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Bleachability and Characterization by Fourier Transform Infrared Principal Component Analysis of Acetosolv Pulps Obtained from Sugarcane Bagasse

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Abstract

Sugarcane bagasse Acetosolv pulps were bleached by xylanase and the pulps classified by using Fourier transform infrared (FTIR) spectroscopy and principal component analysis (PCA). Pulp was treated with xylanase for 4–8 h with stirring at 30°C. Some samples were further extracted with NaOH for 1 h at 65°C. FTIR spectra were recorded directly from the dried pulp samples by using the diffuse reflectance technique. Reduction in kappa number of 69% was obtained after sequence xylanase (4 h)-alkaline extraction. During bleaching the viscosity decreased only 12%. FTIR-PCA showed that the first three principal components (PCs) explained more than 90% of the total variance of the pulp spectra. PC2 × PC1 plot showed that the points related to pulps from sequence xylanase (4 h)-alkaline extraction are different from the other. This group is enlarged by plotting PC3 × PC1 or PC3 × PC2 containing all pulps submitted to alkaline extraction. PC2 and PC3 are the principal factor for differentiation of the pulps. These PCs suffer influence of the ester bands (1740 and 1244 cm^{-1}). On the other hand, the pulps bleached only with xylanase could not be differentiated from the nonbleached pulps.

Index Entries: Xylanase bleaching; Fourier transform infrared spectra of pulps; sugarcane bagasse pulps; Acetosolv pulping.

Introduction

Organosolv pulping of agricultural residues has been studied in both acidic and basic conditions *(1)*. The cellulose in acidic pulps is typically more degraded than in basic ones, and after conventional bleaching

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processes the degradation of the fibers should increase *(2)*. The use of xylanases as a step in the bleaching of acidic pulps can avoid the changes in pH since the pulp is obtained at pH 4.0 to 5.0, corresponding to the maximum activity of xylanases. Treatment of eucalyptus kraft pulps with xylanases has already been investigated and was shown to decrease the kappa number in 11% *(3)*. In the present study we propose the xylanase bleaching of sugarcane bagasse pulp obtained from the Acetosolv process.

Materials and Methods

Pulping and Bleaching

Acetosolv pulping of depithed sugarcane bagasse was carried out as described by Benar *(4)*. After a 2-h cooking time, the pulp was filtered and washed with acetic acid and further with water until reaching pH 4.0. Samples of bagasse Acetosolv pulp (1 g dry) were suspended under agitation in 30.2 mL of water (3.2% consistence) at 30°C for 10 min. Cartazyme HS® (Sandoz) was added at 17.8 U/g of dry pulp. The samples were maintained in a shaker at 30°C for 4–12 h, followed by filtration and washing with 200 mL of distilled water at 30°C for the complete removal of the enzyme. One set of enzymatic bleached pulps was further submitted to alkaline extraction. Samples obtained at different bleaching times (1 g of dry pulp) and original pulp were extracted with 50 mL of 1 mol/L of NaOH at 65°C for 1 h under magnetic stirring. After filtration, the pulps were washed with 50 mL of 1 mol/L of NaOH for 1 h at 65°C and further with distilled water at 65°C until reaching pH 6.0. Chlorite bleaching was also carried out as an example of the classic bleaching process. Dry pulps (1 g) were suspended in 50 mL of water (2% consistence) and heated to 70°C. Sodium chlorite (1.3 mL of 40% aqueous solution) and glacial acetic acid (0.2 mL) were added. The solution was further heated at 70°C for 5 min, and the bleached pulp obtained was exhaustively washed with water. Pulps were oven-dried at 110°C for 15 min and analyzed with respect to kappa number and viscosity by standard methods *(5,6)*.

Hydrolysis of Pulps

One gram of dry pulp (original and bleached) was treated with 10 mL of 72% H_2SO_4 with stirring at 45°C for 7 min. The reaction was interrupted by adding 50 mL of distilled water, the mixture was transferred to a 500-mL Erlenmeyer flask, and the volume reached 275 mL. The flask was autoclaved for 30 min at 1.05 bar for the complete hydrolysis of oligomers. The mixture was filtered and the filtrate (hydrolysate) complete to 500 mL. A 40-mL sample of the hydrolysate was diluted to 50 mL and the pH was adjusted to 2.0 with 2 mol/L of NaOH. After filtration in a Sep-Pak C_{18} cartridge to remove aromatic compounds, the hydrolysate was analyzed in an Aminex HPX-87H column (300 × 7.8 mm) (Bio-Rad) at 45°C by using a Shimadzu chromatograph and refraction-index detector. The mobile phase was 0.005 mol/L of H_2SO_4 at 0.6 mL/min. Sugar concentra-

tions reported as xylan and glucan were determined using calibration curves of pure compounds.

