Appl Biochem Biotechnol (2008) 148:151–161
DOI 10.1007/s12010-007-8120-0

Oxidation in Acidic Medium of Lignins from Agricultural Residues

Gisele Aparecida Amaral Labat · Adilson Roberto Gonçalves

Received: 9 May 2007 / Accepted: 3 December 2007 /
Published online: 3 January 2008

Abstract Agricultural residues as sugarcane straw and bagasse are burned in boilers for generation of energy in sugar and alcohol industries. However, excess of those by-products could be used to obtain products with higher value. Pulping process generates cellulosic pulps and lignin. The lignin could be oxidized and applied in effluent treatments for heavy metal removal. Oxidized lignin presents very strong chelating properties. Lignins from sugarcane straw and bagasse were obtained by ethanol–water pulping. Oxidation of lignins was carried out using acetic acid and Co/Mn/Br catalytical system at 50, 80, and 115 °C for 5 h. Kinetics of the reaction was accomplished by measuring the UV-visible region. Activation energy was calculated for lignins from sugarcane straw and bagasse (34.2 and 23.4 kJ mol^{-1}, respectively). The first value indicates higher cross-linked formation. Fourier-transformed infrared spectroscopy data of samples collected during oxidation are very similar. Principal component analysis applied to spectra shows only slight structure modifications in lignins after oxidation reaction.

Keywords Sugarcane bagasse · Sugarcane straw · Oxidation in acidic medium · Chelating agents · FTIR · PCA

Introduction

Agricultural residues are produced in large quantities throughout the world. Approximately 280 kg residue is produced for each ton of sugarcane in the alcohol industries. In average, 140 kg bagasse and 140 kg straw are generated. Both by-products have high potential of

G. A. A. Labat · A. R. Gonçalves (✉)
Departamento de Biotecnologia (Debiq), Escola de Engenharia de Lorena—EEL/USP, Caixa Postal 116, 12.602–810 Lorena, SP, Brazil
e-mail: adilson@debiq.eel.usp.br
URL: http://www.debiq.faenquil.br/adilson

G. A. A. Labat
e-mail: amaral@alunos.eel.usp.br

energy to be used. For example, the bagasse energy has been already extracted in thermal industries [1].

In energy terms, industries of sugar and alcohol are sustainable, in which about 90% bagasse (calorific power 18.322 kJ kg^{-1}) is used; however, the consumption can be reduced for 70% if the system of steam generation was optimized. Straw can also be mixed to the bagasse to be burned because of high calorific power (18.870 kJ kg^{-1}) [2] but with the drawback of producing more ashes.

Residues proceeding from sugarcane (bagasse and straw) are lignocellulosic materials. These materials are important sources for cellulose, hemicellulose, and lignin. Lignin is a high-complex and cross-linked macromolecule generated by dehydrogenative polymerization promoted by enzymes from hydroxycinnamylic alcohols (*p*-coumarylic, conniferylic, and synapylic). Figure 1 shows three precursors of lignin. The copolymerization from these alcohols furnishes a high-molar mass heterogenic macromolecule, optically inactive and polydisperse [3]. Figure 2 shows a schematic structure of lignin [4].

Currently, methods that use agricultural residues for chemical products obtainment have been studied, as cellulosic pulps production with applications in cardboard packing [5] and the oxidized lignin obtainment, which owns very strong chelating properties [6–8], because of its oxygenated functional groups. This becomes the oxidized lignin with great utility in the industrial effluent treatment. The use of pulps and lignins allows the integral use of the vegetable biomass and makes it possible to obtain products with higher value.

Heavy metals represent one of the major industrial contaminants of the soil and the plant ecosystem [9]. The environment pollution with toxic heavy metals is spreading throughout the world along with industrial progress [10]. The main sources of contamination with heavy metals are fertilizer impurities (Cd) [11]; the use of refuge-derived compost and sewage sludge (Cd and Ni) in irrigation, mining, and mine wastes; discharges from smelters and refineries [12]; tanneries that use chromium for a quality improvement of the leather [13]; and mainly, the use of Cd and Ni on the rise in

