# Ethanol/Water Pulps From Sugar Cane Straw and Their Biobleaching With Xylanase From *Bacillus pumilus*

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# **Abstract**

The influence of independent variables (temperature and time) on the cooking of sugar cane straw with ethanol/water mixtures was studied to determine operating conditions that obtain pulp with high cellulose contents and a low lignin content. An experimental 2² design was applied for temperatures of 185 and 215°C, and time of 1 and 2.5 h with the ethanol/water mixture concentration and constant straw-to-solvent ratio. The system was scaled-up at 200°C cooking temperature for 2 h with 50% ethanol–water concentration, and 1:10 (w/v) straw-to-solvent ratio to obtain a pulp with 3.14 cP viscosity, 58.09 kappa-number, and the chemical composition of the pulps were 3.2% pentosan and 31.5% lignin. Xylanase from *Bacillus pumilus* was then applied at a loading of 5–150 IU/g dry pulp in the sugar cane straw ethanol/water pulp at 50°C for 2 and 20 h. To ethanol/water pulps, the best enzyme dosage was found to be 20 IU/g dry pulp at 20 h, and a high enzyme dosage of 150 IU/g dry pulp did not decrease the kappa-number of the pulp.

**Index Entries:** *Bacillus pumilus*; biobleaching; ethanol/water pulp; organosolv pulping; sugarcane straw; xylanase.

#### Introduction

Brazil is the greatest sugar cane producer in the world followed by India and Australia (1). The sugar cane is cultivated in the southeast and northeast portions of the country, and for 2006–2007, the estimated production is more than 410 million t. Sugar cane straw is the material that is removed before the cane is crushed, and 55% of the sugar cane juice is used to produce alcohol and 45% is used for sugar. One ton sugar cane cultivate produces 140 kg sugar cane straw. Sugar cane straw is inclusive of the dried leaves, fresh leaves, and the tip of plant. Thus, Brazil produces about 40 million t/y of sugar cane straw (2). Most of this residue is burned, thereby losing energy and causing significant pollution. From 2005 onward, environmental concerns and legislation

will forbid the burning of sugar cane fields before harvesting in São Paulo State, making a great amount of sugar cane straw available for other uses. This material has not been used as a source of chemicals, but only as solid fuel (3).

Agricultural fibers constitute an alternative to wood as raw material for making pulp because of their high growth rate and adaptability to various soil types. Spain produces more than 16 million t of major agricultural residues each year. With a yield of 40–50%, this mass would provide more than four times the amount of paper currently obtained from wood fibers in this country (4). The annual production of pulp can hardly have current demand, which is growing dramatically in developing countries and, at lesser extent, in developed countries. This is owing to an increasing shortage of wood raw materials and the gradual deforestation of some areas on the planet. For this reason, the use of alternative nonwood materials such as wheat straw (5,6), hemp (7), flax (8) were used for pulping and paper making.

Traditionally, the cooking process generates large amounts of concentrated waste-water, especially from sulfite and sulfate processes. One solution for this problem is the use of organic solvents. Although their favorable effects on the pulping process are established, the use of this type of solvent for this purpose is recent and is only on the pilot or small industrial scale (9). Prominent among the pulping processes that use organic solvents are those based on alcohols, particularly the Alcell (ethanol/water) (Alcell Technologies Inc., Montreal Canada), MD Organocell (ethanol soda), (Organocell Thyssen GmbH, Planegg, Germany) and alkali sulfite anthraquinone methanol (10–13). Pulping process that uses organic solvents presents several advantages such as: (a) the required equipment is simple (14), (b) byproducts are suitable for further chemical utilization, (c) this technology can be applied for a variety of raw materials, including hardwoods (15), softwoods (16), nonwood materials (5–6), (d) pulps are susceptible to total chlorine free (TCF) bleaching, and (e) (TCF-bleached pulps present high levels of brightness and intrinsic viscosity (17).

