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DOI: 10.2478/s11696-007-0064-x

Ethanol/Water Pulp Enzymatic Pretreatment: Chemical and FTIR-PCA Analyses

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Received 4 January 2007; Revised 1 June 2007; Accepted 6 June 2007

Pulps obtained from ethanol/water cooking of sugarcane bagasse were treated at different times using xylanase enzyme obtained from *Thermomyces lanuginosus* IOC-4145 or commercially (Cartazyme HS, Sandoz Products Ltd.). The enzyme dosage was 18 IU per g of dry pulp and the time varied from 4 h to 12 h. When xylanase from *T. lanuginosus* was used, the kappa number and viscosity improved independently of the processing time used (4 h, 8 h, and 12 h). After chemical evaluation, the obtained pulps were classified using Fourier Transformed Infra-Red Spectroscopy and Principal Component Analysis. The results showed that the first three principal components explained more than 90 % of the total variance of the pulp spectra.

Keywords: ethanol/water pulping, sugarcane bagasse, xylanase bleaching, Fourier transform infrared spectra, principal component analysis

INTRODUCTION

In the last decades, the environmental legislation and the market pressure have forced the pulp and paper industry to reduce and eliminate chlorinated organic compounds in bleaching effluents [1]. Some problems related to the use of chlorine containing bleaching agents are associated with corrosion of storage tanks, formation of toxic and/or mutagenic organic chlorinated compounds, and the increase of chloride and absorbable organic halogen (AOX) levels in bleaching plant effluents [2]. An alternative method applicable in bleaching processes is the enzymatic treatment [3, 4].

Enzymes have been shown to be a biotechnological alternative that can be applied together with conventional sequences in chemical or alternative bleaching [5—7]. The use of enzymes as auxiliaries in the bleaching process passed from laboratory scale at the end of the 80's to industrial applications at present with the advantage of not requesting important modifications in the traditional process [8—10]. Among the enzymes of interest, hemicellulolytic xylanases (xylanases free of cellulase) have been found commercially feasible for pulp bleaching in paper industry [1].

The use of xylanases for pulp treatment offers various benefits like environmental protection and pulp quality improvement. Thus, pretreatment with xylanase facilitates chemical extraction of lignin from pulp reducing consumption of bleaching chemicals and discharge of toxic compounds into the environment [11]. Xylanases catalyze the hydrolysis of xylose—xylose bonds within the xylan chain and solubilize only a fraction of the total xylan [12]. Studies using xylanases in the pretreatment of nonwoody fibers, such as bamboo and bagasse [11—15], have been carried out.

The present study describes the application of enzymatic pretratment of sugarcane bagasse pulps using the xylanase enzyme obtained from *Thermomyces lanuginosus* IOC-4145 fungus and different treatment times. The properties of unbleached pulps were compared to those of pulps obtained after enzymatic pretreatment using commercial enzyme (Cartazyme HS, Sandoz Products Ltd.), and also with pulps obtained after the chemical bleaching processes. In order to classify the pulps submited to different treat-

ments, Fourier transform infra red (FTIR) spectroscopy and principal component analysis (PCA) were used.

EXPERIMENTAL

The pulping was carried out using sugarcane bagasse with ethanol/water mixture ($\varphi_r = 1 : 1$) in a closed and pressurized vessel at 185 °C for 3 h (organosolv process) [15]. The obtained pulp was filtered and dried for the yield determination, and also the kappa number and viscosity were evaluated following the standard TAPPI methods [16, 17].

For enzymatic bleaching (X), pulps of sugarcane bagasse were suspended under agitation in 50 cm^3 distilled water (3 % consistence) at 30 °C for 10 min. Cartazyme HS from the Sandoz and xylanase enzyme obtained from *T. lanuginosus* IOC-4145 fungus [18] were added at 18 IU per g of dry pulp, in separate experiments. The samples were maintained in a shaker at 30 °C for 4—12 h, followed by filtration and washing with 300 cm^3 of distilled water at 30 °C for enzyme removal.