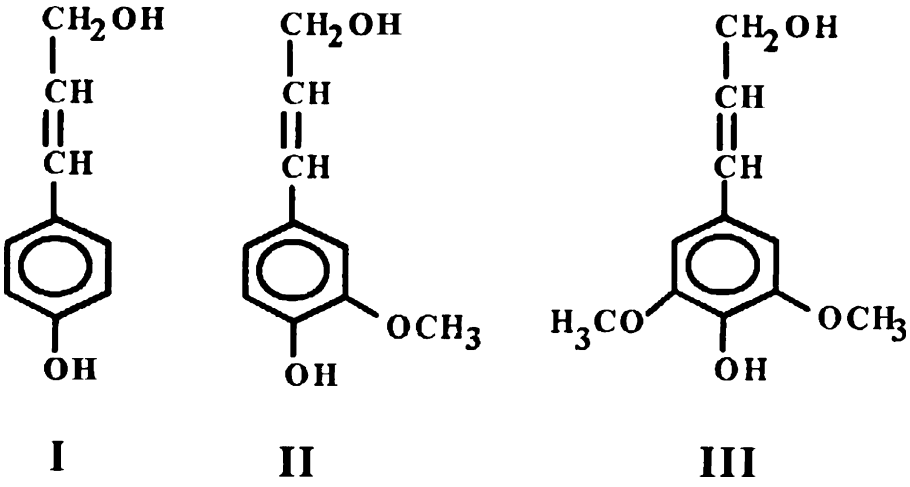

Fig. 1 Structural monomers of lignin: *p*-coumaryl alcohol (*I*), coniferyl alcohol (*II*), and sinapyl alcohol

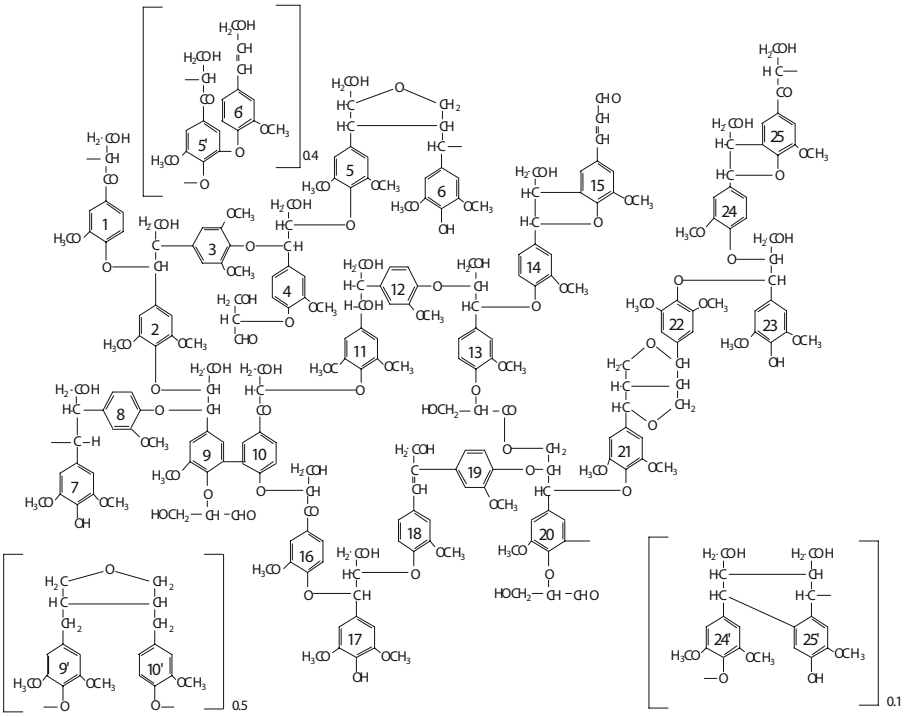

Fig. 2 Schematic structure for lignin (*Fagus sylvatica*)

electroplating, batteries, alloys, pigments, stabilizers for catalysts, and in semiconductors and TV tube phosphors [14].

An important factor is the kinetic study of the lignin oxidation in acidic medium, which is determined by the activation energy and its kinetic reaction.

In this work, lignin oxidation in acetic acid medium with the catalytic system $Co(OAc)_2$/$Mn(OAc)_2$/HBr was carried out. The catalytic system is based on oxidation of alkyl aromatic compounds by O_2. Reaction of cobalt(II) with O_2 forms cobalt(III) bromoacetate, which is catalytically decomposed through homolityc cleavage forming cobalt(II) and radical bromine. In the sequence, radical bromine removes an electron of the aromatic ring of the lignin starting the oxidation of the macromolecule. Manganese(II) ion acts on the system, decomposing the cobalt(III) [15].

$$M^{n+} + ArCH_3 \Rightarrow M^{(n-1)+} + [ArCH_3]^{\bullet +} \tag{1}$$

$$[ArCH_3]^{\bullet +} \Rightarrow ArCH_2^{\bullet} + H^+ \tag{2}$$

Methods of lignin oxidation in acidic media use mainly the Amoco system, used in the auto-oxidation of alkylbenzens into aldehydes and carboxylic acids, catalyzed by cobalt and manganese [16].