Modifications of the production process at the pulping and bleaching stages have been developed. This includes extending the cooking time and introduction of oxygen delignification as a prebleaching step for additional lignin removal. Biological alternatives to minimize the residual hemicellulose and lignin contents in dissolving pulp are also under investigation. Research has been focused on the use of xylanases (18) and white-rot fungi (19) in biobleaching of sulfite pulps as means of improving the selectivity and extent of hemicellulose and lignin removal from dissolving pulp. Xylanase prebleaching technology is now in use at several mills, mainly in Scandinavia and Canada; the main motivating factors for this technology are the economic and environmental advantages that xylanase offers to the bleach plant (20). From western countries many reports about using xylanases from different sources for evaluating their interaction with various kinds of pulps are available (21,22). However, it is necessary to assess and study the processes under specific conditions for different countries

with various kinds of pulps, which are locally available. In Maharashtra, (Índia) where sugar cane is an abundantly grown crop, bagasse is one of the major cheap raw materials available for making paper (23). Extremophilic enzymes, which are active under alkaline conditions and high temperatures, have high potential for industrial application, such as the bleaching process, without any need for cooling or change in pH (24). 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 (25). A treatment with xylanases can improve the chemical extraction of lignin from pulp (26,27). This leads to significant savings of chemicals required for bleaching and to a reduction of toxic chlorine compounds released into the environment. The use of low-cost substrates for the production of industrial enzymes would be expected to greatly reduce production costs (28).

Only a few microorganisms have been identified to have the capability of producing extremophilic xylanases. One of such strains is *Bacillus pumilus* sp. NCIM 59 (29). The most significant feature of the enzyme from this strain is its cellulase-free nature, which is one of the necessary prerequisites for use in the paper and pulp industry. The objectives of this work are to investigate the conditions for sugar cane straw ethanol/water pulping to obtain dissolved pulps and to evaluate the potential of xylanase obtained from *B. pumilus* on sugar cane straw ethanol/water pulps bleaching. Studies of pulping of sugar cane straw and xylanase biobleaching were done for the first time in this article.

## **Materials and Methods**

# Ethanol/Water Pulping in 200-mL Vessel

Whole sugar cane plant was mechanically cut and fresh sugar cane leaves and the tip of plants were kindly provided by "Usina Ester" (Cosmópolis, SP-Brazil). Sugar cane straw was washed with water, sun-dried to 10% moisture, and scissors chopped to a length of 3-5 cm. Pulping was performed in a 200-mL electrically heated horizontal agitation laboratory stainless steel batch cylindrical reactor (digestor was fabricated in São Paulo University, Brazil). In order to determine optimum ethanol/water pulping conditions of sugar cane straw, a  $2^2$  factorial design was used at 150-215°C for 1-4 h. Ethanol concentration of 50% (by volume) and 10:1 (v/w) liquor/straw ratio were maintained constant, according to Gonçalves and Ruzene (30). The pulp was filtered and washed with 2500 mL ethanol/water 1:1 (v/v).

## Ethanol/Water Pulping Scale-Up in 40-L Vessel

The sugar cane leaves, sun-dried to 10% moisture was cut into pieces of approx 3 cm. Scale-up ethanol/water pulping were carried out in a 40-L stainless steel batch cylindrical reactor. An insulated electrical coil arranged

around the reactor provided heating and a manometer was used to read pressure. The reactor rotates around its own axis for agitation. Ethanol/water pulping was performed using ethanol/water mixture 1:1~(v/v) at 10~(v/w) liquid/solid ratio. Reaction time was fixed at 2 h and  $200^{\circ}$ C. After the residence time, the reactor was turned off and cooled for approx 18 h. The pulp was filtered in cotton bag and washed with tap water until wash water was colorless. The moisture content of the washed pulp was 54% (w/w) and it was stored in scaled plastic bags at  $4^{\circ}$ C until use.

# Xylanase Assay

A crude enzyme with high xylanase activity and cellulase-free, produced by bacteria B. pumilus was kindly supplied by Duarte (31). Xylanase activity was determined by incubating 0.1 mL suitable diluted enzyme with 0.9 mL of 1% (w/v) xylan (birchwood xylan, Roth, Karlsrule, Germany) in a pH 8.5 glycine–NaOH buffer for 5 min at 50°C as described by Bailey et al. (32). One unit of xylanase activity was defined as the amount of enzyme that catalyses the release of 1  $\mu$ mol xylose/min of reaction.

