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Sugarcane Bagasse Pulps

Biobleaching with Commercial Cartazyme HS and with Bacillus pumilus Xylanase

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Abstract

Organosolv (ethanol/water and acetosolv) pulps were treated with *Bacillus pumilus* xylanase for 4, 8, and 12 h and compared with commercial Cartazyme HS xylanase–treated pulps. Treatment of ethanol/water pulps with *B. pumilus* xylanase increased viscosity by 40% in 8 h of treatment compared with pulps treated without enzyme. However, acetosolv pulps treated with *B. pumilus* xylanase lost viscosity. Ethanol/water pulps treated with Cartazyme had a viscosity of 18.5 cP in 4 h of treatment. In the acetosolv pulps treated with commercial enzyme, the loss of viscosity was 20% compared with pulps treated without enzyme. Ethanol/water pulps treated with *B. pumilus* and Cartazyme had similar effects: a 44% reduction in kappa number for pulps treated with enzyme followed by alkaline extraction compared with pulps treated with alkaline extraction. In acetosolv pulps treated with *B. pumilus,* the kappa number was from 12 to 18, compared with pulps treated without enzyme, which had a 40% reduction in 4 and 12 h and a 60% reduction in 8 h. Cartazyme-treated acetosolv pulps had a kappa number of 14 in 4 and 8 h of treatment. For 12 h of treatment, the kappa number was 8. Fourier transform infrared spectra of the pulps showed that enzyme-treated pulps had changes in the 1000 cm^{-1} absorption owing to a C-O bond present in esters. Using principal component analysis, it is possible to differentiate the unbleached pulps and enzyme-treated pulps.

Index Entries: *Bacillus pumilus*; sugarcane bagasse pulps; cartazyme; viscosity; xylanase.

Introduction

Xylanase prebleaching technology is now in use at several mills, mainly in Scandinavia and Canada; the primary motivating factors for this

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technology are the economic and environmental advantages that xylanase offers to the bleaching *(1)*. From Western countries, there are many reports of the use of xylanases from different sources for evaluating their interaction with various kinds of pulps *(2,3)*. However, it is necessary to assess and study the processes under conditions specific to different countries with various kinds of pulps locally available. In Maharashtra (India), where sugarcane is the abundantly grown crop, bagasse is one of the cheapest raw materials available for paper making *(4)*.

Extremophilic enzymes, which are active under alkaline conditions and high temperatures, have great potential for industrial application, such as the bleaching process, without any necessity of cooling or changes in pH *(5)*. The large variety of potential applications of these enzymes is the main reason for investigating fungal and bacterial xylanase production. The most important application of xylanases is in the prebleaching of kraft pulp *(6)*. Treatment with xylanases can improve the chemical extraction of lignin from pulp *(6,7)*. This leads to significant savings in chemicals required for bleaching and to a reduction in toxic chlorine compounds released into the environment. The main problems with enzyme bleaching facing the pulp and paper industry are the availability and cost of the enzyme. It is estimated that about 30–40% of the production costs of many industrial enzymes is attributes to the cost of substrate growth *(8,9)*. The use of low-cost substrates for the production of industrial enzymes would be expected to reduce production costs greatly *(10)*.

Currently, most of the commercially available xylanases have been produced by fungi and are active at neutral or acidic pH, and their optimal temperatures for activity are below 45°C *(11)*. Active enzymes at high temperatures and alkaline pH values have great potential because they can be introduced at different stages of the bleaching process without requiring changes in pH or temperature *(6)*.

The production and characterization of the xylanases from *Bacillus* sp. NCIM 59 have been reported *(12)*. The most significant feature of the enzyme is its cellulase-free nature, which is one of the necessary prerequisites for use in the paper and pulp industry.

The aim of the present work was to apply *Bacillus pumilus* xylanase in organosolv bagasse pulps and to compare the results with Cartazyme HS-Sandoz xylanase action.

Materials and Methods

Acetosolv Pulping

Acetosolv sugarcane bagasse pulping was carried out with 93% acetic acid (v/v), with HCl as the catalyst, according to Benar *(13)*. The bagasse/solvent ratio was 1:14 (w/v). Pulping was done at 110°C (temperature of the solvent mixture) for 2 h. The pulp was washed with 93% acetic acid (v/v)

and then washed thoroughly with water until the wash water attained a neutral pH.