Chemical bleaching by alkaline extraction processes was accomplished using the pulps obtained after different treatment times with xylanase (XE) and pulps without any previous enzymatic treatment (E). For this purpose, 3 g of dry pulp were treated with 150 cm^3 1 mol m^{-3} sodium hydroxide (NaOH) at 65 °C for 1 h under magnetic stirring, filtrated, and washed with another 150 cm^3 of distilled water at 65 °C until the suspension reached the pH 6.0.

Chemical bleaching by sodium chlorite ($NaClO_2$) was also accomplished using the pulps of sugarcane bagasse obtained by ethanol/water treatment without further enzymatic action. These pulps were suspended in 150 cm^3 of water (2 % consistence) and heated to 70 °C. Sodium chlorite (3.9 cm^3 of 40 % aqueous solution) and glacial acetic acid (0.6 cm^3) were added and further heated at 70 °C for 5 min. The obtained bleached pulp was exhaustively washed with water.

All the obtained pulp samples were oven-dried at 60 °C for 15 min and analyzed with respect to the kappa number and viscosity by standard methods [16, 17]. All experiments were performed in triplicate. The standard errors or deviations were always found to be lower than 2.7 %

Pulp Characterization

One gram of dried samples of unbleached, pretreated with enzyme (X), or bleached (XE, E, H) pulps was treated with 10 cm^3 72 % H_2SO_4 with stirring at 45 °C for 7 min. The reaction was interrupted by adding 50 cm^3 of distilled water and the mixture (approximately 275 cm^3) was transferred to a 500 cm^3 Erlenmeyer flask. The Erlenmeyer flask was autoclaved for 30 min at 1.05 bar and 120 °C to complete the hydrolysis of oligomers [19]. Afterwards, the mixture was filtered and the volume of the hydrolysate filled up to 500 cm^3 with distilled water. The sample (40 cm^3) of the hydrolysate was diluted to 50 cm^3 and the pH adjusted to 2.0 using 2 mol m^{-3} sodium hydroxide solution.

By passing the solution through a Sep-Pak C_{18} cartridge, aromatic compounds were filtered off and the hydrolysate was analyzed in an Aminex HPX-87H column Bio-Rad (300 × 7.8 mm) at 45 °C using a Shimadzu chromatograph and refraction index detector. The mobile phase was 0.005 mol m^{-3} H_2SO_4 solution with the flow rate of 0.6 cm^3 min^{-1}. Sugar content, reported as xylan and glucan concentrations, was determined using the calibration curves of the respective pure saccharides.

FTIR spectra of the pulp samples were taken directly using the high-attenuated total reflectance technique (HATR). The spectra were recorded (16 scans with 4 cm^{-1} resolution) on a Spectrum One Perkin—Elmer Spectrometer. After a polygonal baseline correction [20], the spectra were normalized by absorption at 901 cm^{-1}, which corresponds to the anomeric carbon atom of the O—C—O group in polysaccharides and suffers no influence of other groups [21]. The spectra were converted to text files using the OMNIC (Nicolet) software. The normalized absorbances in the range of 670—3800 cm^{-1} (783 data points per pulp spectrum) were submitted to principal component analysis (PCA) using the BIOTEC and FAEN programs [22].

RESULTS AND DISCUSSION

Table 1 shows the yield, viscosity, kappa number, and chemical composition of unbleached and chemically bleached (NaOH (E) and $NaClO_2$ (H)) pulps. Both, alkaline and sodium chlorite, extractions originated yields of approximately 75 %. The highest viscosity values (11.9 cP) were observed for the pulps submitted to alkaline extraction.

Table 2 presents the properties of ethanol/water pulps of sugarcane bagasse pretreated with xylanase from two sources, namely endoxylanase from *T. lanuginosus* IOC-4145 and Cartazyme HS (Sandoz Products Ltd.). The yields of pulps obtained after the pretreatment with enzyme (X) and pretreatment with enzyme followed by alkaline extraction (XE) were approximately 91 % and 77 %, respectively.