The easiness of one-electron transfer is directly related to the ionization potential of aromatic hydrocarbons and to electron donor substitutes, such as methoxyl, which decreases the oxidation potential of lignins dissolved in acetic acid [1].

To express the equation of a reaction rate, any kind of measurement proportioned to the concentration can be used. In the systems using constant volume, the action velocity (V) is simply given by

$$V = \frac{\mathrm{dC}}{\mathrm{dt}}, \text{where } C \text{ is the concentration and } t \text{ is the time}$$

The reaction rate of every component is given by the rate variation of its concentration, which is proportional to the time at first-order kinetics. A concentration graphic versus time shows a straight line with inclination k (reaction rate constant) [17].

The lignin oxidation from sugarcane straw and bagasse using Fourier-transformed Infrared spectroscopy (FTIR) followed by principal component analysis (PCA) was also studied.

The aim of this work was to oxidize lignins from agricultural residues, as sugarcane straw and bagasse, aiming heavy metals removal from industrial effluents, determining the activation energy, its reaction kinetics, and analysis of the infrared region.

Materials and Methods

Biomass Origin

Sugarcane bagasse and straw were obtained from Ester Industry of the São Paulo State.

Liquors and Lignins Obtainment

Ethanol–water pulping of sugarcane bagasse [18] and straw [19] were carried out as described by Curvelo and Pereira [20] using optimized temperature and times [18–19].

Lignins were precipitated from liquors by sulfuric acid and ice addition until reaching pH 2. Liquors were filtered, and the lignin was washed with distilled water and oven dried at 60 °C for 24 h for the humidity complete removal.

Oxidations Reactions

A 500-ml round-bottom flask equipped with condenser and magnetic stir was charged with 200 ml glacial acetic acid, 2 g lignin, 1.68 g cobalt acetate (II), 0.2 g manganese acetate (II), and 9.1 ml HBr 33% *w*/*v* under water bath at 50 and 80 °C or silicon oil bath at 115 °C. Oxygen was set at 60 ml min^{-1} flow rate.

Experiments were made in triplicates. The reaction was kept for 5 h. After the reaction temperature reached the desired value, a 2-ml sample was collected at each 30 min until the end of the reaction. One milliliter of this sample was diluted to 10 ml with acetic acid, and 1 ml aliquot of this new sample was diluted to 10 ml with distilled water. Diluted samples were filtered to remove the eventual precipitate (oxidized lignin). The absorbances of the prepared samples were measured in a CINTRA 20 spectrometer at 280 nm.

From the obtained data, graphics were done from the Neperian logarithm of the absorbance versus the reaction time that furnishes, by the straight line equation, the reaction rate constant (k) as angular coefficient. Using the best k values, the Neperian logarithm graphic, as a function of $1/RT$, was done for the determination of the activation energy.

The samples were also submitted to the analysis of the infrared region (400–4,000 cm^{-1}) in a Nicolet Avatar 320 FTIR spectrometer. A 0.15-ml aliquot was dripped on

200 mg KBr. The mixture was homogenized and evaporates in oven (60 °C) for 12 h. Pellets were obtained from the mixture after grinding and spectra recorded with 12 scans with 4 cm^{-1} resolution. The absorbances in the range of 400–4,000 cm^{-1} (935 data points per oxidized lignin spectrum) were normalized by the absorption at 1,510 cm^{-1}, corresponding to the vibrations of aromatic rings, and baseline corrected [21] using OMNIC software. Spectra were converted to text files using OMNIC software (Nicolet). Data were submitted to a statistical analysis from the variance of the intensities through the PCA using the softwares Biotec and FAEN4 compiled in Fortran, which were written based on the work of Scarminio and Bruns [22]. Graphic presentations were easily made with Microsoft Excel 2002.

Results and Discussion

Kinetics and Activation Energy

Experiments of lignin oxidation were performed in acetic acid medium in the presence of oxygen and catalysts $Co(OAc)_2/Mn(OAc)_2/HBr$.