Ethanol/Water Pulping

Ethanol/water pulping of sugarcane bagasse was performed in a 200-mL closed vessel, using an ethanol/water mixture of 1:1 (v/v), a bagasse-to-solvent ratio of 1:10 (m/v), and a 2.5-h cooking time. The pulp was filtered and washed with 2500 mL of ethanol/water according to Gonçalves and Ruzene *(14)*.

Xylanase Assay

Xylanase from *B. pumilus* was supplied by Duarte et al. *(11)* and was assayed as described by Bailey et al. *(15)* by incubating the diluted enzyme solution (glycine buffer, pH 8.5) at 50°C for 5 min using a substrate solution of 1% (w/v) birchwood xylan (Roth, Karlsruhe, Germany). One unit of xylanase activity was defined as the amount of enzyme that catalyzes the release of 1 μmol of xylose equivalents/min of reaction.

Xylanase Pretreatment of Pulp

Samples of acetosolv or ethanol/water pulp with 3% pulp consistency were incubated in Erlenmeyer flasks in a shaker at 50°C for 4, 8, and 12 h. One set of samples was incubated with 18 IU of xylanase from *B. pumilus*, and another set of samples was incubated with the same charge of commercial enzyme (Cartazyme HS; Sandoz). After incubation, the pulp was filtered in a Büchner funnel and washed thoroughly with distilled water. The wet enzyme-pretreated bagasse pulp (3 g dry wt) was placed in an Erlenmeyer flask and treated with 2% NaOH at 60°C for 1 h. The pulp was filtered and washed with distilled water. One set of samples was incubated without enzyme and another set was treated with NaOH using the same conditions as just described (control pulps). Xylanase-bleached pulps were compared with the control pulps.

Estimation of kappa Number

A pulp sample was exposed to the action of 0.1 *N* $KMnO_4$ at 25°C for 10 min. The reaction was stopped by adding excess KI solution, and the consumed $KMnO_4$ was determined from the results of back-titrating the liberated iodine with standard sodium thiosulfate. The kappa number was obtained from the volume in milliliters of 0.1 *N* $KMnO_4$ consumed per gram of pulp *(16)*.

Determination of Viscosity

Viscosity was determined by dissolving bagasse pulp in cuprietylenediamine and measuring the viscosity of a 0.5% solution with an Ostwald Fensk viscometer *(17)*.

Determination of Chemical Compounds of Pulp

One gram of dry pulp was treated with 10 mL of 72% H_2SO_4 with stirring at 45°C for 7 min. The reaction was interrupted by adding 25 mL of distilled water, the mixture was transferred to a 250-mL Erlenmeyer flask, and the volume was adjusted to 140 mL. The flask was autoclaved for 30 min at 1.05 bar for complete oligomer hydrolysis. The mixture was filtered and the filtrate (hydrolysate) was made up to 250 mL. A 20-mL sample of the hydrolysate was diluted to 25 mL, and the pH was adjusted to 2.0 with 2 mol/L of NaOH. After filtration through 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, Hercules, CA) at 45°C 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 concentrations reported as xylan and glucan were determined using calibration curves of pure compounds. Lignin was determined by gravimetric analysis *(18)*.

Fourier Transform Infrared and Principal Component Analysis of Bleached and Unbleached Pulps

Fourier transform infrared (FTIR) spectra were directly obtained from the bleached and unbleached refined pulps utilizing the attenuated reflexion technique under the conditions described by Faix et al. *(19)*. Spectra were recorded (16 scans) on a Perkin-Elmer spectrometer. After polygonal baseline correction *(19)*, the spectra were normalized by the absorption at 900 cm^{-1}, which corresponds to the anomeric carbon atom of the O-C-O group in polysaccharides and is not influenced by other groups *(20)*. The spectra were converted to text files using OMNIC software (Nicolet), and normalized absorbances in the range of 650–4000 cm^{-1} were submitted to principal component analysis (PCA) calculations using BIOTEC and FAEN programs compiled in FORTRAN, which were written in our laboratory based on the work of Scarminio and Bruns *(21)*. Graphic presentations were readily made using Microsoft EXCEL 5.0.

