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Research article

Activated carbons preparation from yellow mombin fruit stones for lipase immobilization

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ABSTRACT

Activated carbons were prepared from yellow mombin fruit stones as carbon precursor and subsequently used as support for lipase immobilization. Carbons were prepared by chemical activation method using phosphoric acid or potassium hydroxide as activating agents. The influence of the activating agent and the carbonization temperature on the physical and chemical properties of carbons was investigated. Although carbon specific surface area increased with increasing activation temperature, there was a reduction in the number of surface functional groups. The activation with phosphoric acid resulted in higher yields and favored a higher surface area and pore volume of carbon. The optimum conditions for lipase immobilization onto activated carbons were carried out varying contact time, pH, initial enzyme concentration, and temperature. The immobilized enzymes showed good activity in both carbons, and can be considered a promising alternative as support for lipase immobilization.

1. Introduction

Activated carbon (AC) is a carbonaceous material characterized by having an internal surface area and highly developed porosity and various functional groups on the surface [1]. It has been widely used as adsorbent [2] in catalysis or as catalyst support [3], and purification and separation processes [4].

Despite being a very efficient material, the cost of activated carbon often restricts its use, thus, there is a growing interest in alternative sources to reduce production costs. The products used in carbon synthesis have high carbon value and low levels of inorganic compounds, as well as solid waste from agriculture (peels and seeds, wood and bagasse), which are effective raw materials for activated carbons preparation [3,5]. The nature of the carbon precursor material as well as the methods and process conditions for activation will determine the chemical properties and pore structure of the activated carbon [6].

There are basically two methods to produce activated carbon: physical activation and chemical activation. The first involves the primary carbonization of the precursor material (lignocellulosic material) followed by activation at high temperature, from 800 °C to 1100 °C, under gas flow such as water vapor, carbon dioxide or a mixture of them [7]. In contrast, the chemical activation consists in the impregnation of the precursor material with dehydrating agents, followed by heat treatment with temperatures between 400 °C and 900 °C, with carbonization and activation performed simultaneously. Zinc chloride (ZnCl), phosphoric acid (H_2PO_4), sodium hydroxide (NaOH) and potassium hydroxide (KOH) are examples of chemical agents, which has been widely employed in the activation process from lignocellulosic materials. Some important advantages of chemical activation in relation to the physical activation are low pyrolysis temperature, shorter activation time and higher yield, allows obtaining materials with higher surface area and incorporation of functional groups on the surface [7,8].

Carbons produced from different agricultural sources have been activated in different ways to maximize their surface area and increase their adsorption capacity, allowing them to be used in a wider range of processes [5,9]. However, activated carbon as support for lipase immobilization has been little studied.

Lipases (triacylglycerol acylhydrolases, EC3.1.1.3) comprise a group of hydrolytic enzymes which act generally in the organic-aqueous interface, catalyzing the hydrolysis and the synthesis of esters formed by glycerol and long chain fatty acids [10,11]. Due to their versatility, these enzymes have potential applications in various industries such as dairy, alimentary, pharmaceutical, and oleo chemical industries, wastewater treatment, formulation of detergents, biosurfactants synthesis, among others [12,13].

Despite the great catalytic efficiency of lipase, its application as biocatalyst is limited by factors related to cost and enzyme stability limits, and an alternative is to use the enzyme in the immobilized form. Lipases may be immobilized by different methods namely adsorption, cross-linking, adsorption followed by cross-linking, multipoint strong attachment and physical entrapment [14]. Adsorption is the most common

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immobilization method due to its low cost, and few deleterious effects on enzyme activity and selectivity [15,16]. In this technique, the enzyme is immobilized on a solid support by low energy interactions, such as van der Waals or hydrophobic interactions, ionic and hydrogen bonding, among others. Various materials can be used for this purpose and the choice of material depends on its morphology and surface chemistry, such as surface area, shape, pore distribution and size, hydrophobic/hydrophilic surface character, presence of surface charges, chemical stability and costs [17,18].

Activated carbon may be a promising support for lipase immobilization due to its physical and chemical properties. Thus, the production of activated carbon from residue of food industry becomes relevant, because of the demand for effective support for lipase immobilization, with low costs. The stones of yellow mombin have proven as an alternative raw material for production of activated carbon. This species has been economically exploited in Brazil, especially in the North and Northeast, generating large amounts of waste. Given the above, this study aimed to produce activated carbon using the stones of yellow mombin as carbon precursor material under different synthesis conditions, and used them as a support for lipase immobilization by the adsorption method.

2. Materials and methods

2.1. Production of the precursor material

The yellow mombin stones were used as carbon precursor material in the synthesis of activated carbons. They were kindly provided by a pulp fruit industry located at the state of Bahia, Brazil. The fruit stones were washed and dried by natural convection, crushed using disk mill and sieved on a sieve with opening of 420 μm. The resulting material was used in the carbon synthesis.

