XII Seminário Brasileiro de Tecnologia Enzimática
ENZITEC 2016

Lignocellulosic Materials as Alternative Supports for Enzyme Immobilization: Physico-chemical Characterization of Pretreated Coconut Fiber and Corn Cob Powder

Guilherme Peixoto [1*], Thaís Milena de Souza Bezerra [2], Juliana Cristina Bassan [1], Clariana Zanutto Paulino da Cruz [2], Fernando Masarin [1] and Rubens Monti [1]

[1]Universidade Estadual Paulista - Faculdade de Ciências Farmacêuticas
Caixa Postal 14800-903 Araraquara - SP - E-mail: peixotog@fcfar.unesp.br
[2]Universidade Estadual Paulista - Instituto de Química
Caixa Postal 14800-060 Araraquara - SP

ABSTRACT

Coconut Fiber (CF) and Corn Cob Powder (CCP) presented distinctive characteristics from those found in traditional supports. These characteristics were useful for the immobilization of enzymes like laccase, peroxidase and proteases. The physico-chemical characterization described in this report helped to reveal innovative properties of these sources and encourage the use of lignocellulosic materials as valuable low cost supports for enzyme immobilization.

Keywords: coconut fiber; corn cob powder; supports; characterization; accessible volume.

INTRODUCTION

Lignocellulosic wastes are the main by-product of agroindustrial activities. The large generation of these materials and the inefficient solid waste management are potential sources of environmental impacts. As a consequence, many efforts have been made to implement their use in the production of value added chemicals (Herrera et al., 2013). These materials present a valuable composition including polysaccharides (cellulose, hemicellulose) and an aromatic polymer (lignin). The biocompatibility and easy derivatization of lignocellulosic materials are characteristics that make them suitable for the immobilization of industrial enzymes. These "alternative supports" also present large amount of easily accessible hydroxyl units that allow the attachment of a great variety of functional groups, changing some properties according to the enzyme needs (Sjöström, 1981). The first report of alternative supports was the lipase immobilization in rice straw by Castro et al. (2001). Later, the corncob was employed in the immobilization of soybean peroxidase, showing high activation and reuse rate (Galárraga et al., 2013). However, important properties of CCP and CF were not assessed up to now, such as porosity, pore size and surface area. These properties affect the amount of immobilized enzyme and possibly the steric hindrance to the substrate, as well as the distribution of the active groups in the internal and external surfaces of the support. Therefore, the aim of this work is the physico-chemical characterization of two alternative supports derived from coconut fiber and corncob powder.

MATERIAL AND METHODS

Coconut fiber (CF) and Corncob powder (CCP) treatments

The coconut fiber was firstly soaked with H_2SO_4 2% followed by NaOH 2% at 1:10 ratio with thermal decompression. Corn cob powder was soaked with 70% ethanol with thermal decompression, followed by an alkaline treatment with NaOH 2M (24h; 140 rpm).

Scanning Electron Microscopy (SEM)

The SEM was performed using a TOP COM-SM-300 device at voltages ranging from 10 to 20 kV. The samples were prepared in a BAL-TEC SCD050 sputter coater. Gold sputter coating was performed for 80 s at 25 ◦C and 41 mA in vacuum.

Chemical Composition of Supports

Approximately 3 g of the milled sample was treated with 95% ethanol for 6 hours in a Soxhlet apparatus. Ethanol-extracted samples were hydrolyzed with 72% sulfuric acid at 30°C for 1 h (300 mg of sample and 3 mL of sulfuric acid). The acid was diluted with the addition of 79 mL of deionized water, and the mixture was heated at 121°C and 1 atm for 1 h. The soluble lignin concentration in the filtrate was determined by the measurement of absorbance at 205 nm. The concentration of monomeric sugars in the soluble fraction was determined by high-performance liquid chromatography (HPX87H column at 45 °C and an elution rate of 0.6 mL/min with 5 mM H2SO4).

Determination of Pore Distribution

The porosity expressed as the volume of pores/g of support was determined by the solute exclusion technique as already reported in the literature (Júnior et al., 2013; Stone and Scalan, 1968). Six dextran probes were used for 24h at 25◦C, ranging from 20 to 553 A (1.5%, w/w). Afterward, the concentration of the probe in the supernatant was determined using a HPLC's refractive index detector with water as mobile phase at 0.4 mL/min and injections of 20 µL.

Supports Activation

The activation methodology was modified from Guisán (1988).

RESULTS AND DISCUSSION

Scanning Electron Microscopy

SEM data suggest that all treatments were efficient in the removal of the external surface layers that can hinder the derivatization of the fibers and, consequently, the immobilization of enzymes (Figure 1).

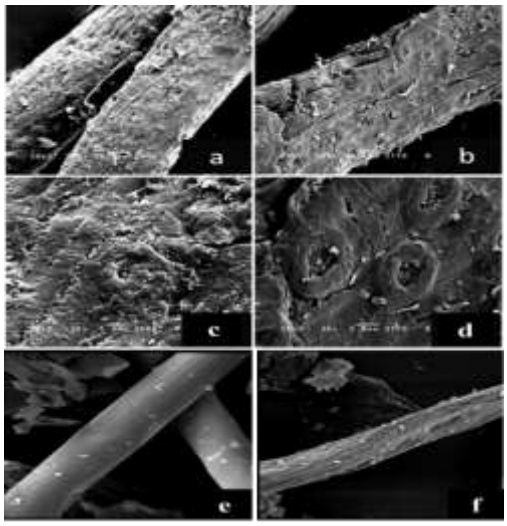

Fig. 1. Scanning electron microscopy of CF and CCP after chemical pretreatments: (a, c) CF natural; (b) thermal decompression of CF in NaOH 2%; (d) Thermal decompression of CF in H_2SO_4 2%; (e) CCP natural; (f) thermal decompression of CCP in ethanol 70% and treatment with NaOH 2M.