For the kinetic study, an approach of the lignin oxidation reaction was made for a reaction of pseudo-first order, as shown in Eq. 3.

$$\text{Lignin} + [O_2] \xrightarrow{\text{cat}} \text{oxidized lignin} \tag{3}$$

The kinetic equation that represents this process independently of the oxygen concentration is given by Eq. 4.

$$V = \frac{-d[\text{lignin}]}{dt} = \frac{d[\text{oxidized lignin}]}{dt} = -k.[\text{lignin}] = k.[\text{oxidized lignin}] \tag{4}$$

The integrate shows

$$\text{Ln}[\text{oxidized lignin}] = kt + C_0 \tag{5}$$

$$\text{LNA}_{280} = Kt + C \tag{6}$$

where C_0 is the concentration of the original lignin that is constant, k is the rate constant, and t is the time. Using the Beer–Lambert's Law, the concentration of the oxidized lignin can be substituted by the absorbance at 280 nm (Eq. 6).

By this way, the logarithm of the absorbance in relation to the reaction time has as angular coefficient the reaction rate constant and the value of the linear coefficient C related to the lignin initial concentration.

The points with possible experimental errors were removed from the graphics for a better result analysis and calculated the reaction rate constant with its respective R^2 values (Table 1 and 2) based on pseudo-first order kinetics.

Values of k varied from 1.10×10^{-2} h^{-1} to 15.3×10^{-2} h^{-1} for the oxidation reaction of lignin from sugarcane straw, whereas, for oxidation reactions of lignin from sugarcane bagasse, it varied from 10.07×10^{-2} to 56.61×10^{-2} h^{-1}.

For the calculation of the activation energy, the average of the rate constants was used for each temperature, and the average of the temperature during the experiment that oscillated at ±2 °C.

Table 1 Values of rate constants and R^2 for the oxidation reaction of lignins from sugarcane straw.

Temperature (°C)	Experiment 1, k (h^{-1}) 10^{-2} (R^2)	Experiment 2, k (h^{-1}) 10^{-2} (R^2)	Experiment 3, k (h^{-1}) 10^{-2} (R^2)
115	9.90 (0.8777)	15.3 (0.9358)	12.7 (0.9522)
80	6.20 (0.9732)	1.20 (0.8893)	1.70 (0.7922)
50	1.10 (0.9711)	1.90 (0.9970)	8.00 (0.9312)

The graphic made with Neperian logarithm of k as a function of $1/RT$, where R is the universal gas constant (8.314 J mol^{-1} K^{-1}) and T is the average experimental temperature (in Kelvin), has as an angular coefficient the activation energy (Ea), as observed in the equation below:

$$\mathrm{Ln\ k} = \mathrm{LnA} - \frac{\mathrm{Ea}}{\mathrm{RT}}$$

A certain discrepancy between the values of k at the same temperature can be observed. This may be caused by the fact that the oxidation medium is not totally homogeneous, turning difficult the occurrence of an identical replication or by the difference in the oxygen amount dissolved in the medium. A difference in the linearity coefficient values was found: 8.6556 for the first (straw) and 6.5263 for the second (bagasse), a 24.6% lower value (Figs. 3 and 4). These values are related to the concentration of the original lignin; however, they are different probably by the same fact of the discrepancy between the k values.

Activation energy calculated for the oxidation reaction of lignin from sugarcane straw was 34,239 J/mol (R^2=0.9676; Fig. 3), a value higher than that found in the oxidation reaction of lignin from sugarcane bagasse, which is 2,3378 J/mol (R^2=0.9721; Fig. 4).

The differences between the activation energy and linearity coefficient values can be related to the fact that the lignin from straw presents higher cross-linked formation. This fact can be also corroborated by the colors of the original lignins. Lignin from sugarcane straw presents a darker color indicating to possess more C–C linkages.

FTIR Analysis

The spectra had the baseline corrected by the polygonal method [21]. The used region was from 400 to 4,000 cm^{-1}, and the spectra were normalized for the 1,510 cm^{-1} (characteristic vibration of aromatics), originating 390 variables for both used lignins. Spectra were recorded in a Nicolet Avatar 320 FTIR spectrometer. Spectra were converted to text file files using OMNIC software (Nicolet).

Spectra in Fig. 5 were obtained by FTIR relative to the experiment performed at 80 °C for lignin from sugarcane bagasse. The peaks at 1,600 and 1,506 cm^{-1} correspond to the

Table 2 Values of rate constants and R^2 for the oxidation reaction of lignins from sugarcane bagasse.

