0273-2289/06/129–132/320–325/$30.00

Obtainment of Chelating Agents Through the Enzymatic Oxidation of Lignins by Phenol Oxidase

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Abstract

Oxidation of lignin obtained from acetosolv and ethanol/water pulping of sugarcane bagasse was performed by phenol oxidases: tyrosinase (TYR) and laccase (LAC), to increase the number of carbonyl and hydroxyl groups in lignin, and to improve its chelating capacity. The chelating properties of the original and oxidized lignins were compared by monitoring the amount of Cu^{2+} bound to lignin by gel permeation chromatography. The Acetosolv lignin oxidized with TYR was 16.8% and with LAC 21% higher than that of the original lignin. For ethanol/water lignin oxidized with TYR was 17.2% and with LAC 18% higher than that of the original lignin.

Index Entries: Lignin; enzymatic oxidation; polyphenoloxidase; chelating agents.

Introduction

In the past years there was an increased interest in the use of agricultural residues, like as the sugarcane bagasse, seeking less harmful techniques to the environment as well as the technological development, to obtain products of higher aggregated value *(1)*.

The lignin has many aromatic rings with high electronic density. Having the capacity to complex with metallic ions from aqueous solution this property can be used in the decontamination of industrial effluents.

The chemical or enzymatic oxidation of the lignin can promote the introduction of carbonyl groups and other functional group that have high electronic density, increasing the chelating power of this macromolecule *(2)*.

Enzymatic oxidation is more selective and in this work phenol oxidases (PO) were used. PO are oxidoreductase enzymes which catalyze reactions involving direct activation of oxygen; those enzymes do not need any other cellular component act, being easier to be used. The two major groups of phenol oxidases are the tyrosinases and the laccases. Tyrosinases are produced by several types of organisms, such as mushrooms, bacteria

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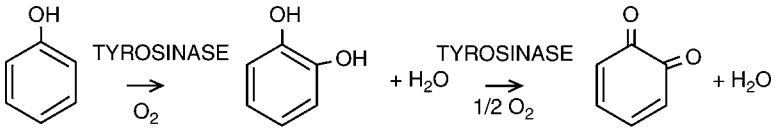
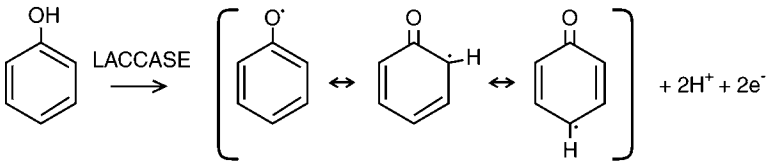
and superior plants being linked to the external membrane of the cells or soluble inside of the cellular medium *(3)*. This enzyme catalyzes two reactions: The insert of one oxygen in the orto position to the phenolic hydroxyl, and the oxidation of o-dihydroxyphenols to o-quinones *(2)*.



The laccase enzyme is produced by mushrooms and it can catalyze the oxidation of phenolic compounds to the correspondent form of free radical that is highly reactive, making possible the entry of oxygen molecules *(3)*.



In this work, lignins were oxidized by tyrosinase extracted from potatoes and by commercial laccase.

Materials and Methods

The enzyme tyrosinase (TYR) was extracted from potatoes and the laccase (LAC), was obtained from extracts of mushrooms (commercial enzyme NOVOZYM 51003, used as received). The potato extract was obtained by the grinding of 65 g of potatoes with 100 mL of 0.05 mol/L phosphate buffer pH 7.6. The homogeneous solution was filtered and centrifuged and the solution was immersed in an ice-bath and agitated, adding slowly 5 g of ammonium sulfate. The precipitate obtained was filtered and dried under a stream of nitrogen by 12 h and finally dissolved in 10 mL of phosphate buffer.

Lignin isolated from Acetosolv and ethanol/water pulping of sugarcane bagasse was oxidized by TYR and LAC using O_2 and glycerol in the homogenous phase (3:1 [v/v] 0.05 mol/L phosphate buffer: dioxane solution at pH 7.6, measured after mixing) for 5 h. Samples were taken every 30 min and analyzed by UV in a Cintra 200 spectrometer. Oxydized lignin was recovered after precipitation with HCl.