Fourier Transform Infrared Principal Component Analysis of Bleached and Unbleached Pulps

Fourier-transform infrared (FTIR) spectra were obtained directly from the bleached and unbleached refined pulps utilizing the diffuse reflectance technique (DRIFT), under the conditions described in ref. 7. Spectra were recorded (64 scans) in a Nicolet 520 spectrometer. After polygonal baseline correction *(7)*, the spectra were normalized by the absorption at 900 cm^{-1}, which corresponds to the anomeric carbon atom of the group O-C-O in polysaccharides and suffers no influence from other groups *(8)*. Spectra were converted to text files using OMNIC software (Nicolet). The normalized absorbances in the range of 400–4000 cm^{-1} (1866 data points per pulp spectrum) were submitted to principal component analysis (PCA) calculations using the BIOTEC and FAEN programs compiled in FORTRAN, which were written in our laboratory based on the work of Scarminio and Bruns *(9)*. Graphic presentations were easily made with Microsoft EXCEL 5.0.

Results and Discussion

Table 1 gives the viscosity values, kappa number, and yields for the pulps obtained in the xylanase treatment, alkaline extraction, and sequence xylanase-alkaline extraction. For comparison, results from the treatment of the bagasse Acetosolv pulp with sodium chlorite are also given.

Unbleached and nonextracted pulps were not refined before viscosity measures, and the presented results cannot be directly compared with those of extracted pulps. Viscosity values for alkaline-extracted pulps were greater than those for the other pulps, probably owing to losses of lower molecular weight carbohydrate fractions, as can be seen by the yields in Table 1.

Alkaline extraction is responsible for the solubilization of lignin fragments released after pulping or xylanase action. Alkaline extraction without the use of xylanase reduced the kappa number from 28 to 13.4, but the pulp yield was only 85%.

Enzymatic treatment extended to 12 h did not improve the bleaching efficiency, as can be seen in Table 1. Viscosity values were maximum with 4 h of xylanase treatment and the decrease in kappa number from 4 to 12 h was only 8%. Xylanase treatment followed by alkaline extraction was the best sequence. These values cannot be compared with the bleaching results achieved by chlorite treatment because with the same values of viscosity, the kappa number for xylanase-alkaline-extracted pulp was still 28% higher.

The decrease in kappa number followed a pattern along different enzymatic treatment times. Reduction in kappa number from 28 to 8.6 was achieved by using the better experimental condition: 4 h of enzymatic treat-

Table 1
Viscosity, Kappa Number, and Yields of Different Treated Pulps

Sequence[a]	Viscosity (cP)	Kappa number	Yield (%)
Original	10.7	28.0	—
Chlorite bleaching	11.5	6.7	72.5
X (4 h)	9.0	25.4	92.0
XE (4 h)	11.5	8.6	89.2
X (8 h)	8.7	23.9	98.1
XE (8 h)	10.1	9.9	89.0
X (12 h)	7.1	23.4	88.4
XE (12 h)	11.1	10.9	87.5
E	11.0	13.4	85.2

[a]X, xylanase; E, alkaline extraction; XE, xylanase followed by alkaline extraction.

Table 2
Carbohydrate Composition of Pulps and Xylan/Glucan Ratio

Pulp[a]	Cellobiose (%)	Glucose (%)	Total glucan (%)	Xylan (%)	Xylan/glucan ratio	Acetyl groups (%)
Original	1.32	72.20	73.52	9.40	0.13	4.02
X	—	60.74	60.74	8.01	0.13	4.25
XE	4.01	67.20	71.21	6.48	0.09	<1.0
E	—	—	56.90	4.40	0.08	<1.0

[a]X, xylanase treated (4 h); XE, xylanase treated (4 h) followed by alkaline extraction.

ment followed by alkaline extraction. A tendency of the kappa number to increase when the time was increased was observed. The recondensation of the lignin over the fibers can occur, diminishing the action of the alkaline extraction. A decrease of 12% in the viscosity values after 4 h of treatment was also observed.

Carbohydrate quantification of the pulps was performed to evaluate possible selective degradation of pulp constituents. Table 2 gives the results of the quantification of dimers and monomers of cellulose and xylans as well as the xylan/glucan ratio.

