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Evaluation of sugarcane straw-derived activated carbon as an effective support for lipase immobilization

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ABSTRACT

Lipases are recognized as the most important group of biocatalysts in biotechnology. To be largely applied, enzyme immobilization is necessary since it facilitates the reuse and operational stability of the biocatalysts. This work aimed to evaluate the performance of the activated carbon obtained from sugarcane straw (SAC) as a support for lipase immobilization from *Aspergillus niger* 11T53A14. SAC presents a high surface area and protein adsorption capacity, analyzed by N_2 physisorption and Langmuir isotherm, respectively. Different initial protein concentration was studied (0.1–0.4 mg mL^{-1}), and results revealed an immobilization yield of 89 % when the protein concentration was 0.1 mg mL^{-1}, leading to the highest hydrolytic activity (300.2 U g^{-1}). These results indicated the potential of activated carbon synthesized from the sugarcane straw as a promising support for enzyme immobilization.

Keywords: biocatalyst. physical adsorption. *Aspergillus niger*. hydrolysis.

1 INTRODUCTION

Lipases are enzymes responsible for catalyzing the hydrolysis of triglycerides into glycerol and free fatty acids. In an aqueous-restricted reaction medium, these enzymes catalyze esterification, interesterification, and transesterification reactions[1]. This enzyme exhibits great biotechnological potential, mainly due to thermal stability, pH, and organic solvent tolerance, as well as chemo-, regio- and enantioselectivity[2]. Enzyme immobilization is a tool that enables easy biocatalyst recovery and reuse and can improve some enzyme features, such as stability, activity, specificity, selectivity, and resistance to inhibitors and chemical reagents[3,4]. Different methods can be used for immobilization, but physical adsorption is the most used since it is simple and cheap. Besides, this type of immobilization does not promote significant changes in enzyme three-dimensional structure since it is spontaneously immobilized in a preferential and energetically favorable orientation, not causing denaturation[5].

Various materials can be used as support for lipase immobilization. These include magnetic nanoparticles, polymers, mesoporous materials, and nanomaterials[4,6]. Activated carbon (AC) is a porous carbonaceous material with a high surface area and thermal, chemical, and mechanical resistance[7]. AC is obtained by carbonizing and activating the carbon precursor material through chemical or physical methods. It is considered an advantageous alternative due to the lower production costs than other porous matrices when produced from lignocellulosic materials, such as agro-industrial residues. Brazil is one of the largest ethanol producers in the world, using sugarcane as feedstock. Due to the replacement of manual by mechanized harvest, an increase in sugarcane straw as residue is observed[8]. In this way, sugarcane straw is an alternative raw material for AC production. Few reports use this agro-industrial residue as a precursor for carbon-activated production.

This study aimed to immobilize lipase from *Aspergillus niger* 11T53A14 on sugarcane straw-activated carbon (SAC). The support was characterized by N_2 physisorption, and the effect of enzyme concentration on immobilization efficiency and catalytic activity was also analyzed to identify the ideal condition for applying SAC as enzyme support.

2 MATERIAL & METHODS

The *Aspergillus niger* 11T53A14 strain was provided by the culture collection of Embrapa Food Technology Collection, Rio de Janeiro, Brazil. The crude enzymatic lipase extract was produced by solid-state fermentation (SSF). The media was composed of wheat bran (96.48 w/w%), spent coffee grounds (3.52 w/w%) as an inducer, supplemented with 0.6 % nitrogen (ammonium sulfate) and 55 % moisture (110 mL for 100 g of 0.1 mol L^{-1} HCl solution). A spore suspension (10^7 spores mL^{-1}) was used to inoculate the media, and fermentation was carried out at 32 °C for 40 h. The activated carbon was obtained from sugarcane straw (SAC) after impregnation with H_3PO_4 and activation. The precursor was impregnated with H_3PO_4 (85 w/w%) at room temperature and dried in a rotary evaporator for 24 h, at 60°C, for 2 h. The material obtained was kept at 100 °C for 72 h, followed by activation in a conventional tubular reactor under N_2 flow (60 mL min^{-1}) at 500 °C, with a heating rate of 10 °C min^{-1} for 2h[8].