Viscosity values of bleached or pretreated pulps (Table 2) were higher than those reported for unbleached pulps (Table 1) indicating that there was no degradation of fibers, in others words, the polymerization degree of cellulose was maintained and the fiber length was preserved. The reagents used in the bleaching sequences partially remove lignin without reducing the viscosity values. Thus, no decrease of viscosity of the bagasse pulps pretreated with xylanase was ob-

Table 1. Properties of Unbleached and Bleached Pulps without Enzymatic Pretreatment

Pulp	Yield	Viscosity	Kappa number number	Glucan	Xylan	Xylan to glucan ratio	Total lignin	Acetyl
	%	cP		%	%		%	%
Unbleached	54.4*	9.8	42.8	69.8	10.7	0.15	9.9	2.5
Alkaline extraction (E)	76.6	11.9	14.1	81.9	5.9	0.07	4.5	0
Sodium chlorite (H)	74.6	9.8	5.6	73.8	11.6	0.15	5.9	0

*Calculated in relation to sugarcane bagasse mass.

Table 2. Properties of Pretreated and Bleached Ethanol/Water Pulps

Bleaching sequence	Bleaching time	Endoxylanase from *T. lanuginosus*			Cartazyme		
		Yield	Viscosity	Kappa number	Yield	Viscosity	Kappa number
	h	%	cP		%	cP	
X	4	92.0	10.2	39.3	91.6	10.1	38.5
XE	4	77.6	12.0	14.6	78.7	11.3	13.8
X	8	90.7	10.3	38.7	91.8	9.1	35.0
XE	8	77.6	11.6	13.9	77.8	11.1	13.7
X	12	91.3	10.4	38.9	90.8	10.7	38.3
XE	12	76.7	11.9	14.3	76.9	12.2	14.6

Table 3. Chemical Composition of Pretreated and Bleached Ethanol/Water Pulps

Bleaching sequence	Bleaching time	Endoxylanase from *T. lanuginosus*					Cartazyme				
		Glucan	Xylan	Xylan/glucan Ratio	Total lignin	Acetyl	Glucan	Xylan	Xylan/glucan Ratio	Total lignin	Acetyl
	h	%	%		%	%	%	%		%	%
X	4	71.7	10.7	0.15	9.8	2.6	55.2	8.1	0.15	8.8	0
XE	4	81.6	6.3	0.08	5.3	0	68.2	5.0	0.07	3.7	0
X	8	71.9	10.6	0.15	9.7	2.5	59.6	9.0	0.15	10.6	0
XE	8	81.1	6.4	0.08	5.2	0	58.1	4.6	0.08	3.3	0
X	12	71.3	10.3	0.15	11.1	2.3	62.9	9.2	0.15	9.2	0
XE	12	81.9	6.5	0.08	5.0	0	60.7	4.7	0.08	3.3	0

served, even at prolonged incubation period of 12 h (Table 2).

In comparison to unbleached pulps (Table 1), reductions of the kappa number by about 8.2 % and 10 % was observed for pretreated pulps after 4 h of enzymatic treatment using endoxylanase from *T. lanuginosus* and Cartazyme, respectively (Table 2). This fact indicates that the enzyme obtained from *T. lanuginosus* fungus exhibits practically the same efficiency in the organosolv pretreatment of pulps as the commercial enzyme (Cartazyme). *Gonçalves* and *Ruzene* [15] reported similar results using acetosolv pulps.

Table 2 shows that enzymatic treatment carried out for 12 h did not improve the bleaching efficiency (viscosity and kappa number values). High value of the kappa number was observed in the case of the sample pretreated with xylanase (X) indicating that some residual lignin still remained in the pulps. On the other hand, the kappa number decreased by about 67 % after bleaching with xylanase followed by alkaline extraction (XE), showing that the residual lignin was removed from the pulps. Apparently, the kappa number equal to 14 was the limit value of the ethanol/water pulp after bleaching with xylanase followed by alkaline extraction independently of the treatment time applied (Table 2). The results indicate that neither the kappa number nor the viscosity were affected by the treatment time.