Chelated complexes were quantified using gel permeation chromatography with a Sephadex G-10 and the mobile phase was composed of 0.58 g NaCl, 1.21 g hydroymethylaminomethane (Tris-buffer) and 0.128 g of $CuCl_2 \cdot 2H_2O$ in 800 mL and the pH was adjusted to 8.0. Samples were prepared with 17 mg of original or oxydized lignins

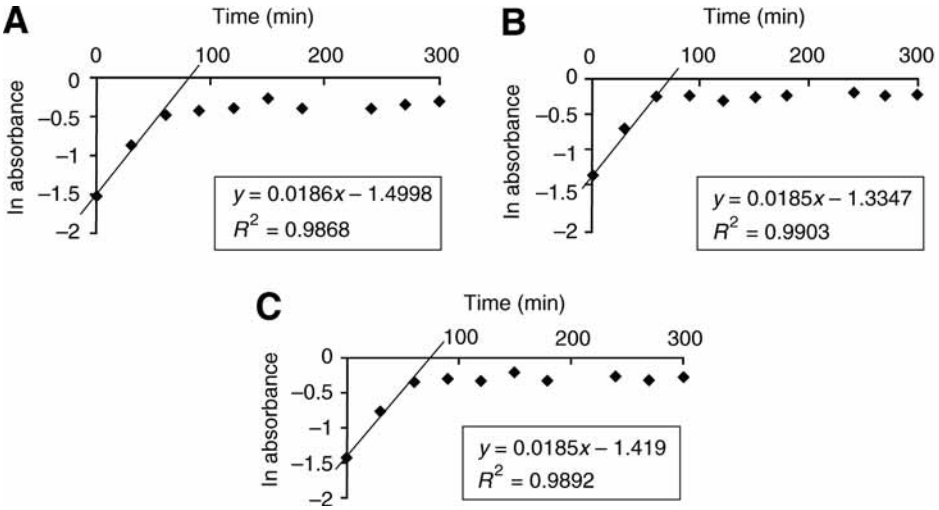


Fig. 1. ln (absorbance) in function of the time at 265 **(A)**, 280 **(B)** and 320 nm **(C)** for the oxidation of lignin with TYR.

in 10 mL of a 0.04 mol/L NaOH. Samples were applied to the top of the 35-cm column (1 cm internal diameter), using 0.5 mL of the solution. Fractions were collected during the elution of about 1 mL every 5 min. A volume of 0.5 mL of each fraction was added to 5 mL of sodium diethyldithiocarbamate (0.1019 g in 100 mL) and the mixture was completed to 25 mL with distilled water. The absorbance of the complex obtained was measured at 450 nm in a Cintra 200 spectrometer. A blank experiment was performed using 0.5 mL of 0.04 mol/L NaOH and the negative area obtained was considered in the presented calculations. For the calibration curve 5 standard Cu^{2+} solutions were used. Calibration method was validated with 95% confidence level and the data presented are significative also in 95% confidence level.

Results and Discussion

Variation of absorbance values was adjusted to a pseudo-first order kinetics for the oxidation and graphics of ln (absorbance) in function of the time were obtained. Figure 1 shows graphics for the enzymatic oxidation of the lignin using TYR enzyme and Fig. 2 graphics for LAC enzyme. It was verified that the coefficients found for the reaction catalyzed by the TYR enzyme were four times higher and also more uniform in relation to that found for the reaction catalyzed with LAC enzyme. The mean for TYR was 0.0185/min and for LAC the means was 0.0044/min.

For the chromatographic determination of copper ions retained for the lignin chromatogram as showed in Fig. 3 was obtained. The lignin is a larger molecule, and therefore its speed is faster than the mobile phase. The lignin is complexed with copper ions and at the end of the column,

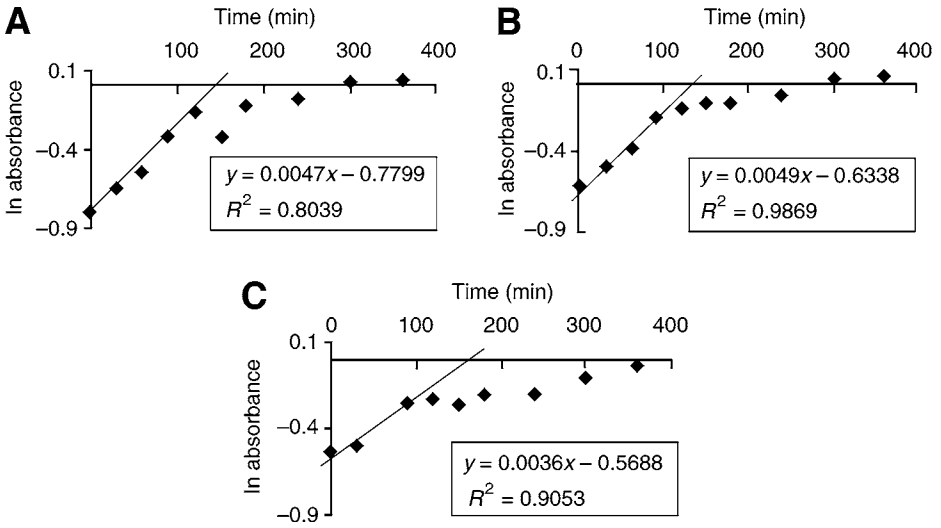


Fig. 2. ln (absorbance) in function of the time at 265 **(A)**, 280 **(B)**, and 320 nm **(C)** for the oxidation of lignin with LAC.

