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Running Title: Xylanase Biobleaching Effects

Effect of Xylanase Pretreatment Procedures for Nonchlorine Bleaching

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The pretreatment of softwood kraft pulps with endo-xylanase expressed from Streptomyces lividan[pIAF18] was shown to have beneficial effects for bleaching with selective oxygen-based bleaching reagents. Optimal biobleaching results were achieved with ozone which exhibited enhanced bleaching selectivity and brightness gains. Enzyme pretreatment also improved brightness and delignification of peracetic acid bleached pulps. The potential role of xylanase in future commericial applications is discussed.

Keywords: *Xylanase: pulp pretreatment; biobleaching; bleaching; ozone; peracetic acid; performic acid; caro's acid.*

Introduction

Advances in the application of biotechnology to the pulp and paper industry have developed significantly over the past few years.[1] To date, one of the most successful applications has been the use of *endo*-(1,4)-β-D-xylanase as a pretreatment for chlorine and chlorine dioxide bleaching sequences.[2] Based on numerous laboratory[3] and mill studies,[4] it is now becoming well established that pretreatment of kraft pulps with xylanase significantly enhances the bleachability of these pulps for chlorine-based bleaching sequences such as (CD)EDED or DEDED (C:chlorine; D:chlorine dioxide; E: caustic extraction; see Table I for a complete listing of bleaching abbreviations).[5] The most significant bleaching benefits found from xylanase pretreatment regimes include higher brightness ceilings, reductions in the amounts of bleaching chemicals needed to achieve high brightness, and reduced amounts of organo-chlorine compounds in bleach plant effluents. In addition, all of these benefits are incurred without significant capital costs or changes in the bleaching process.

The pretreatment of kraft pulps with xylanase followed by high chlorine dioxide substitution in the early stages of pulp bleaching provides a facile method of addressing current environmental concerns. Nonetheless, it remains uncertain if these practices will be acceptable in the future as more stringent environmental regulations are drafted. In response to these environmental concerns, a variety of nonchlorine bleaching procedures are being investigated. In this study, we have examined the effect of xylanase pretreatment on bleaching efficiency for a variety of nonchlorine-based bleaching reagents, including performic acid, peracetic acid, peroxymonosulfate acid, and ozone. All of these reagents are under active investigation as possible bleaching chemicals for nonchlorine bleaching of kraft pulps and could potentially benefit from a xylanase pretreatment procedure.

Materials and methods

Materials

Hydrogen peroxide (30% by wt.), formic acid, peracetic acid (32% by wt), and the triple salt of $KOSO_4 \bullet KHSO_4 \bullet K_2SO_4$ (trade name, Oxone™) were commercially purchased and used without further purification. Commercial xylanase, produced by batch fermentation of *Streptomyces lividans*[pIAF18], was kindly provided by ICI Biological Products. Xylanase activity was determined as reducing sugars released from the water-soluble portion of oat-spelt xylan (1%) following Miller's procedure.[6] Bleaching studies employed industrial, never-dried, softwood kraft pulp (Kappa number 23.0). Prior to bleaching and/or enzyme treatment, the extractives from the pulp were removed following TAPPI method T264. The extractives were removed from the pulp to simplify subsequent effluent characterization studies which are ongoing.

Methods

Xylanase Treatment of Pulps

All xylanase treatments were carried out on 88 gr batches of extractives-free, softwood kraft pulp (Kappa number 22.3) at 6% consistency. Prior to addition of the enzyme, the pulp slurry pH was adjusted to ca 5.5-6.0 with 1 M H_2SO_4. Enzyme was added at a dose of 7,480 IU (2.75 ml, xylanase activity/ml: 2,713 IU/ml) to the pulp slurry and then sealed in a polyethylene bag. This mixture was immersed in a constant temperature bath (50°C) for 2 h. During the xylanase pretreatment reaction, the pulp sample was kneaded every 15 min to ensure uniform distribution of the enzyme. The pulp was then washed with water and suction dried to approximately 15% consistency. The xylanase treated pulps were immediately bleached or stored at 4°C for no longer than one week.