Table 3 shows the chemical composition of ethanol/water pulps pretreated with xylanase (endoxylanase from *T. lanuginosus* and Cartazyme) for different bleaching periods. A quantification of the pulps carbohydrate composition was carried out to evaluate possible selective degradation of constituents. Comparing the pulp pretreated with xylanase with the unbleached pulp, the glucan amount was maintained after bleaching with the enzyme obtained from *T. lanuginosus*, while a decrease of this parameter (21 % approximately) was found when the pulp was bleached with Cartazyme.

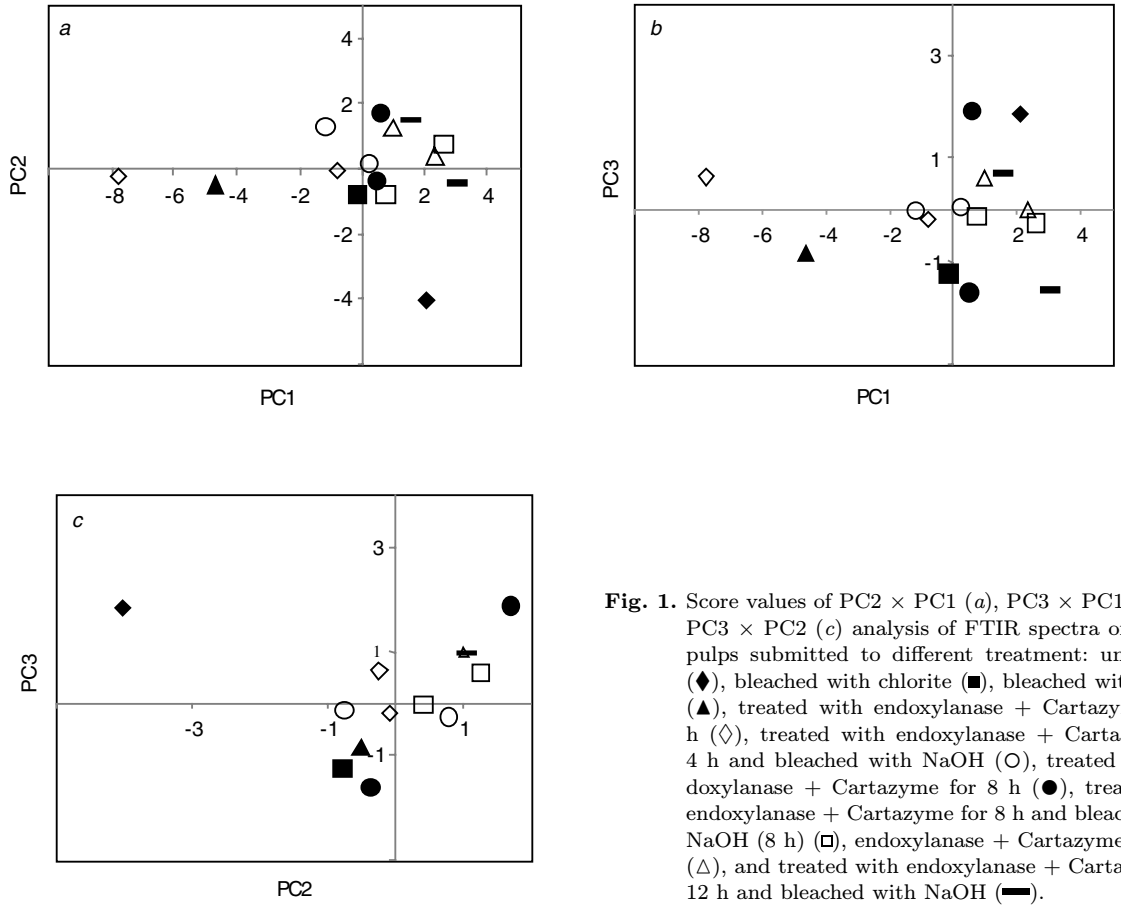

Fig. 1. Score values of PC2 × PC1 (*a*), PC3 × PC1 (*b*), and PC3 × PC2 (*c*) analysis of FTIR spectra of bagasse pulps submitted to different treatment: unbleached (⧫), bleached with chlorite (■), bleached with NaOH (▲), treated with endoxylanase + Cartazyme for 4 h (◊), treated with endoxylanase + Cartazyme for 4 h and bleached with NaOH (○), treated with endoxylanase + Cartazyme for 8 h (●), treated with endoxylanase + Cartazyme for 8 h and bleached with NaOH (8 h) (□), endoxylanase + Cartazyme for 12 h (△), and treated with endoxylanase + Cartazyme for 12 h and bleached with NaOH (▬).

Small differences were detected in the amount of xylan when the results of unbleached pulps were compared with those of pulps bleached with the enzyme obtained from *T. lanuginosus* fungus. The amount of xylan was preserved even after the enzymatic treatment. Only after the alkaline extraction, the xylan content decreased, which means that xylanase started the xylans fragmentation, however, the fragments were not easily released.

The total amount of lignin did not decrease after the pretreatment for 4 h with xylanase originating from fungus, however, when using Cartazyme, the total lignin content decreased by 11 %. Table 3 shows that the pretreatment time did not have any significant effect on the ethanol/water pulping when it was varied within the range of 4—12 h.

The amount of total lignin decreased only after the alkaline extraction and this reduction was higher than that observed for the pretreatment with Cartazyme. Alkaline extraction makes the solubilization of both lignin and xylan fragments feasible**.** Analyzing the values of the kappa number, viscosity, and the pulp chemical composition, it was found that the enzyme from fungus presents practically the same efficiency as Cartazyme (commercial enzyme) and there are no significant changes when different treatment times with xylanase are employed (4 h, 8 h, and 12 h). It can be concluded that 4 h is a sufficient time period for the pretreatment of pulp with xylanase from *T. lanuginosus*.