# Xylanase Pretreatment of Pulp

Before pulping, sugar cane straw pulp was treated with the crude enzyme *B. pumilus*. Optimization of enzyme loading for biobleaching was carried out by treating pulp with varying xylanase charges, ranging from 5 to 50 IU/g of dry pulp for 2 h and 150 IU/g for 20 h pH 8.5 in glycine–NaOH buffer. Samples of ethanol/water pulp with 3% pulp consistency were incubated in transparent plastic bags in a water bath at 50°C with intermittent kneading. Control samples were treated under the same conditions without enzyme. After incubation, the pulp slurry was filtered through a Büchner funnel, and the pulp was washed thoroughly with distilled water. The wet enzyme pretreated straw pulp (3 g dry weight) was placed in Erlenmeyer flask and treated with 1% (w/w) NaOH at 60°C for 1 h. The pulp was filtered and washed with distilled water. A set of samples was treated with NaOH, under the same condition described as used as a control medium.

# Estimation of Kappa-Number

A sample of the pulp (0.3–0.35 g dry pulp) was exposed to 0.1 N KMnO $_4$  at 25°C for 10 min. The reaction was stopped by adding excess KI solution, and the KMnO $_4$  consumed was determined by back-titrating the liberated iodine with standard sodium thiosulfate. The  $\kappa$ -number so obtained, was the volume in milliliter of 0.1 N KMnO $_4$  consumed per gram of pulp and used to measure residual lignin in the pulp (33).

#### Determination of Viscosity

Viscosity was determined by dissolving sugar cane straw pulp in cupriethylenediamine and measuring the viscosity of 0.5% solution with an Ostwald Fensk viscometer (34).

# Determination of Pulp Chemical Compounds

The chemical composition of the sugar cane straw and the pulps treated with xylanase were determined by acid hydrolysis, a method developed at the Laboratory of Biomass Conversion (São Paulo University, São Paulo). Approximately 2.0 g of milled sugar cane straw (Manesco and Ranieri knife mill to pass through a 0.5-mm screen) or 1.0 g of pulp was hydrolyzed with 72% sulfuric acid at 45°C for 7 min. The acid was diluted to a final concentration of 5% (addition of 140 mL of water) and the mixture heated at 125°C/1 atm for 30 min. The residual material was cooled and filtered through fast-filtration paper filter. The solid was dried to constant weight at 105°C and determined gravimetrically as insoluble lignin. The soluble lignin concentration in the filtrate was determined by measurement of the absorbance at 205 nm and using the value of 1101/g/cm as the absorptivity of soluble lignin (35). The concentrations of monomeric sugars in the soluble fraction were analyzed using an Aminex HPX-87H column (300 × 7.8 mm<sup>2</sup>) (Bio-Rad, Hercules, CA) at 45°C with a Shimadzu chromatograph and refraction-index detector (RI-RID-10A, Shimadzu, Tokyo, Japan). The monosaccharides present in hydrolyzates were converted to percent polysaccharides: D-glucose to glucan, D-xylose to xylan, and D-mannose to mannan. The monosaccharide peak areas were converted using standard equations ascertained with the appropriate internal standards (curves standards of D-glucose, D-xylose, D-mannose, L-arabinose, and acetic acid). The monosaccharide weights were converted to polysaccharide percentage by considering hydrolyzate sample dilution, water of hydrolysis factors, and the original sample dry weight. The factors used to convert sugar monomers to anhydromonomers were 0.90 for glucose and 0.88 for xylose and arabinose. The acetyl content was calculated as the acetic acid content multiplied by 0.7. These factors were calculated based on water addition to polysaccharides during acid hydrolysis (36).