Temperature (°C)	Experiment 1, k (h^{-1}) 10^{-2} (R^2)	Experiment 2, k (h^{-1}) 10^{-2} (R^2)	Experiment 3, k (h^{-1}) 10^{-2} (R^2)
115	56.61 (0.9106)	28.13 (0.9995)	34.14 (0.9725)
80	14.83 (0.8588)	29.77 (0.8083)	25.11 (0.8947)
50	10.61 (0.9171)	11.09 (0.9396)	10.07 (0.9165)

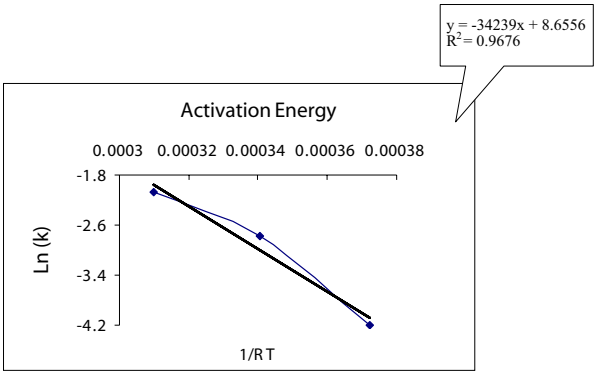

Fig. 3 Activation energy calculated for the oxidation reaction of lignins from sugarcane straw

C = C and aromatic nucleus; the region between 1,200 and 1,110 cm^{-1} is relative to the C–O bands, and the region between 800 and 400 cm^{-1} is relative to the bands of aromatic substitutes.

These spectra and those of other temperatures are very similar because they present the same picks with similar intensities. For a better evaluation of the results, spectra were submitted to analysis by PCA.

The Biotec program originated these variables for each treated spectrum, which were stored in a data file in the insert sequence of the spectra.

The program FAEN2 was used to calculate and, after treatment of the spectra, ten principal components (PCs) were originated, which contain the information of the analyzed spectra. The FAEN2 program provides each principal components with its percentage of total explained variance.

The principal component 1 PC1 explains (64.96 and 75.13%) the variance of the original data, PCs 2 and 3 explain (16.95 and 12.54%) and (11.23 and 3.43%) for straw and bagasse, respectively. The other PCs provide little explanation of the original data (6.45 and 7.96%). Figure 6 shows a graph of the variance explained in each PC; the arrow indicates the ideal number of principal components to represent the original data. These figures show that the PC3 does not have a very high variation of the explained variance. Applying the PCA was possible to reduce the original dimension of the data for three PCs.

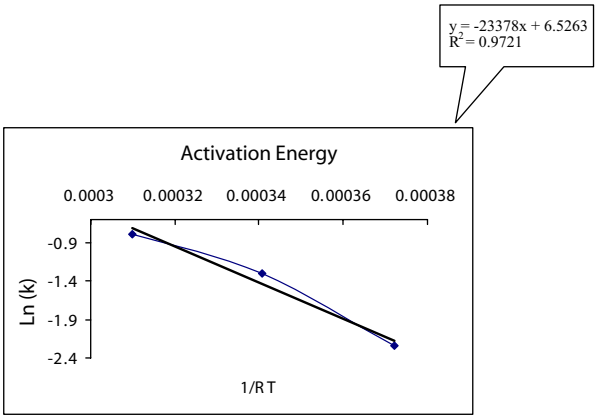

Fig. 4 Activation energy calculated for the oxidation reaction of lignins from sugarcane bagasse

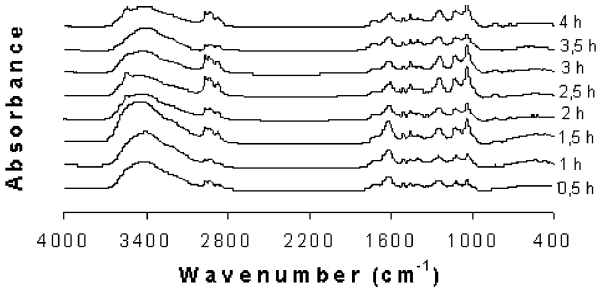

Fig. 5 FTIR spectra of the samples of the experiment performed at 80 °C for lignin from sugarcane bagasse

The first three principal components explain more than (93.14 and 91.1%) of the total variance of the system, utilizing lignin from sugarcane straw and bagasse, respectively. This means that the 390 variables for each temperature can be reduced to only three with more than 90% confidence level. Each spectrum can be reduced to a single point, as shown by PC1 × PC2 and PC1 × PC3 graphics (Figs. 7 and 8), where differences can be evaluated.