Results and discussion

Viscosity and kappa Number of Pulps

The viscosities of ethanol/water pulps treated with *B. pumilus* xylanase were from 10 to 14 cP. The better time of enzymatic treatment was 8 h, for which the viscosity was 14 cP. In the Cartazyme-treated pulps, the viscosities obtained were from 9.5 to 18.5, and the higher viscosity was obtained for 4 h of treatment. The viscosity of the pulps treated with *B. pumilus* and Cartazyme xylanases followed by alkaline extraction was 20 cP (Fig. 1A). Mediating the standard deviation, control pulps, *B. pumilus*, and commercial xylanase–treated pulps showed a similar viscosity in 8 and 12 h of treatment. The variance of treatment time did not affect the viscosity in the acetosolv

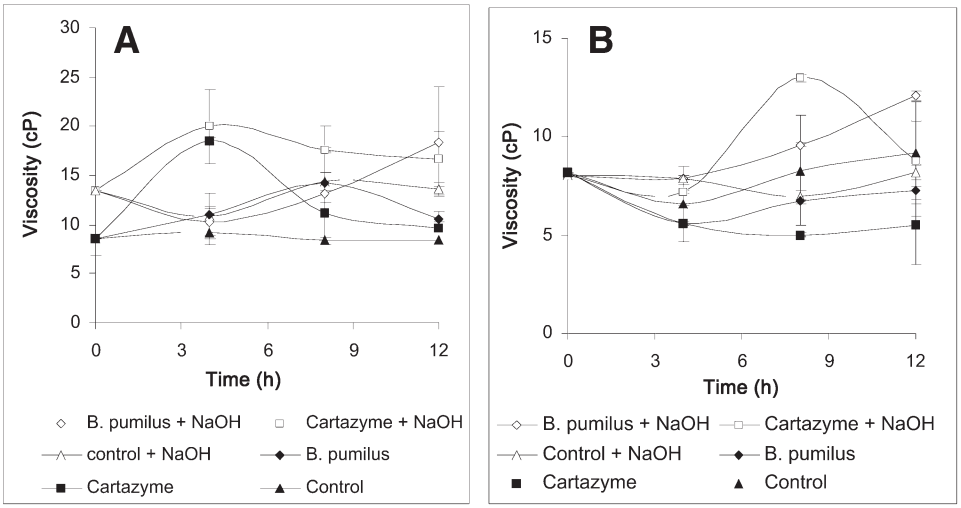

Fig. 1. Viscosity of unbleached Cartazyme and *B. pumilus* sugarcane bagasse pulps: **(A)** ethanol/water pulps; **(B)** acetosolv pulps.

pulps treated with Cartazyme (6 cP). Compared with the control pulps, the treatment of acetososlv pulps with Cartazyme reduced the viscosity 20% at the three times studied. The treatment of acetosolv pulps with Cartazyme followed by alkaline extraction achieved the maximum viscosity, 13 cP (Fig. 1B).

Extending the treatment time decreased the kappa number of ethanol/water pulps treated with *B. pumilus* xylanase (Fig. 2A). Comparison of the ethanol/water pulps treated with *B. pumilus* xylanase and pulps treated without enzyme revealed a reduction in kappa number of 11–30%. In the alkaline extraction of the ethanol/water pulps enzymatically treated with *B. pumilus,* the positive effect of the enzyme can be observed. Comparison of the pulps treated with xylanase and control pulps showed a 44% reduction in kappa number after 8 h of treatment. The same effect was observed for the ethanol/water pulps treated with commercial enzyme. The increase in the treatment time promoted a decrease in kappa number. Ethanol/water pulps treated with Cartazyme experienced on effect similar to that of *B. pumilus*–treated pulps. In 8 h of treatment, the ethanol/water pulps treated with Cartazyme presented a 42% reduction in kappa number compared with control pulps.