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

Fourier transform infrared (FTIR) spectra were obtained directly from the bleached and unbleached refined pulps utilizing attenuated reflexion technique, under the conditions described by Faix (37). Spectra were recorded (32 scans) in an Avatar-320-FT-IR Nicolet spectrometer (Nicolet Instrument Corporation, Madison, WI). After polygonal baseline correction (37), the spectra were normalized by the absorption at 900 cm<sup>-1</sup>, which corresponds to the anomeric carbon atom of O–C–O group in polysaccharides and suffers no interference from other groups (38). Spectra were converted to text files using OMNIC software (Nicolet), and normalized absorbances in the range of 650–4000 cm<sup>-1</sup> 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 (39). Graphic presentations were easily made with Microsoft EXCEL 5.0.

# **Results and Discussion**

Optimization of Sugar Cane Straw Ethanol/Water Pulping

In order to determine the optimum conditions for sugar cane straw ethanol/water pulping to obtain bleached pulps with appropriate dissolving pulp compositions, the temperature and the time of pulping were studied with a 2<sup>2</sup> factorial design over a temperature range of 150–190°C and a time-span of 1–4 h for an ethanol/water mixture of 50% (by volume), and a sugar cane straw-to-solvent ratio of 1 : 10 (m/v). At  $150^{\circ}$ C, no pulp was formed more than 1–4 h. Similarly, no pulp was obtained at 170°C and 2.5 h. However, at 190°C, the pulps obtained between 1 and 4 h were very dark. Based on these results, we decided to perform another experimental design 2<sup>2</sup> over a temperature range of 185–215°C and a time period of 1.5–2.5 h with replicate at the middle point (maximum temperature plus minimum temperature divided by two). Once again, the ethanol/water concentration and biomass-to-solvent ratio were held constant. The second experimental design was carried out in three replicate determinations of the independent variables of the pulping carried out in the 200-mL vessel, as shown in Table 1.

The pulping yield was highest (55.6%) at the highest temperature evaluated. However, pentosan was still degraded (2.3%), and the lignin amount increased to 33%. Pentosan degradation was correlated with the lower viscosity (4.4 cP) for the pulp. At 185°C and 2.5 h, the yield dropped to 47.6%, and the kappa-number, and the glucan and lignin contents were lower than obtained at 200°C, whereas the pentosan content and viscosity were highest. For paper pulp, 185°C and 2.5 h of pulping were optimum conditions for sugar cane straw ethanol/water pulping. Because ethanol/water-dissolving pulps must have a maximum of 10% pentosan (40), we decided the better time and temperature of pulping were 2 h and 200°C, respectively.

The system was scaled-up to a 40-L reactor with sugar cane straw ethanol/water pulping performed for 2 h at 200°C based on the results in Table 1. Now the ethanol/water pulping yield was 50%, and the chemical composition of the pulp obtained was 61.7% glucan, 3.2% pentosan, 31.5% lignin, and 4.1% ash. The viscosity and kappa-number were 3.14 cP and 58.09, respectively. Degradation of the pentosan resulted in a lower viscosity of 3.14 cP compared with the 200-mL vessel at 200°C for 2.5 h. Moriya et al. (41) used pulping conditions of an ethanol/water mixture of 1 : 1 (v/v), a bagasse-to-solvent ratio of 1 : 10 (m/v), temperature 185°C, and a 2.5 h cooking time to obtain a pulp viscosity of 8.64 cP and a kappa-number of 50 for sugar cane bagasse. Using these same conditions for pulping of sugar cane straw resulted in a pulp viscosity of 10.6 cP and a kappa-number 54.5.

Table 1
Conditions Used in the Ethanol Pulping of Sugar Cane Straw and Experimental Results for the Yield, Properties Kappa-Number, Viscosity, and the Chemical Composition of the Pulps Obtained

$X_{\mathrm{T}}$	$X_{t}$	YI (%)	KN	VI (cP)	GL (%)	PE (%)	LI (%)
+1	+1	55.6	61.4	4.4	60.3	2.3	33.0
-1	-1	53.7	64.4	5.4	59.6	2.5	33.3
-1	+1	47.6	54.5	10.6	58.6	11.9	26.1
-1	-1	45.2	59.1	12.2	57.5	14.2	25.6
0	0	51.7	61.0	6.9	62.2	6.0	26.8
0	0	52.8	64.2	7.5	63.6	6.5	25.7

 $X_{\rm T}$ , temperature (+1 = 215°C, 0 = 200°C, -1 = 185°C);  $X_{\rm t}$ , time (+1 = 2.5 h, 0 = 2 h, -1 = 1.5 h); YI, yield; NK, kappa-number; VI, viscosity; GL, Glucan; PE, pentosan; LI, total lignin.