Further, the unbleached, pretreated with enzymes, and bleached pulps were classified using the Fourier transform infrared spectroscopy. FTIR spectral data are widely used for characterization of pulps and wood [20] and can be used as a tool for the analysis of chemical modifications of bagasse or other lignocellulosic materials. In this work, the effects of the enzymatic action were firstly evaluated by comparing the FTIR spectra (obtained by the HATR technique) of unbleached bagasse pulps with those of pretreated bagasse pulps. The results were quite similar, and small differences were evaluated by PCA. After the PCA treatment, the three first principal components (PCs) explained more than 90 % of the spectra total variance, *i.e.* the 783 variables (data points of each spectrum) can be reduced to 3 PCs with a 90 % confidence level.

Figs. 1*a*—1*c* present the PCs' graphics for the 19 FTIR spectra obtained taking into account 15 samples of unbleached, pretreated, and bleached pulps.

In Fig. 1*a* (PC2 × PC1) the group of pulps pretreated with xylanase was differentiated from that cor-

responding to the pulps bleached with NaOH (alkaline extraction), and it cannot be differentiated from the group of pulps bleached with xylanase followed by alkaline extraction. In Fig. 1*b* (PC3 × PC1), the pulps pretreated with xylanase and pulps bleached with xylanase followed by alkaline extraction are distanced from the pulps bleached with chlorite and with NaOH. In Fig. 1*c* (PC3 × PC2), the differenciation between the pulps pretreated with xylanase or bleached with NaOH after the pretreatment with xylanase and those bleached with chlorite or NaOH only is presented. Thus, the PCA evaluation of pulps showed that the pulps pretreated with xylanase submitted or no to the subsequent alkaline extraction and the unbleached pulps differ substantially from the pulps which underwent another bleaching sequence.

Acknowledgements. The authors acknowledge financial support from the FAPESP and the CNPq.

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