The relative amount of xylan was preserved even after xylanase treatment. Only after alkaline extraction was a reduction in xylan detected, which means that xylanase acts over xylans but the fragments formed are not easy released. Alkaline extraction makes solubilization of both lignin and xylan fragments feasible. Removal of glucan and xylan (decreased pulp yield) caused changes in xylan/glucan ratio.

Figure 1 shows the FTIR spectra of the unbleached and three bleached pulps (with chlorite, xylanase, and xylanase + alkaline extraction). The spectra are quite similar and the differences among them were evaluated by PCA.

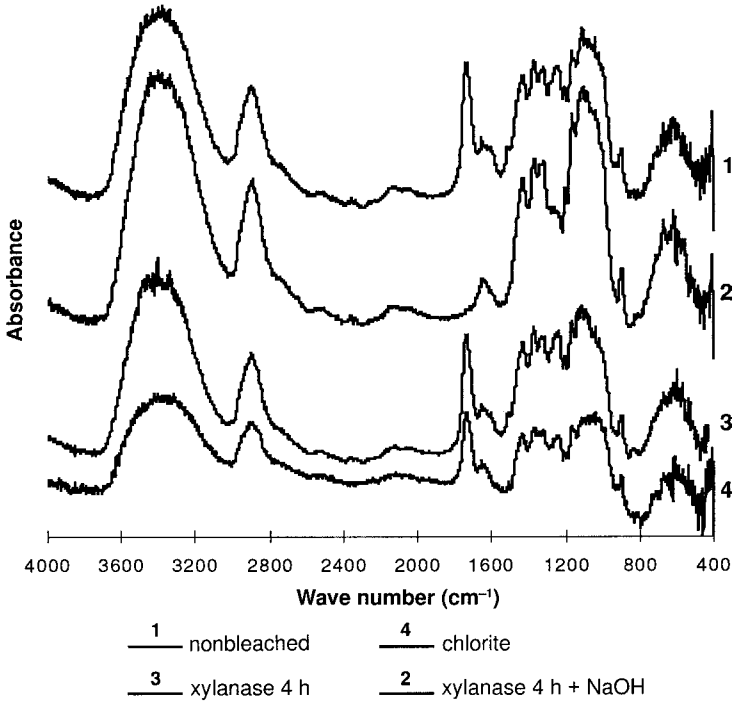

Fig. 1. FTIR spectra of the pulps.

The first three principal components (PCs) explain more than 90% of the total variance of the system. This means that the 1866 properties (data points) of each spectrum can be reduced to only 3 with 90% confidence. The principal components are obtained by the linear combination of the 1866 data, by also discarding the regions that do not change among the samples, and the results are score values. Figures 2–4 show the PC's graphics for the 23 FTIR spectra obtained from 6 different assemblies (bleached and unbleached pulps).

In Fig. 2 (PC2 × PC1), three of the points corresponding to the FTIR of bleached pulps with xylanase (4 h) followed by alkaline extraction are grouped in a different way with respect to the other points (highlighted by the ellipse). In Fig. 3, a larger group is evidenced containing FTIR spectra of bleached pulps with xylanase at different times and followed by alkaline extraction. This group can be differentiated from the others, since the closer the groups, the more similar the corresponding spectra.

Score points corresponding to the unbleached pulp form a group with small dispersity in the PC3 × PC2 plot (Fig. 4), and the pulps extracted with NaOH form a well-defined group. The points corresponding to more drastic treatment (chlorite and xylanase for 8 h) are very different with respect to the unbleached pulp.

This finding suggests that PC2 and PC3 are the principal factors for the differentiation between the pulps' spectra. This is better analyzed by the loading values of each PC (Fig. 5). From Fig. 5 the influence of infrared