Thus, sugar cane straw pulp gave a higher viscosity than that for sugar cane bagasse pulp, but the kappa-number of sugar cane straw pulp was higher than that for sugar cane bagasse pulp.

# Effect of Xylanase Concentration on Bleach Boosting and Paper Properties

The chemical composition of unbleached (control) pulp and different xylanase concentration-treated pulps are shown in Table 2. Xylanase treatment had an influence on content of cellulose and a slight influence on lignin content, which indicated that the treatment alone could not remove lignin and depolymerize cellulose of sugar cane straw pulp effectively. With 10 IU/g of xylanase, the pentosans content decreased, indicating that hemicellulose was degraded by xylanase as expected.

The ethanol/water pulps treated with xylanase were next bleached with NaOH in a single stage. The results of the chemical composition of the pulps treated with xylanase followed by alkaline extraction are shown in Table 3. A long treatment time of 20 h and high enzyme charge were not effective in biobleaching of sugar cane straw pulp. According to Christov and Prior (17), accessibility problems arise for dissolving pulp because chemical bleaching apparently removed more accessible portions of xylan from the cell walls, leaving the remaining part in locations that were less accessible to xylanase. Senior et al. (42) reported that prolonged incubation times were not as effective as a series of subsequent short treatments of pulp using xylanase in which about 50% of xylan could be removed.

Sugar cane straw pulps treated with 5 and 10 IU/g of enzyme resulted in a 3.3 cP viscosity, a marginal increase compared with unbleached pulp, and increasing the enzyme charge decreased viscosity. Pulps treated with xylanase followed by alkaline extraction gave higher viscosity than that treated only with enzyme, but only 20 IU/g gave a viscosity increase

Table 2 Chemical Composition of Unbleached and Xylanase Bleached Pulps With Different Enzyme Dosages

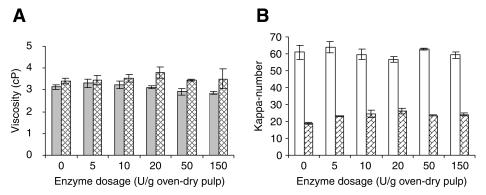
Enzyme dose (U/g)	0	5	10	20	50	150
Glucan (%)	$61.7 \pm 2.1$	$66.5 \pm 0.7$	$68.9 \pm 1.2$	$64.2 \pm 2.6$	$64.7 \pm 1.5$	$61.8 \pm 3$
Pentosan (%)	$3.2 \pm 0.3$	$3.2 \pm 0.1$	$2.4 \pm 0.1$	$3.1 \pm 0.2$	$3.4 \pm 0.2$	$2.8 \pm 0.2$
Lignin (%)	$31.5 \pm 2.4$	$29.0 \pm 1.8$	$28.9 \pm 2.4$	$28.5 \pm 1.9$	$26.9 \pm 0.8$	$27.9 \pm 0.1$
Ash (%)	$4.1\pm0.3$	$3.5\pm0.5$	$3.4 \pm 0.3$	$3.3 \pm 0.1$	$3.2\pm0.1$	$3.4\pm0.3$

Table 3 Chemical Composition of Unbleached, Xylanase Bleached Pulps With Different Enzyme Dosages Followed by Alkaline Extraction