The kappa numbers of acetosolv pulps treated with *B. pumilus* xylanase were from 12 to 18 (Fig. 2B). The lowest kappa number was obtained after 8 h of treatment. Comparison of the enzymatic treatment of pulps and control pulps revealed a 40% reduction in kappa number after 4 and 12 h of treatment, and a 60% reduction after 8 h of treatment. In the acetosolv pulps treated with *B. pumilus* followed by alkaline extraction, the reduction in kappa number was 31–70% compared with control pulps. The major reduction was obtained in 4 h of treatment. The kappa number of acetosolv pulps treated with Cartazyme was 14 in 4 and 8 h of treatment, and when

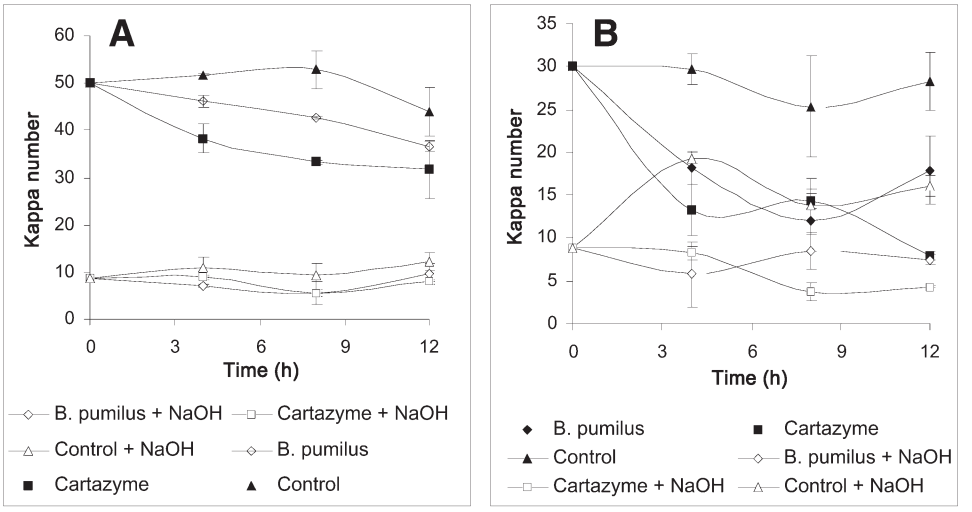

Fig. 2. The kappa number of unbleached Cartazyme and *B. pumilus* sugarcane bagasse pulps: **(A)** ethanol/water pulps; **(B)** acetosolv pulps.

the treatment time was extended to 12 h, the resulting value decreased to 8. The treatment with commercial xylanase had beneficial effects: the reduction in kappa number was 57% in 4 h and 73% in 8 and 12 h, compared with pulps treated without enzyme. The acetosolv pulps treated with Cartazyme followed by alkaline extraction presented the lowest kappa number (4) after 8 h.

In ethanol/water pulps, the treatment with *B. pumilus* enzyme had positive effects in relation to viscosity, and the kappa number was reduced to levels similar to those obtained with Cartazyme. In the acetosolv pulps, treatment with *B. pumilus* xylanase showed a reduction in kappa number to levels similar to those obtained with Cartazyme, but with loss in viscosity.

Bim and Franco *(6)* treated commercial hardwood kraft pulp with *B. pumilus* and observed 2.5 units (24%) of reduction in kappa number compared with nonbleached pulp. In this report, xylanase treatment had no effect on the viscosity of the pulp, indicating that xylanase did not affect the pulp fibers. Dhillon et al. *(7)* observed no decrease in the viscosity of eucalyptus kraft pulp treated with *B. circulans* AB16, indicating that the enzyme did not affect the cellulose chain and, thus, the quality of paper. The cellulose chain length is indicated in terms of viscosity. Nonspecific endoglucanases were observed to reduce the viscosity of softwood kraft pulp, indicating the degradation of cellulose chains *(22,23)*. However, Kulkarni and Rao *(4)* reported no decrease in the viscosity of bagasse pulps treated with xylanase from the *Bacillus* NCIM 59, even after a long incubation period of 12 h.

Chemical Composition of Enzyme-Treated Pulps

Varying the treatment time did not change the chemical composition of *B. pumilus*– and Cartazyme xylanase–treated pulps (Table 1). These pulps presented similar chemical compositions.