Physical and Chemical Characterization of Pulps

The Kappa number was determined by $KMnO_4$ titration of the pulp following standard Tappi method T-236. Brightness values for the pulp samples were measured by preparing optical handsheets (TAPPI standard method T-218) and measuring the percent light reflectance at 457 nm following Tappi method T-452 and/or T-534. The viscosity of the pulp was measured employing a capillary viscometer as described in Tappi method T-230.

Bleaching Procedures

Bleaching Conditions for Oxone, Performic Acid, and Peracetic Acid

Prior to bleaching with either performic acid or peracetic acid, the pulps were treated with acetic acid following Sundquist's procedure[7] for removing trace metals. The pulp samples bleached with oxone were first treated with Na_2SO_3 and DTPA (0.4% charge) to remove trace metals.

Pulp samples (25 g, OD:oven-dry weight) were placed in a polyethylene bag containing water and a stabilizing reagent (see Table II for further detail). After thoroughly mixing the pulp by kneading, the pulp slurry was preheated to 70° C. The bleaching reagent was then rapidly added to the pulp, and the sample bag was sealed, kneaded, and then immersed in a constant temperature water bath. During the bleaching reaction, the pulp sample was kneaded every 15 min to ensure a uniform distribution of the bleaching reagent throughout the sample. Upon completion of the bleaching reaction (see Table I for further experimental detail), the pulp was removed from the water bath, and its contents were washed with distilled water (4 x 500 ml). The pulp was then suction dried and analyzed for lignin content (Kappa #), brightness, and viscosity.

A portion of the bleached pulp (12 g, OD) was then diluted with 0.12 N NaOH solution to 1% consistency, sealed in a polyethylene bag, and warmed to 70° C for one hour. The extracted pulp was then filtered and washed with distilled water (4 x 500 ml).

Ozone Bleaching Conditions

Ozone bleaching studies were carried out at 30-35% consistency after prewashing the pulp with a 1 M H_2SO_4 solution. Pulp samples (50 gr, oven dry weight) were then treated with ozone by means of a modified roto-evaporator designed to deliver ozone directly onto the pulp. A Welsbach ozone generator was used to provide ozone/oxygen gas for each bleaching experiment. Ozone gas concentrations were monitored by measuring the concentration of ozone before and after contact with the pulp. Ozone concentrations were determined by bubbling the gas through an aqueous 10% KI solution and then titrating the solution with 0.10 N sodium thiosulfate solution. Detailed descriptions of the ozone bleaching experiments are summarized in Table III.

Results and discussion

The effects of xylanase pretreatment on oxygen-based bleaching reagents were studied with a southern softwood kraft pulp. The pulp was pretreated with a xylanase, cellulase-negative solution following well-established literature procedures.[2] As summarized in Table III, treatment of the brownstock increased the viscosity and brightness of the pulp by 6% and 12%, respectively. Enzyme treatment also removed minor amounts of lignin decreasing the kappa value of the pulp by 3%. The improvements in the viscosity presumably occur due to an enrichment in high molecular weight polysaccharides, which occurs when xylan is selectively removed.[8] The gain in brightness and the loss of lignin after enzyme treatment have been previously noted in the literature and are attributed to the loss of lignin-carbohydrate complexes.[9]

Our initial bleaching studies focused on evaluating the biobleaching effect on oxone,[10] peracetic acid,[11] and performic acid.[7] Following well-established literature bleaching procedures for these reagents, the control and xylanase-treated pulps were bleached and then extracted with 0.12 M NaOH solution. After caustic extraction the kappa number, viscosity, and brightness values were determined following standard procedures, and these results are summarized in Table IV. The results of these studies indicated that the enzyme pretreatment procedure had no beneficial effects for performic acid bleaching. The data acquired for the oxone bleaching reactions were of more interest, since these studies, summarized in Table IV, indicated that the biobleaching process enhanced overall delignification of the pulp by 7% over the nonenzyme-treated pulp sample. Unfortunately, the gains observed in delignification were not extended to either changes in the brightness or viscosity values of the bleached and extracted pulps.