In graphs of PC1 × PC2 and PC1 × PC3, it is important to analyze the similarities and differences between the experiments carried out at different temperatures. Close points mean that the samples are similar, and spaced points mean that they are different. These graphs show that different temperatures for the oxidation reaction led to different behaviors. With the temperature increase, the score values acquired higher dispersion in the data, with exception of some points. This behavior can be better observed in the graphs of the lignin from straw. For example, in the graph of PC1 × PC2 for lignin from sugarcane bagasse, the temperature of 50 °C presented the very close scores values with exception of only three points, and the temperature of 115 °C presented distant scores values, indicating that the temperature possesses influence on the reaction of lignin oxidation in acidic medium. The ellipses show the highest concentration of the score values for the different temperatures.

The behavior of the scores values is influenced by the loadings graphics (Fig. 9). The more distant the loading value is from zero, the larger the contribution of this variable for the principal component in study.

Loadings graphs present bands more distinct from 1,170 cm^{-1}; therefore, in the region from 400 to 1,170 cm^{-1}, the sampling presented peaks with many noises, mainly for principal component 1.

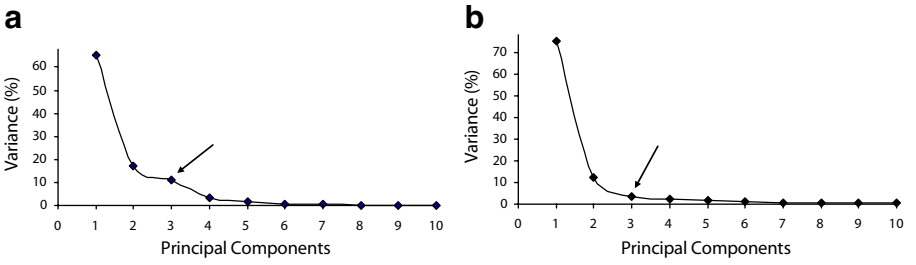

Fig. 6 Variance explained for each principal component for the oxidation reaction with lignins from **a** sugarcane straw and **b** sugarcane bagasse

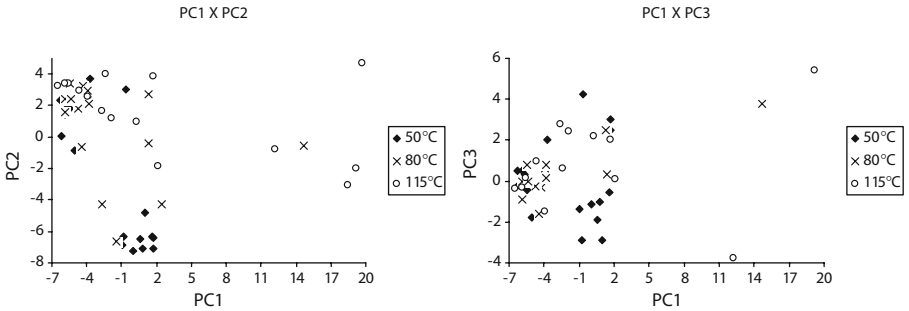

Fig. 7 Scores values of PC1 × PC2 and PC1 × PC3 from FTIR spectra of samples of lignin from sugarcane straw

The principal component 1 has a large percentage of variance (65 and 75%) for straw and bagasse, respectively, indicating to possess higher information of system.

In Fig. 9a and b, the principal component 1 presents strong contribution in the bands at 1,610, 1,630, and 1,650 cm^{-1}, characteristic of C = C aromatics and nonconjugated C = O. Figure 9a presents bands at 1,730 cm^{-1}, characteristics of C = O.

In Fig. 9b, PC3 seems to have a larger contribution on the system because of the present bands between 1,610 and 1,630 cm^{-1}. This behavior can be explained because of the variance of the sampling, by the fact that oxidation medium is not totally homogeneous and by the accumulated mistakes from the retreat of the aliquots to making pellets for reading in FTIR [23].

The existence of the C = C bands can be related to the fact that the oxygen is a soft oxidant and does not cleavage aromatic rings from lignin, and the C = O bands can be related to the lignin oxidation.

The studies carried out in this work have indicated that the original lignins obtain approximately 20% removal of heavy metal [24]. Using the oxidized lignin is intended to reach a higher value for the heavy metals removal.