Table 1
Chemical Compounds of Ethanol/Water Pulps Treated with *B. pumilus* and Commercial Xylanase

	B. pumilus			Cartazyme		
Treatment[a]	Glucan (%)	Xylan (%)	Total lignin (%)	Glucan (%)	Xylan (%)	Total lignin (%)
X (4 h)	72.0 ± 1.7	9.0 ± 2.1	16.5 ± 2.8	72.2 ± 0.7	6.8 ± 0.1	18.5 ± 1.5
XE (4 h)	85.9 ± 0.6	5.5 ± 0.9	7.3 ± 0.8	87.8 ± 0.5	5.0 ± 0.1	6.5 ± 0.2
X (8 h)	71.0 ± 1.8	8.7 ± 1.6	17.7 ± 1.1	73.1 ± 0.1	7.5 ± 2.3	17.2 ± 1.4
XE (8 h)	87.0 ± 0.7	7.1 ± 0.7	4.6 ± 1.3	86.7 ± 3.0	5.8 ± 0.3	6.9 ± 1.0
X (12 h)	65.5 ± 1.7	8.6 ± 0.8	16.5 ± 2.8	70.2 ± 3.2	9.4 ± 1.6	18.2 ± 0.3
XE (8 h)	87.5 ± 0.8	6.2 ± 0.6	5.6 ± 0.9	86.4 ± 0.7	5.7 ± 0.2	7.2 ± 0.8

[a]X, xylanase; E, alkaline extraction.

Table 2
Chemical Compounds of Acetosolv Pulps Treated with *B. pumilus* and Commercial Xylanase

	B. pumilus			Cartazyme		
Treatment[a]	Glucan (%)	Xylan (%)	Total lignin (%)	Glucan (%)	Xylan (%)	Total lignin (%)
X (4 h)	73.3 ± 0.3	5.3 ± 0.1	16.0 ± 1.0	61.3 ± 0.3	10.9 ± 0.1	20.7 ± 0.4
XE (4 h)	89.4 ± 3.9	3.5 ± 0.7	4.8 ± 0.8	79.3 ± 3.5	9.4 ± 1.4	9.1 ± 3.3
X (8 h)	73.3 ± 0.6	6.6 ± 1.4	14.9 ± 0.7	70.4 ± 2.7	7.9 ± 1.5	16.3 ± 1.7
XE (8 h)	89.9 ± 0.7	3.8 ± 0.2	4.6 ± 0.8	84.9 ± 4.3	7.8 ± 2.9	5.5 ± 1.0
X (12 h)	71.4 ± 0.8	4.9 ± 0.4	18.9 ± 0.7	68.3 ± 2.7	9.0 ± 0.1	16.7 ± 1.0
XE (8 h)	89.8 ± 0.1	2.9 ± 0.4	5.3 ± 0.3	87.9 ± 4.2	6.5 ± 0.3	4.0 ± 1.2

[a]X, xylanase; E, alkaline extraction.

From Table 2, it can be seen that prolonging the treatment time did not affect the chemical composition of the enzymatically treated pulps. Cartazyme-treated pulps showed degradation of glucan when compared with *B. pumilus*–treated pulps. Cellulose degradation affects the viscosity of the pulps. Cartazyme-treated pulps had lower viscosity than *B. pumilus*–treated pulps. The *B. pumilus*–treated pulps contained degraded xylan compared with Cartazyme-treated pulps.

FTIR and PCA of Pulps

In Fig. 3 A, the ethanol/water pulps treated with *B. pumilus*, Cartazyme, and Cartazyme followed by alkaline extraction have similar FTIR spectra. The spectra of enzymatically treated pulps are different from the spectra of control pulps (pulps treated without enzyme). In the spectra of the pulps treated with enzyme, there is a large band at 1000 cm^{-1}, which corresponds