Enzyme dose	0	5	10	20	50	150
(U/g) Glucan (%)	$70.1 \pm 0.6$	$74.5 \pm 2.2$	$75.4 \pm 1.5$	$73.2 \pm 2.2$	$75.6 \pm 0.6$	71 ± 2.7
Pentosan (%)	$2 \pm 0.1$	$2.3 \pm 0.1$	$2.1 \pm 0.1$	$2 \pm 0.1$	$2.2 \pm 0.1$	$1.8 \pm 0.1$
Lignin (%)	$13.1 \pm 2.3$	$12.3 \pm 2.5$	$11.4 \pm 0.1$	$11.7 \pm 1.3$	$13.5 \pm 2.8$	$12.9 \pm 0.3$
Ash (%)	$2.7 \pm 0.1$	$3 \pm 0.6$	$2.8 \pm 0.2$	$2.8 \pm 0.1$	$3 \pm 0.1$	$2.8 \pm 0.3$

(Fig. 1A). Enzyme charges of 10 and 20 IU/g of xylanase decreased the kappa-number of sugar cane straw pulps, and pulp treated with 20 IU/g presented the lowest kappa-number (57). The kappa-number of the pulps treated with different enzyme dosages followed by alkaline extraction were virtually the same (Fig. 1B).

Bisson et al. (43) evaluated xylanase from *Thermomyces lanuginosus* SSBP on bagasse soda pulp, varying the charge over a range of 5–150 IU and found that increasing the xylanase treatment from 50 to 150 IU/g only changed the kappa-number reductions by 0.5 and 0.6 points, respectively. The kappa-number of the control and xylanase pretreated pulps after DED bleaching were the same. Although kappa-reduction has been attributed to lignin removal, hexeneuronic acid has been shown to account for a significant fraction of the oxidizable components in Kraft pulps (44). Jeffries and Davis (45) showed that there is an excellent correlation between xylanase activity and hexeneuronic acid; however, kappareduction did not correlate directly with either of these factors. These



■ Enzyme 

Enzyme followed alkakine extraction 

Enzyme 

Enzyme followed alkaline extraction

**Fig. 1.** Pulps treated with different enzyme dosages and followed by alkali extraction. **(A)** Viscosity and **(B)** kappa-number.

findings indicate that xylanase pretreatment can result in the release of other compounds, which influence the kappa-number determination (44).

Jiang et al. (46) studied the biobleaching boosting effect of recombinant xylanase B from the hyperthermophilic Thermotoga maritima on wheat straw soda-anthraquinone pulp and found that an increase in enzyme dosage from 50 to 150 UI/g dry pulp did not decrease the kappa-number in the pulps, but did increase brightness by 4.7%. International Organization for Standardization (ISO) whereas decreased tensile index by 2% and broke length by 2.5%. Ideally, it would be desirable for xylanase treatment to decrease the kappa-number and increase viscosity, resulting in an increase in the viscosity per kappa-number ratio for pulps treated with xylanase. The variation in viscosity with kappa-number (i.e., the selectivity of the process) is shown in Fig. 2. Pulps treated with low enzyme dosage (5 IU/g) presented same seletivity compared with that, control pulp and pulps treated with 20 IU/g presented the higher seletivity (Fig. 2A). The alkali extraction of xylanse-treated pulps provided a similar selectivity in the pulps treated with different enzyme dosage (Fig. 2B). In comparison, Roncero et al. (4) treated wheat straw soda pulps by a TCFbleaching sequence using ozone and xylanase, giving a similar selectivity to the unbleached pulp but low selectivity in the peroxide stage.

#### FT-IR and PCA of the Pulps

FT-IR spectra of unbleached, xylanase biobleached, and xylanase followed by alkali extraction pulps were recorded and corrected for the 1860 and 780 cm<sup>-1</sup> fingerprint regions and normalized to the 902 cm<sup>-1</sup> C-O-C region. PCA is a statistical program and in PCA bidimensional plot, one FT-IR spectra of the pulp represent one point. In the bidimensional plot, the points that are near represent similar pulps. Spectra of the pulps treated with different enzyme charge, unbleached pulp, and xylanase