The results of peracetic acid bleaching studies were of greater interest since the xylanase pretreatment procedure provided a significant biobleaching effect in regards to delignification and brightness gains. As summarized in Table IV, depending upon the amount of peracetic acid employed during bleaching, the xylanase pretreated pulps exhibited a 0-7% additional loss in lignin while maintaining viscosities comparable to the control pulps. Furthermore, the enzyme-treated pulps exhibited higher brightness values after bleaching and caustic extraction. The bioboosting effect of xylanase was most efficient with a low charge of lignin which could be attributed to the extent of lignin oxidation during bleaching. Since potential future bleaching applications of peracetic acid would employ relatively low charges of this reagent, our results clearly suggest that the xylanase pretreatment could enhance the bleaching efficiency of peracetic acid.

The final bleaching reagent examined in this preliminary study was ozone. Due to current bleaching interests in this reagent, we elected to monitor the biobleaching effect after ozone treatment and following caustic extraction. Prior to ozone bleaching, the pulp samples were acidified to a pH of 5 or 2. At the latter pH, two series of bleaching experiments were performed employing a 0.6% charge and a 1.2% charge of ozone. The pH 5 pulp sample was bleached with a 0.6% charge of ozone. Although it is well known that the optimal pH for ozone bleaching[12] is between 2-3, we desired to determine if the xylanase pretreatment procedure could alter the preferred pH range for ozone bleaching.

The control and xylanase-treated pulps were bleached at high consistency in a modified roto-evaporator especially designed to maximize ozone contact with the pulp. The results of the ozone bleaching studies are summarized in Table V, and these data demonstrate that xylanase pretreatment of the softwood kraft pulp impacts beneficially on delignification, brightness, and viscosity changes. The biobleaching procedure enhanced the overall delignification effect of the 0.6% charge of ozone by 17% while reducing the loss in viscosity by 11%. Comparable results were also achieved when employing a 1.2% charge of ozone. The biobleaching procedure also afforded higher brightness values for the ozone bleached and extracted pulps. These results indicate that not only does the xylanase pretreatment procedure improve delignification by ozone, but it also enhances the physical properties of the bleached and extracted pulps. The viscosity results are of significant value given the overall concern that ozone bleached pulps exhibit lower strength properties than chlorine and/or chlorine dioxide bleached pulps.[13] Examination of the kappa numbers after bleaching and caustic extraction for the 0.6 and 1.2% charge of ozone (pH 2.5) reveals that the bioboosting effect of xylanase is not diminished with the larger charge of ozone. These results differ from the bleaching data obtained with peracetic acid, where the biobleaching effect was shown to be sensitive to the amount of reagent employed. A comparison of the ozone bleaching data acquired at a pH of 2.5 and 5.0 with a 0.6% charge of ozone suggests that the optimal pH range for ozone bleaching of xylanase-treated pulps remains at a pH level of 2-3. Nonetheless, even at this unfavorable pH of 5, the enzyme-treated pulp responds more favorably to ozone.

The results of our xylanase/ozone studies extend the preliminary data that Allison and Clark recently presented.[14] Their research focused on applying a xylanolytic/cellulase mixture to pulps prior to bleaching with either ZED or (DC)ED. The results of these studies indicated that the enzyme pretreatment procedure enhanced delignification and brightness levels throughout the bleaching sequence. The changes in viscosity were not as favorable presumably due to the presence of cellulase in the enzyme mixture. Our results indicate that by employing a cellulase-negative, xylanase mixture all the biobleaching effects reported by Allison and Clark[14] can be achieved without additional loss of viscosity caused by the enzyme procedure.