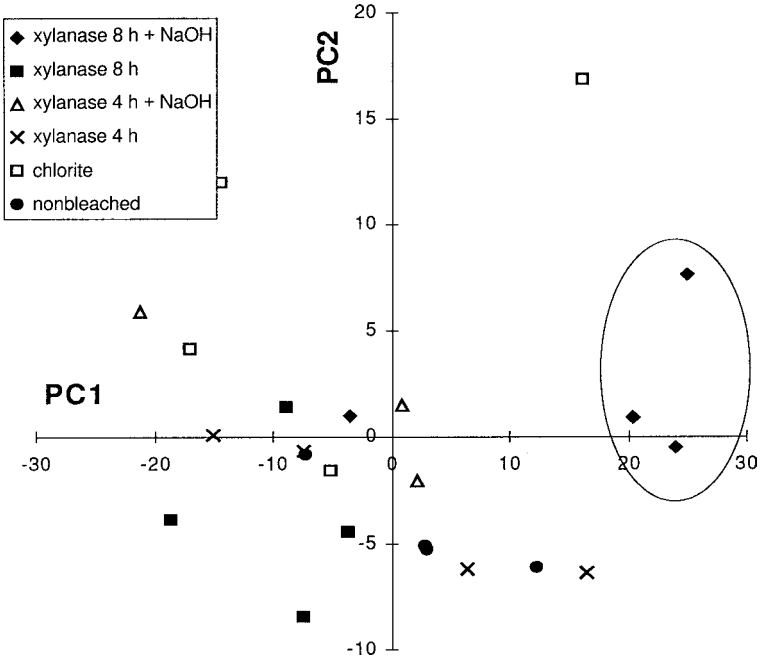

Fig. 2. Score values of PC2 × PC1 from FTIR spectra of bleached and unbleached bagasse pulps.

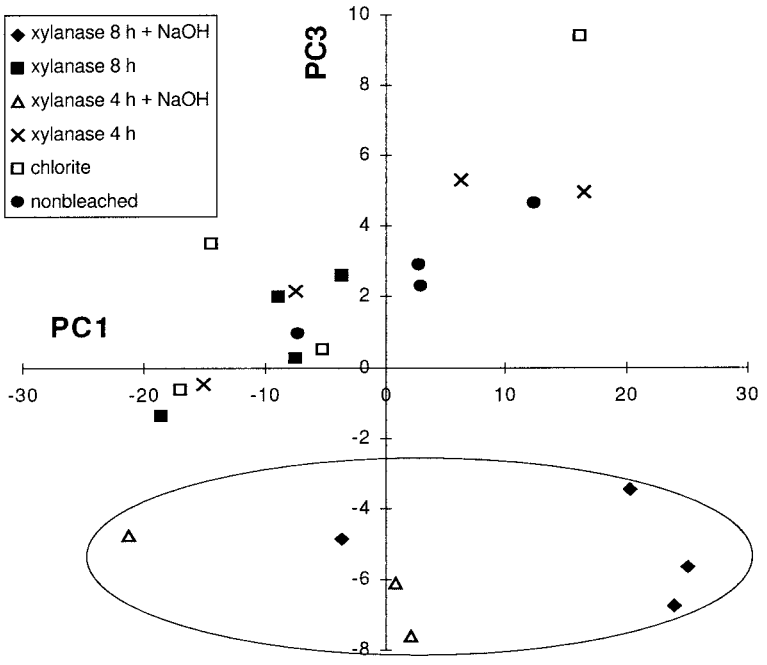

Fig. 3. Score values of PC3 × PC1 from FTIR spectra of bleached and unbleached bagasse pulps.

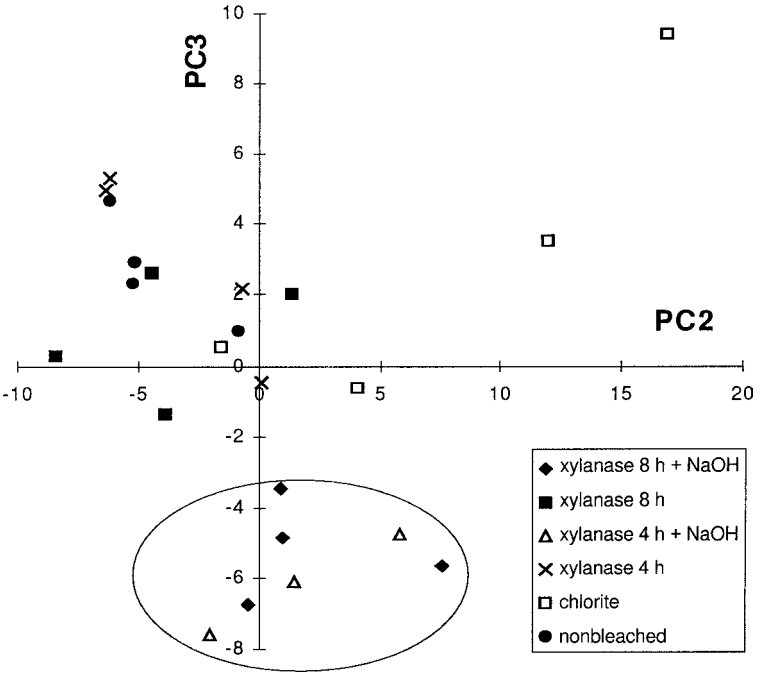

Fig. 4. Score values of PC3 × PC2 from FTIR spectra of bleached and unbleached bagasse pulps.