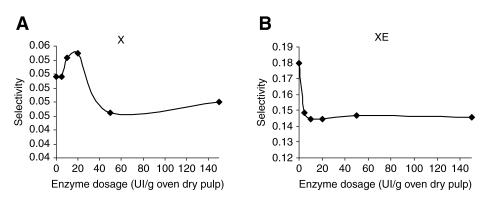
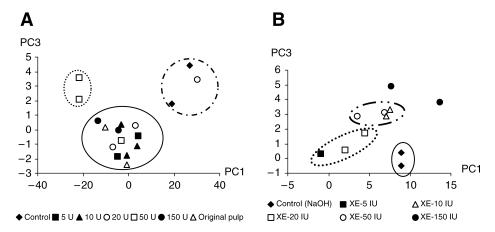
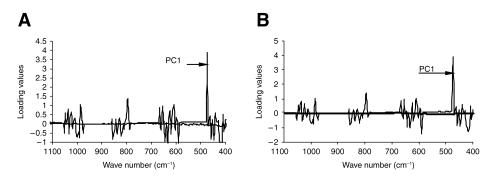


Fig. 2. (A) Sugar cane straw ethanol/water pulps treated with different enzyme dosages and (B) xylanase followed by alkali extraction pulps.



**Fig. 3. (A)** Score values (PC3×PC1) of the pulps treated with different enzyme dosages and **(B)** score values (PC3 × PC1) from FTIR spectra of unbleached, xylanase bleached, and xylanase followed by alkali extraction sugar cane straw ethanol/water pulps.

followed by alkali extraction-treated pulps were very similar, so FT-IR spectra were better analyzed by PCA, as shown in Fig. 3. Pulps treated with different enzyme charge, three groups of pulps could be identified: unbleached pulps (control pulps) are differentiated (highlighted by noncontinuous ellipse), the pulps treated with 20 UI/g enzyme dosage were highlighted by trace ellipse and in the other group were the pulps treated with different enzyme dosages (Fig. 3A). Thus, it was possible to confirm that the pulp treated with 20 IU/g is different from the other pulps and presented reduction in kappa-number. Chemical analysis and the physical properties of pulps treated with different enzyme loading did not provide many differences between the pulps, but in the PCA analysis it was possible to differentiate the pulps treated with xylanase followed by alkali extraction. In Fig. 3B, PC3 × PC1 plot, four groups of pulps were differentiated: control



**Fig. 4.** Loading values of PC1 of FTIR spectra of sugarcane straw ethanol/water pulps **(A)** xylanase-treated pulps with different enzyme dosages and **(B)** xylanase followed by alkali-extraction pulps.

pulps were highlighted by continuous ellipse, the pulps treated with 5 and 20 IU/g enzyme dosage were highlighted by trace ellipse, in the middle of PCA plot were pulps treated with 10 and 50 IU/g, and up the plot were pulps treated with higher enzyme dosage (150 IU/g). The first three PCs explain more than 93% of the total variance of the system, PC2 and PC3 being the principal factors for the differentiation between pulp spectra. This is better analyzed by the loading values of each PC (Fig. 4). From Fig. 4, for the sugar cane straw ethanol/water pulps, the influence of infrared bands on PC scores can be evaluated showing that PC1 was influenced by C–O (1000 cm<sup>-1</sup>) bonds present in the esters.

#### **Conclusions**

The temperatures and times required for pulping of sugar cane straw by ethanol/water mixtures were more severe than that needed for the sugar cane bagasse. Furthermore, the pulp obtained had low pentosan (3.2%) and high lignin (31.5%) contents. Thus, the viscosity of the pulp was low (3.14 cP) and the kappa-number was high (58.09). Xylanase enzyme appeared to be effective for loadings less than 20 IU/g. However, it did not produce a direct delignification effect on pulp. A high enzyme dosage (150 IU/g dry pulp) and long time (20 h) of xylanase treatment of the pulp did not decrease the kappa-number of the pulp. There were insignificant differences in the physical properties of the enzyme-treated pulp compared with the reference pulp. Different enzyme dosages resulted in different chemical compositions for the pulps that can be identified through PCA.

# Acknowledgments

This work was supported by Conselho Nacional do Desenvolvimento Científico e Tecnológico, Fundação de Amparo à Pesquisa do Estado de

São Paulo and Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (all Brazilian Agencies).

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