Conclusions

The use of agricultural residues allows the integral use of biomass for production of materials with higher value. Pulping reactions generate cellulose that is destined for

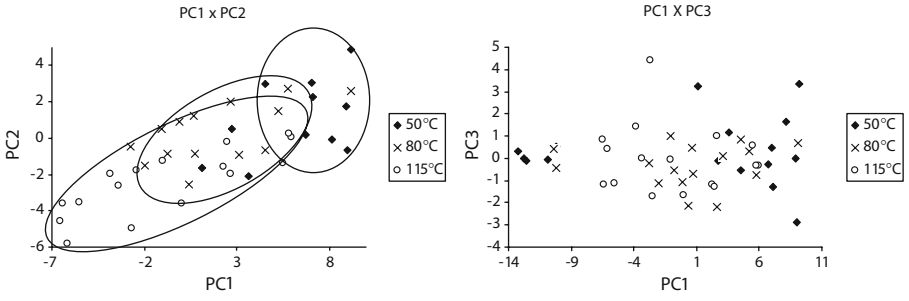

Fig. 8 Scores values of PC1 × PC2 and PC1 × PC3 from FTIR spectra of samples of lignin from sugarcane bagasse

manufacture of paper and lignins, which when being oxidized, becomes efficient in the treatment of effluent with heavy metals.

The technique of UV/visible was sufficiently viable in this work; therefore, it can supply kinetic information of the oxidation process.

The infrared spectroscopy is very important to understand what occurs with the spectrum of lignin after oxidation; therefore, it is an almost direct measure of carbonyls and hydroxyls groups responsible for the chemical modifications.

PCA is widely used in complex biotechnological processes as the lignin oxidation. This tool makes possible the differentiation between samples of oxidized lignins.

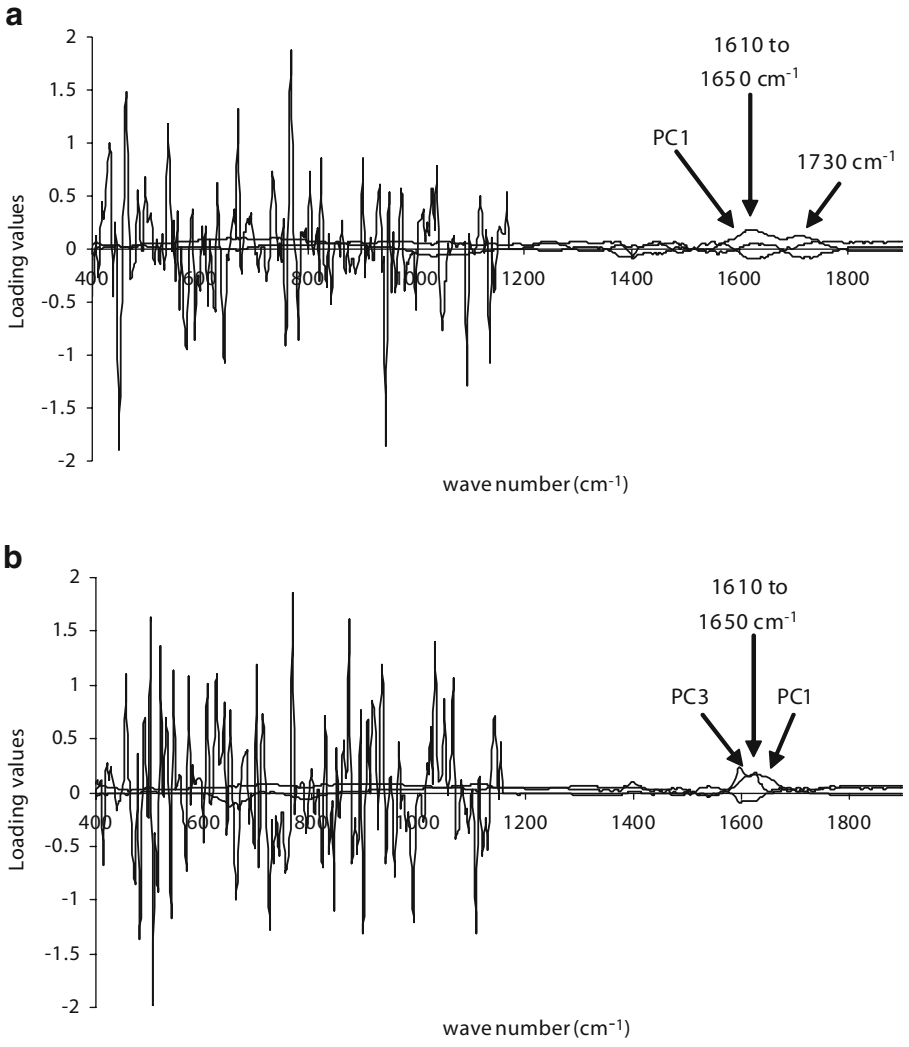

Fig. 9 Loadings values of PC1, PC2, and PC3 of FTIR spectra for the oxidation reaction of lignin from **a** sugarcane straw **b** sugarcane bagasse

