAPPI Press, Atlanta, GA.

TAPPI T560 om-96 (1996). "CIE whiteness and tint of paper and paperboard (using d/0, diffuse illumination and normal viewing)," TAPPI Press, Atlanta, GA.

Thakur, V. V., Jain, R. K., and Mathur, R. M. (2012). "Studies on xylanase and laccase enzymatic prebleaching to reduce chlorine based-chemicals during CEH and ECF bleaching," *BioResources* 7(2), 2220-2235. DOI: 10.15376/biores.7.2.2220-2235

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