Different lipase concentrations (0.1 - 0.4 mg mL^{-1}) were prepared for immobilization using sodium phosphate buffer 25 mmol L^{-1} (pH 7.0). Enzymatic solution (15 mL) was added to 0.15 g of SAC and kept under mild stirring at 25 ± 1 ºC for 3h. The immobilization process was accompanied by the analysis of protein concentration[9] and enzyme activity. The hydrolytic activity of soluble and immobilized lipase was determined by a titrimetric method based on quantifying fatty acids released after the enzymatic hydrolysis of olive oil emulsion. The fatty acids were quantified through titration with 50 mM NaOH using an automatic

titrator (Mettler Toledo T50). One unit (U) of lipase activity was defined as the amount of enzyme that produces 1 μmol of fatty acids per minute of reaction under assay conditions.

The specific area, pore volume, and pore mean size of the activated carbon from sugarcane straw (SAC) were determined using N_2 physisorption (Micromeritics, Model ASAP 2020 Plus). The BET method was used to determine the specific area and pore volume, while the BJH method determined the pore mean size.

3 RESULTS & DISCUSSION

Table 1 shows the textural properties of SAC used as support. It is observed that SAC presented a high BET area (2070 m²/g) and pore volume (1.5 cm³/g), which can promote increased surface contact for enzyme adsorption, enhancing the immobilization efficiency. Generally, activated carbons with pore volume and large pore size are preferred for enzyme immobilization, as they provide greater diffusion and accessibility for the enzyme to bind and interact with the carbon surface[10].

Table 1 Textural properties of activated carbon. BET specific area (A_{BET}), pore volume (V_{pore}), and mean pore size (D_{pore}).

Sample	A_{BET} (m²/g)	V_{pore} (cm³/g)	D_{pore} (Å)
Sugarcane straw-activated carbon (SAC)	2070.0	1.5	43.9

The amount of enzyme that must be loaded on the support is one of the main characteristics to be considered in the preparation of a biocatalyst by immobilization[11]. Adsorption data are conveniently represented by isotherms, which help determine the support's immobilization capacity. The ability of the SAC to adsorb lipase was evaluated using the nonlinear Langmuir isotherm model. The adsorption isotherm represents the equilibrium amount of protein adsorbed (Qe) as a function of its equilibrium concentration (Ce). The Langmuir isotherm model (Figure 1) adjusted well to the experimental data, showing a high determination coefficient (Table 2) and concerning the parameter RL, which represents the inverse of the ratio of adsorption/desorption constants (1/1+(K*Qm)), values below 1 were obtained, indicating a favorable adsorption phenomenon for *A. niger* lipase, and demonstrating the viability of this enzyme immobilization on activated carbon.
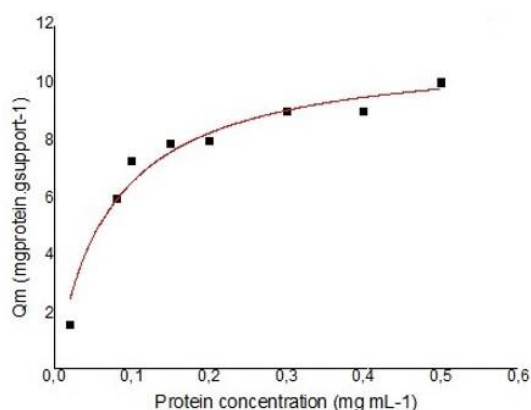

Figure 1 Lipase immobilization capacity by adsorption on activated carbon. Immobilizations were carried out at room temperature (25 ± 1 °C) for 2 h, using 15 mL of enzymatic solution (phosphate buffer 25 mmol L^{-1} pH 7.0) and 0.15 g of support.

Table 2 Parameters of Langmuir isotherm for adsorption of *A.niger* lipase onto SAC.