CONCLUSIONS

In summary, these results demonstrate that xylanase pretreatment of softwood kraft pulps can enhance the bleaching efficiency of several nonchlorine-based bleaching reagents including ozone. These studies and a recent report by Eriksson[15] demonstrating that xylanase can activate kraft pulp toward bleaching with hydrogen peroxide suggest that future applications of this enzyme will not be focused solely on chlorine and chlorine dioxide bleaching. It is anticiapted that as new oxygen-based bleaching technology is transferred from the laboratory to the paper mill the application of a xylanase pretreatment procedure will be extensively employed to reduce the cost of these bleaching reagents and to enhance the physical performance of the fully-bleached pulps.

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Table I. List of bleaching abbreviations.

Abbreviation	Bleaching Reagent
C	Chlorine
DMD	Dimethyldioxirane
D	Chlorine Dioxide
E	Caustic Extraction
O	Oxygen Delignification
P	Hydrogen Peroxide
PFA	Performic Acid
PPA	Peracetic Acid
Z	Ozone
X	Pretreatment with Xylanase

Table II. Bleaching conditions employed for Oxone, performic, and peracetic acid treatments of softwood kraft pulp.

Bleaching Reagent:	Oxone[a]	Performic Acid[b]		Peracetic Acid[c]		
Charge (gr/gr OD)	8%	3.9%	7.8%	2.0%	3.95	4.4%
Stabilizer Employed (gr/gr OD pulp %)	--	2.5% Citric Acid		2.5% Citric Acid		
Bleaching Temperature (C)	70°	80°	80°	80°	80°	80°
Bleaching Time (hr)	1	3	3	3	3	3

[a] Hammann's bleaching procedure[10]; [b]performic acid was generated in-situ following the procedure described by Poppius et al.[7] employing a 50% formic acid bleaching solution; [c]employed commercial 32% by weight solution of peracetic acid in dilute acetic acid.

Table III. Bleaching conditions employed for ozone treatments of softwood kraft pulp.

Bleaching Reaction	Charge of Ozone Applied to the Pulp	Initial pH of Pulp
I	1.2%	2.5
II	0.6%	2.5
III	0.6%	5.0

Table IV. Results from bleaching softwood kraft pulp with oxone, performic, and peracetic acid.

Bleaching Treatment	Kappa	Tappi Brightness	Viscosity/cP
Brownstock	22.3	24.0	14.2
Xylanase-treated Brownstock	21.7	26.8	15.1
X(PFA-3.9% charge)E	14.3	--	--
(PFA-3.9% charge)E	14.0	--	--
X(PFA-7.8% charge)E	8.6	35.1	5.3
(PFA-7.8% charge)E	8.8	34.0	5.6
X(PAA-2.0% charge)E	7.2	51.4	7.6
(PAA-2.0% charge)E	8.8	49.4	7.8
X(PAA-3.9% charge)E	5.4	51.4	7.4
(PAA-3.9% charge)E	6.3	48.7	7.9
X(PAA-4.4% charge)E	5.8	51.2	8.9
(PAA-4.4% charge)E	6.2	49.8	8.5
X(Oxone-8%)E	14.1	29.0	9.5
(Oxone-8%)E	15.1	29.2	9.0

Table V. Characterization of xylanase biobleaching effects on ozone bleaching of kraft softwood pulp.

Pulp	Pulp Properties		
	Kappa	Viscosity/cP	Tappi Brightness
Brownstock	22.3	14.2	24
Xylanase treated brownstock	21.7	15.1	27
Bleached with 1.2% ozone/pH:2.5			
-Xylanase-treated	9.9	--	--
-Control	11.6	--	--
followed by caustic extraction[a]			
-Xylanase-treated	6.3	10.3	48
-Control	7.6	9.4	44
Bleached with 0.6% ozone/pH:2.5			
-Xylanase-treated	15.2	--	--
-Control	15.8	--	--
followed by caustic extraction[a]			
-Xylanase-treated	11.1	12.1	38
-control	12.5	11.4	36
Bleached with 0.6% ozone/pH:5.0			
-Xylanase-treated	15.0	--	--
-Control	16.1	--	--
followed by caustic extraction[a]			
-Xylanase-treated	12.2	12.2	36
-Control	13.9	11.7	32

[a]extracted with a 0.12 M NaOH solution for 1 h at 70°C.

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