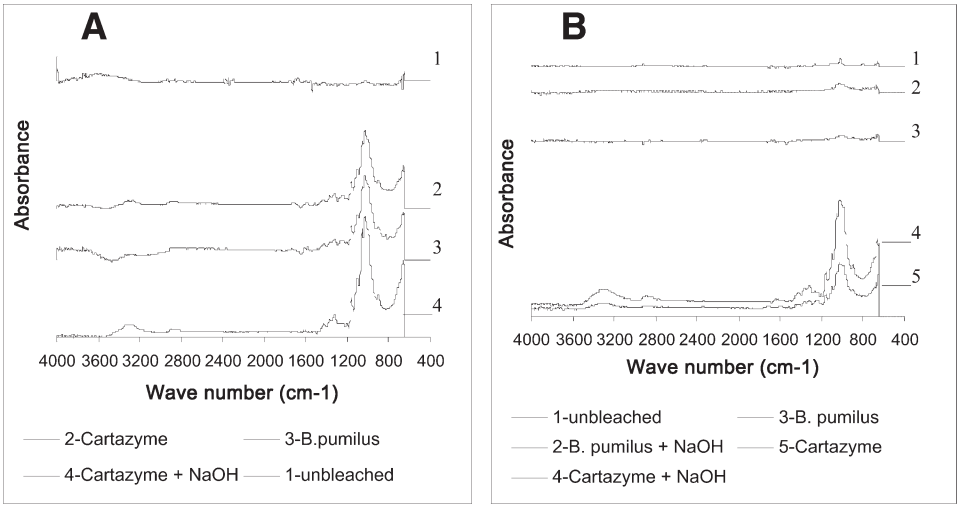

Fig. 3. FTIR spectra of sugarcane bagasse pulps: **(A)** ethanol/water pulps; **(B)** acetosolv pulps.

to C-O bonds. The spectrum of the control pulp is similar to that of the ethanol/water control pulp (Fig. 3A).

Acetosolv pulps treated with Cartazyme and treated with commercial xylanase followed by alkaline extraction have spectra similar to that of the ethanol/water-treated enzyme pulps. The spectra of acetosolv pulps treated with *B. pumilus* are different from that of the Cartazyme-treated pulps. The spectra of acetosolv pulps treated with *B. pumilus* showed few differences compared with that of unbleached pulps (Fig. 3B).

FTIR spectra are better analyzed by PCA. In the PC2 × PC1 plot in Fig. 4A, unbleached pulps are differentiated (enclosed in the oval) from the biobleached pulps. In the unbleached pulps, there are diffences in the pulps treated at different times. Control pulps, treated without enzyme but with alkaline extraction, are similar in relation to treatment time, showing that the treatment time has no effect on the pulps. The unbleached pulps are different from biobleached pulps, as seen in Fig. 4 B, where the group treated with enzyme and treated with enzyme followed by alkaline extraction is enclosed in the dashed oval. Ethanol/water pulps treated with *B. pumilus* and Cartazyme were similar.

In the PC2 × PC1 plot in Fig. 5A, two groups are identified, highlighted by the dashed oval: (1) six points representing the control pulps (unbleached and pulps treated only with alkaline extraction); and (2) biobleached pulps, without differences between *B. pumilus* and Cartazyme-treated pulps.

In the PC3 × PC2 plot in Fig. 5B, three groups are identified: unbleached pulps, Cartazyme-treated pulps, and *B. pumilus*–treated pulps with 12 h of treatment. The unbleached pulps treated during different times are not greatly different. There were few differences between the pulps treated with

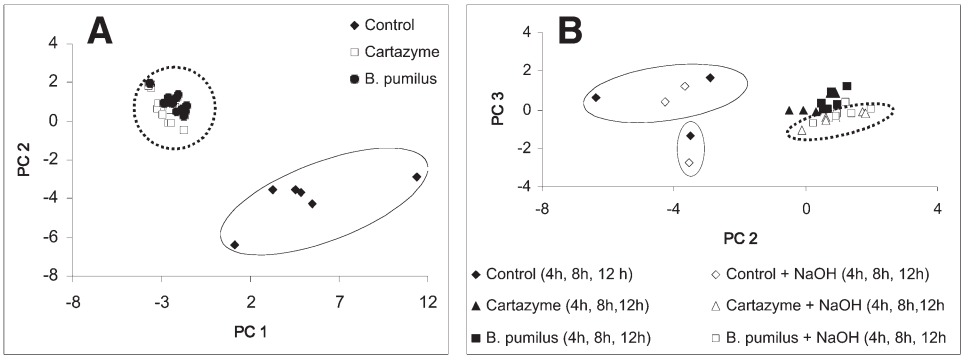

Fig. 4. (A) PC2 × PC1 and **(B)** PC3 × PC2 score values from FTIR spectra of *B. pumilus*–bleached and Cartazyme xylanase–bleached and unbleached ethanol/water bagasse pulps.