The studies indicated that lignin possess strong chelating properties; however, with the oxidation, the chelating properties of the lignin become more evident and could be an alternative in the treatment of industrial effluents containing heavy metals.

Acknowledgment The authors acknowledge financial support from FAPESP, CNPq, and Lignocarb–ALFA Program.

Reference

1. Lobo, P. C., Jaguaribe, E. F., Rodrigues, J., & da Rocha, F. A. A. (2007). *Applied Thermal Engineering*, *27*, 1405–1413.
2. Ripoli, T. C. C., Molina Jr, W. F., & Ripoli, M. L. C. (2000). *Science in Agriculture*, *4*(57), 677–681.
3. Fengel, D., & e Wegener, G. (1989). In *Wood: Chemistry, ultra structure, reactions* (pp. 132–181). Berlin: Walter de Gruyter.
4. Nimz, H. H. (1974). *Angewandte Chemie International edition in English*, *13*, 313.
5. Costa, S. M. (2005). DR thesis, Departamento de Biotecnologia/FAENQUIL, Lorena, Brazil.
6. Gonçalves, A. R., & Luz, S. M. (2000). In *Catalizadores y Adsorventes Iberoamericanos para la Remoción de Metales Pesados de Efluentes Industriales*. P. Á. Garcia (Ed.), Ediciones Cyted (pp. 159–168). Madrid
7. Gonçalves, A. R., Luz, S. M. (2001a). Poster presentations, proceedings, Guaratinguetá, pp. 345–342, Brazil.
8. Gonçalves, A. R., Luz, S. M. (2001b). Poster presentations, proceedings, Guaratinguetá, pp. 266–269, Brazil.
9. Ghoshroy, S., Freedman, K., Lartey, R., & Citovsky, V. (1998). *Plant Journal*, *13*, 591–602.
10. Dönmez, G., & Aksu, Z. (1999). *Proceedings in Biochemistry*, *3*, 135–142.
11. Schickler, H., & Caspi, H. (1999). *Physiologia Plantarum*, *105*(1), 39–44.
12. Chaoui, A., Mazhoud, S., Ghorgbal, M. H., & El Ferjani, E. (1997). *Plant Science*, *121*(2), 139–147.
13. Jordão, C. P., Da Silva, A. C., Pereira, J. L., & Brune, W. (1999). *Química Nova*, *22*, 47–52.
14. Kefala, M. I., Zouboulis, A. I., & Matis, K. A. (1999). *Environmental Pollution*, *94*, 283–293.
15. Partenheimer, W. (1991). *Journal of Molecular Catalysis*, *67*, 35–46.
16. Sheldon, R. A., & Kochi, J. K. (1981). pp. 121–133, 315–328. New York: Academic.
17. Levenspiel, O. (2000). In *Engenharia das Reações Químicas*. São Paulo: Edgard Blücher, pp. 21–22.
18. Ruzene, D. S. (2005). DR thesis, EEL/USP, Lorena, Brazil.
19. Moriya, R. Y., Gonçalves, A. R., & Duarte, M. C. T. (2006). 28th Symposium on Biotechnology for Fuels and Chemicals, EUA.
20. Curvelo, A. A. S., & Pereira, R. (1995). 8, Helsinki 1995. Proceedings. V.2, pp. 473–478.
21. Faix, O. (1992). S. Y. Lin and C. W. Dence (eds.), Springer, Berlin, pp. 83–109.
22. Scarminio, I. S., & Bruns, R. E. (1989). *Trends in Analytical Chemistry*, *8*, 326–327.
23. Benar, P., Mandelli, D., Ferreira, M. M. C., Schuchardt, U., & Gonçalves, A. R. (1999). *Journal of Wood Chemistry and Technology*, *19*, 155–165.
24. Gonçalves, A. R., & Ventura, T. R. (2003). Proceedings. VII Encontro de Iniciação Científica, São José dos Campos, SP, Brazil.