Chemical Composition

After alkaline and acid treatments of CF, the residual solids corresponded to 36.6% and 37.6% of the initial CF, respectively. For CCP the residual solids corresponded to 52.6% of the original weight (Table 1 and 2). The solids yield after the pretreatment indicate extensive dissolution of the supports components in both cases. Chemical composition of treated materials and mass balance indicate that waxy materials were almost completely removed. The glucan fraction was mostly retained in all treatments. Lignin or polyphenols were removed in the alkaline treatments, whereas xylan and

arabinosyl groups were mainly removed in CF and strongly decreased in CCP. The acetyl groups were fully removed from all supports.

Table 1. Quantification of chemical constituents of coconut fiber

Sample	Treatment yield (%)	Chemical composition[a]						Mass balance[b]					
		Total Lignin	Glucan	Xylan	Arabinosyl Groups	Acetyl Groups	Extratives[c]	Total Lignin	Glucan	Xylan	Arabinosyl Groups	Acetyl Groups	Extratives[c]
Untreated	100	13.6 ± 0.2	16.0 ± 0.2	8.1 ± 0.1	2.4 ± 0.0	1.4 ± 0.1	47.2	13.6 ± 0.2	16.0 ± 0.2	8.1 ± 0.1	2.4 ± 0.0	1.4 ± 0.1	47.2
Steam/Alkali	36.6 ± 0.1	17.8 ± 0.1	48.0 ± 0.9	16.9 ± 0.4	2.9 ± 0.1	0.1 ± 0.0	1.0	6.6 ± 0.1	17.7 ± 0.5	6.3 ± 0.2	1.1 ± 0.0	0.0 ± 0.0	0.4
Steam/Acid	37.6 ± 0.1	49.0 ± 0.1	39.3 ± 0.4	2.5 ± 2.2	0.1 ± 0.0	0.7 ± 0.1	2.0	18.8 ± 0.0	15.1 ± 0.2	1.0 ± 1.2	0.0 ± 0.0	0.2 ± 0.1	0.7

[a] g/100 g of coconut fiber.
[b] g/100 g of initial coconut fiber.
[c] Ethanol soluble compounds.

Table 2. Quantification of chemical constituents of corn cob powder

Sample	Treatment yield (%)	Chemical composition[a]						Mass Balance[b]					
		Total Lignin	Glucan	Xilan	Arabinosyl Groups	Acetyl Groups	Extractives[c]	Total Lignin	Glucan	Xilan	Arabinosyl Groups	Acetyl Groups	Extractives[c]
Untreated	100	12.73 ± 0.03	28.02 ± 0.05	24.16 ± 0.16	7.15 ± 0.02	2.32 ± 0.02	6.12	12.73 ± 0.03	28.02 ± 0.05	24.16 ± 0.16	7.15 ± 0.02	2.32 ± 0.02	6.12
Steam/Alkali	52.6 ± 0.02	8.01 ± 0.18	47.62 ± 0.87	22.08 ± 0.20	7.43 ± 0.01	0.03 ± 0.03	0.97	4.21 ± 0.02	25.04 ± 0.03	11.61±0.08	3.91±0.01	0.01 ±0.01	0.51

[a] g/100g of corn cob powder.
[b] g/100g of initial corn cob powder.
[c] Ethanol soluble compounds.

Porosity

The alkaline pretreated CF presented higher porosity showing more accessibility to the probes than the acid pretreated CF. In the range of laccase diameter, the accessible volume was 1.33 mL/g and 0.64 mL/g for the alkaline and the acid pretreated CF, respectively. The porosity of the pretreated CCP presented an accessible volume of 1.57 mL/g to trypsin molecules.

Supports Activation

The activation of the alternative supports was comparatively higher than those obtained with agarose, implying in up to 200 µmols aldehyde groups /g of support (Mateo et al., 2006).

CONCLUSIONS

The new alternative supports presented in this report . The useful physico-chemical properties and low cost of these supports can bring new alternatives to important industrial enzymatic processes.

REFERENCES

Herrera, M.; Havlík, P.; Valin, H.; Notenbaert, A.; Rufino, M. C.; Thornton, P. K.; Blümmel, M.; Weiss, F.; Grace, D.; Obersteiner, M. Biomass use, production, feed efficiencies, and greenhouse gas emissions from global livestock systems. v. 110 (52), p. 20888–20893, 2013.

Bezerra, T. M. S.; Bassan, J. C. Santos, V. T. O.; Ferraz, A.; Monti, R. Covalent immobilization of laccase in green coconut fiber and use in clarification of apple juice. Process Biochemistry 50 (2015) 417–423.

Ferraz A, Baeza J, Rodriguez J, Freer J. Estimating chemical composition of biodegraded pine and eucalyptus by DRIFT spectroscopy and multivariate analysis. Bioresource Technology. 2000;74:201-212.

Galárraga, JCV; Santos, AF; Bassan JC; Goulart AJ; Monti R. Bromophenol blue discoloration using peroxidase immobilized on highly activated corncob powder. Rev. Ciênc. Farm. Básica Apl., v. 34(3), p.321-326, 2013.

Mateo C, Palomo JM, Fuentes M, Betancor L, Grazu V, López-Gallego F, et al. Glyoxyl agarose: a fully inert and hydrophilic support for immobi- lization and high stabilization of proteins. Enzyme Microb Technol v.39, p. 274–80, 2006.