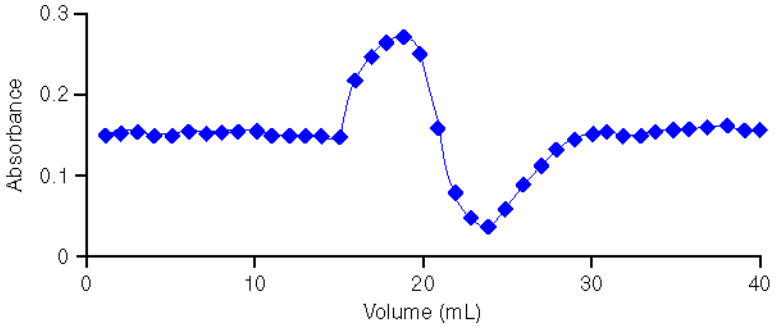


Fig. 3. Chromatogram for the elution of lignin with cupper ions.

a larger concentration of this ion will exist (positive area), and consequently a smaller concentration after the total passage of the lignin (negative area).

Theoretically the areas (positive and negative) should have the same value, and arithmetic mean the two areas was used, discounting the negative area due to the blank with NaOH.

Concentration at Cu^{2+} complexed by lignins Acetosolv original (LANO), oxidized by LAC (LAON) and by the TYR enzyme (LAOB) are displayed in Table 1. Through the Table 1 it was possible to observe that LAON presented an increase of 16.8% in the chelating power in all of the replicates in relation to LANO. The larger chelating power of 21% higher was obtained by the LAOB. In spite of both enzymes treatment presented an increase of chelating power, it was observed that the values obtained by

Table 1
Cu^{2+} Complexed by Acetosolv Lignin

Lignin		Area (absorbance units times volume [mL])	Corresponding Cu^{2+} mass (g)	mg Cu^{2+}/mg lignin	
				Replicates	Mean
	1	0.5121	0.1692	9.95	
LANO	2	0.5986	0.2203	12.96	11.9 ± 1.7
	3	0.5973	0.2195	12.91	
	1	0.6101	0.2271	13.36	
LAON	2	0.6516	0.2516	14.8	13.9 ± 0.8
	3	0.6183	0.232	13.65	
	1	0.5798	0.2092	12.31	
LAOB	2	0.6499	0.2506	14.74	14.4 ± 1.9
	3	0.6873	0.2727	16.04	

Table 2
Cu^{2+} Complexed by Ethanol/Water Lignin

Lignin		Area (absorbance units times volume [mL])	Corresponding Cu^{2+} mass (g)	mg Cu^{2+}/mg lignin	
				Replicates	Medium
	1	0.6646	0.2409	14.17	
LENO	2	0.5149	0.1738	10.22	12.2 ± 2.0
	3	0.5782	0.2082	12.25	
	1	0.6531	0.2525	14.85	
LEON	2	0.6206	0.2333	13.72	14.3 ± 0.6
	3	0.6387	0.2440	14.35	
	1	0.6863	0.2721	16.01	
LEOB	2	0.8801	0.3867	not used	14.4 ± 2.3
	3	0.5949	0.2181	12.83	

LAON were more uniform, as it can be observed in the value of the standard of mean values. Concentration at Cu^{2+} complexed by lignins ethanol/water original (LENO), oxidized by LAC (LEON) and by the TYR enzyme (LEOB) are displayed in Table 2. Through the Table 2 it was possible to observe again that LEON presented an increase of 17.2% in the chelating power in all of the replicates in relation to LENO. A similar chelating power (18%) was also obtained by the LEOB. The data presented confirm previous results with acetosolv lignins oxidized by TYR *(1,2)*.

Conclusions

The lignins were oxidized with success by TYR and LAC considering that the measure of absorbance was, theoretically, the increase of carbonyls

and hydroxyls in the lignin. In both cases, the oxidize lignins were tested as for the chelating power, which increased due to the oxidation, and the best results of retention of copper ions were obtained by the lignin oxidized by the extracted enzyme of potatoes (tyrosinase). However, the results of the lignin oxidized by the commercial enzyme NOVOZYM 51003 (laccase) were more uniform. Comparing Acetosolv and ethanol/water lignins, the second one had the higher chelating power.

Acknowledgments

The authors wish to thank the Brazilian agencies Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional do Desenvolvimento Científico e Tecnológico (CNPq).

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