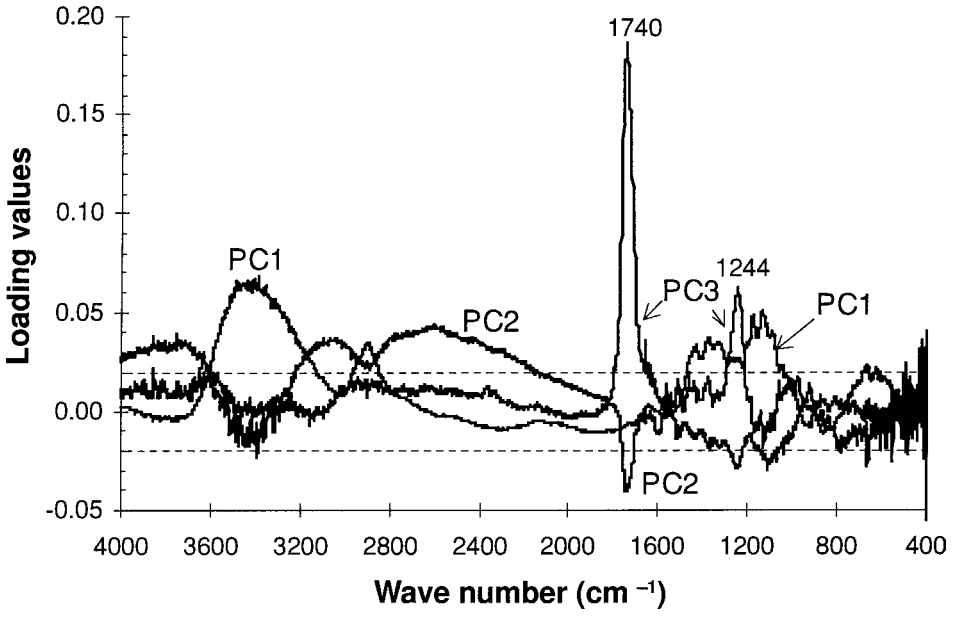

Fig. 5. Loading values of PC1, PC2, and PC3 of FTIR spectra of bleached and unbleached bagasse pulps.

bands on PC scores can be evaluated. The plot of PC3 is influenced by carbonyl (1740 cm^{-1}) and C-O (1244 cm^{-1}) bonds, characteristic of esters. The difference between the pulps can be explained by the acetyl groups (ester) formed during the Acetosolv pulping that are removed during the alkaline extraction. This fact is also corroborated by the chemical analysis previously discussed, with a decrease in the acetyl groups.

Acknowledgments

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References

1. Raymond, A. Y. and Akhtar, M. (1998), *Environmentally Friendly Technologies for the Pulp and Paper Industry*, Wiley, New York.
2. Minor, J. L. (1996), in *Pulp Bleaching, Principles and Practice*, Dence, C. W. and Reeve, D. W., eds., Tappi, Atlanta, pp. 25–57.
3. Milagres, A. M. F., Erismann, N. M., and Durán, N. (1992), in *Proceedings of 2nd Brazilian Symposium on the Chemistry of Lignin and Other Wood Components*, Durán, N. and Espósito, E., eds., UNICAMP, Campinas-Brazil, pp. 372–376.
4. Benar, P. (1992), MS thesis, Instituto de Química, UNICAMP, Campinas, Brazil.
5. TAPPI, Technical Association of the Pulp and Paper Industry. (1982), *TAPPI Standard Methods*, T 230 om-82.
6. TAPPI, Technical Association of the Pulp and Paper Industry. (1985), *TAPPI Standard Methods*, T 236 cm-85.
7. Faix, O., Böttcher, J. H., and Bertelt, E. (1992), in *8th International Conference in Fourier Transform Spectroscopy Proceedings SPIE 1575*, Heise, M., Korte, E. H., and Siesler, H. W., eds., The International Society for Optical Engineering, San Diego, CA, pp. 428–430.
8. Morohoshi, N. (1991), in *Wood and Cellulosic Chemistry*, Hon, D. N. S. and Shiraishi, N., eds., Marcel Dekker, New York, pp. 331–392.
9. Scarminio, I. S. and Bruns, R. E. (1989), *Trends Anal. Chem.* **8,** 326, 327.