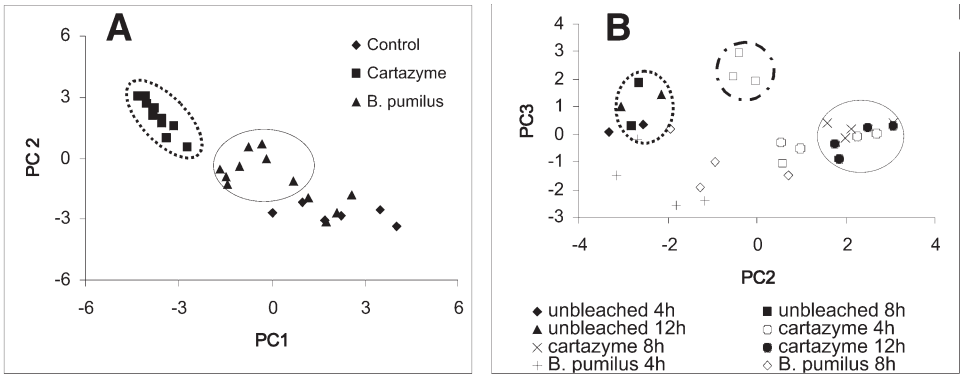

Fig. 5. (A) PC2 × PC1 and **(B)** PC3 × PC2 score values from FTIR spectra of *B. pumilus*–bleached and Cartazyme xylanase–bleached and unbleached acetosolv bagasse pulps. (□ is for *B. pumilus* for 12 h.)

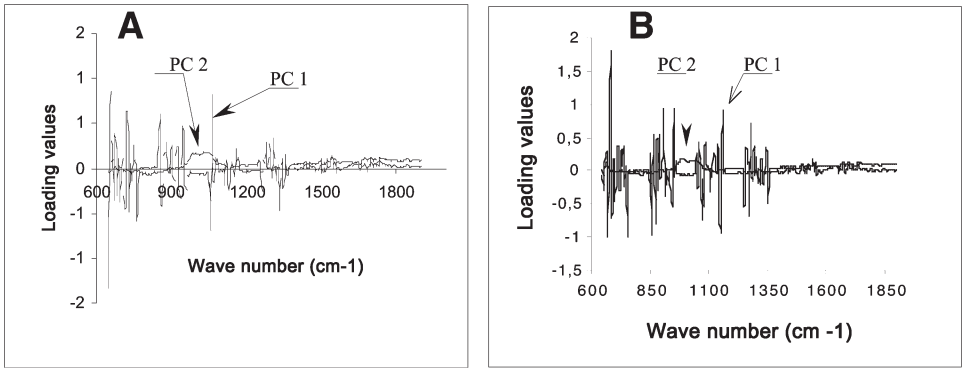

Fig. 6. (A) Ethanol/water and **(B)** acetosolv loading values of PC1 and PC2 from FTIR spectra of *B. pumilus*–bleached and Cartazyme xylanase–bleached and unbleached bagasse pulps.

Cartazyme during different times. Pulps treated with *B. pumilus* for 4 and 8 h are near control pulps.

The first three principal components (PCs) explain more than 93% of the total variance of the system, PC2 and PC3 being the principal factors for the differentiation between the pulp spectra. This is better analyzed by the loading values of each PC (Fig. 6).

From Fig. 6A, in the ethanol/water pulps, the influence of IR bands on PC scores can be evaluated. The plot of PC2 is influenced by C-O bonds (1000 cm^{-1}), also present in esters. From fig. 6B, in the acetosolv pulps, it can be seen that PC2 is also influenced by C-O (1022 and 1294 cm^{-1}) bonds.

Conclusion

Treatments of ethanol/water pulps with *B. pumilus* and Cartazyme were beneficial, increasing the viscosity when compared with unbleached pulps. Both xylanases had a similar reduction in kappa number. In acetosolv pulps, *B. pumilus* increased the viscosity of the pulps and reduced the kappa number to the same extent as Cartazyme-treated pulps. However, acetosolv pulps treated with Cartazyme showed a decrease in viscosity, compared with unbleached pulps.

FTIR spectra of unbleached, Cartazyme-bleached, and *B. pumilus*–bleached pulps showed that the pulps are different, making it possible to differentiate the treatment times of the pulps.

Acknowledgments

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