Enzyme	Qm (mg$_{protein}$ g$_{support}^{-1}$)	K (mL mg$_{protein}^{-1}$)	RL	R²
A. niger lipase	11.1 ± 0.6	14.4 ± 2.9	0.006	0.95

Different initial protein concentrations were studied to evaluate its effects on immobilization by physical adsorption. It was observed that the increase in enzyme concentration led to increased adsorptive capacity until it reached a maximum value of 9.0 mg protein g support^{-1} (Figure 2a). The maximum immobilization yield (89%) was obtained with a low protein concentration (0.1 mg L^{-1}). The lipase immobilization can be influenced by the interaction protein/support. When there is a low protein/support (mg protein/ mg support), some adsorption sites may be available, and some interaction (protein/support) can be observed. On the other hand, at high initial protein concentrations, the number of available binding sites on the solid support is saturated, resulting in fewer binding sites being available for additional lipase molecules to adsorb. Moreover, higher enzyme concentrations enable more protein-protein interactions, which can lead to conformational changes and, consequently, a more pronounced decrease in hydrolytic activity[12].
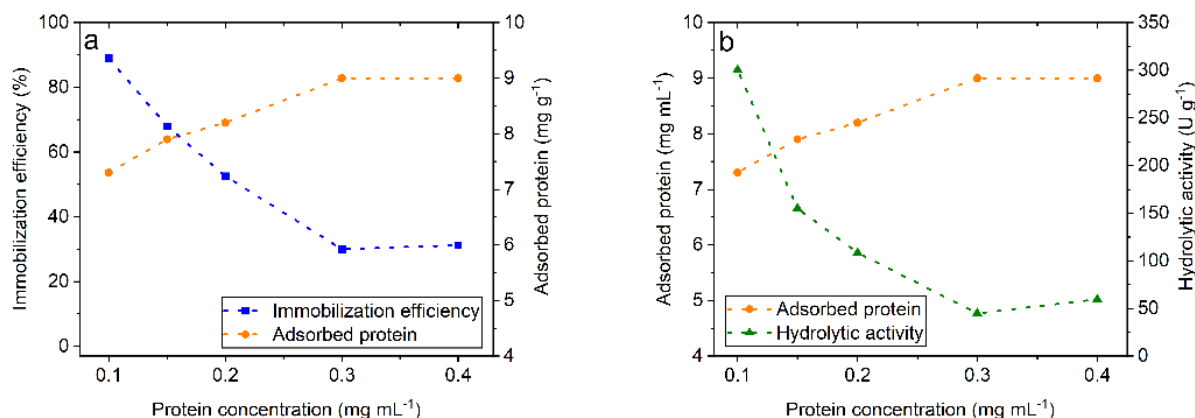
Figure 2 Effect of initial protein concentration in immobilization efficiency (a) adsorbed protein (a,b) and hydrolytic activity of immobilized derivatives (b). Immobilizations were carried out at room temperature (25 ± 1 °C) for 2 h, using 15 mL of enzymatic solution (phosphate buffer 25 mmol L^{-1} pH 7.0) and 0.15 g of support.

Regarding the hydrolytic activity of immobilized lipase (Figure 2b), the highest activities were obtained in the lowest enzyme concentration studied, with a maximum (300.2 U g^{-1}) obtained when 0.1 mg mL^{-1} of protein was used in immobilization. The decreasing enzyme activity values with increased lipase loading indicate that, although most of the protein had been adsorbed onto the support surface, few remained active. The optimal enzyme activity depends on both the support effects in enzyme conformation and the diffusional limitations due to enzyme concentration on the support's surface[11]. The overloading of enzymes causes some unfavorable protein-protein interactions, leading to multilayers or clusters of molecules' enzymes on the support surface[11,13,14]. Pashangeh et al.[13] also studied the effect of the initial concentration of *Rhizopus oryzae* lipase on covalent immobilization using amino-functionalized magnetic nanoparticles as supports. The authors varied the initial lipase concentration (0.1-0.3 mg mL^{-1}) and observed that relative activity reached a maximum value when 0.1 mg mL^{-1} lipase was used. According to them, because the binding sites on the support surface are limited, the overloading of enzyme molecules causes the expansion of some unfavorable protein-protein interactions on the nanoparticle's surface. In addition, diffusion of substrate and product can be hindered, which also can reduce immobilized enzyme activity.

4 CONCLUSION

Immobilizing lipase on activated carbon was a very fast and simple process, leading to an 89% of immobilization yield when 0.1 mg mL^{-1} of *A. niger* lipase was used in the process. This enzyme concentration also leads to the highest activity (300.2 U g^{-1}) in olive oil hydrolysis catalyzed by the immobilized derivative. The sugarcane straw-activated carbon can support lipase immobilization due to its low cost and favorable physicochemical characteristics, producing an efficient biocatalyst that can be applied in biotechnological processes.

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