The composition of the precursor material was 1.96% ash, 9.7% moisture, 9.47% lignin, 14.87% cellulose, and 8.23% hemicellulose. The low moisture and ash contents, as well as the lignocellulosic fraction of this residue make this residue a precursor material with great potential for preparing the activated carbon, since the composition of the lignocellulosic material has a direct influence on carbon porosity [19,20].

2.2. Activated carbon preparation

Two methodologies were used, with modifications on the activating agents and carbonization temperatures. The yield of activated carbon calculated by Eq. (1).

$$\text{Yield}\ (\%) = (W_f/W_0) \times 100 \quad (1)$$

where W_f and W_0 (g) are the weights of activated carbon and dried precursor material.

2.2.1. Chemical activation using phosphoric acid - H_3PO_4

The residue was impregnated with phosphoric acid (mass fraction: 85%) in the weight ratio 1.5:1 (activating agent/precursor), and dried at 105 °C for 24 h. Then, the material was carbonized in a muffle furnace under nitrogen flow (50 mL·min^{-1}) at a heating rate of 5 °C min^{-1} up to 450 °C or 500 °C for 1 h. Finally, the activated carbon was cleaned with hot water for several times until the filtrate reaching pH 7.0. The AC was dried at 105 °C for 24 h and sieved on a sieve with opening of 420 μm.

2.2.2. Chemical activation using potassium hydroxide - KOH

The precursor material was impregnated with potassium hydroxide in the weight ratio 1.5:1 (activating agent/precursor), and dried at 105 °C for 48 h. Then, the material was carbonized in a muffle furnace under nitrogen flow (50 mL·min^{-1}) at a heating rate of 5 °C min^{-1} up to 450 °C or 500 °C for 1 h. The sample was immersed in HCl 0.2 mol·L^{-1} and heated to boiling for 30 min under reflux to accelerate the neutralization step, since the potassium chloride formed during the reaction is more soluble and hence easier to be removed. After acid washing, the samples were cleaned with hot water for several times until the filtrate reached pH 7.0, to eliminate the ions dispersed over the carbon surface [21]. The AC was dried at 105 °C for 24 h and sieved on a sieve with opening of 420 μm.

2.3. Characterization of prepared activated carbons

The ash content was determined according to the methodology proposed by the AOAC [22]. The functional groups on the surface of activated carbons were analyzed using Fourier transform infrared spectroscopy (FTIR). Direct readings were performed by attenuated total reflectance (ATR) in the infrared region of 4000–500 cm^{-1}. The point of zero charge was determined by the method known as "experiment of 11 points" [23]. The morphology of the actived carbons was determined by scanning electron microscopy. For this, carbons samples produced were fixed in appropriate supports (stub) and directly metalized with a thin layer of gold. Then, the samples were conducted for analysis in a scanning electron microscope (Zeiss, Modelo DSM940). Adsorption-desorption nitrogen isotherms at 77 K were obtained in a Micromeritcs equipment, model ASAP 2020. Before the measurements, samples were subjected to a pre-treatment stage consisting of heating to 393 K. The specific surface area was determined by the Brunauer-Emmett-Teller method (BET) isotherm equation and pore size distribution was determined from the desorption isotherm using the BJH method, while the volume of micropores was determined by t-plot analysis.

2.4. Lipase immobilization on AC

Lipase from porcine pancreas type II Sigma (40% protein and 100–500 U/mg protein) was used for the immobilization tests. The optimum conditions for the lipase immobilization onto activated carbons were carried by varying contact time (10–200 min), pH (3.0–8.0) of the reaction medium, working temperature (10–50 °C) and initial enzyme concentration (2000–6000 mg·L^{-1}). For that, aliquots of 5 mL of lipase solution were added to tubes containing 0.1 g of each support. The tubes were kept under constant stirring (20 rpm) on an orbital shaker. A BOD chamber (Logen Scientific LG340FT220-RBC) was used for temperature control.

To determine the contact time required to reach adsorption equilibrium of lipase immobilization in AC, was used a solution with initial protein concentration of 4000 mg·L^{-1} in sodium acetate buffer (0.1 mol·L^{-1}, pH 3.5) at 30 °C. At each predetermined time, the tubes were removed and centrifuged. Equilibrium of the process was achieved when the concentration remained constant with time. The pH effect was investigated by using a solution with initial protein concentration of 4000 mg·L^{-1} at 30 °C. The pH values of the initial lipase solution were: 3.0, 3.5, 4.0, 5.0, 6.0, 7.0, and 8.0. Sodium acetate buffer (0.1 mol·L^{-1}) pH 3.0 to 5.0 and sodium (0.1 mol·L^{-1}) pH 6.0 to 8.0 were used in the same conditions. The effect of temperature on lipase immobilization process were performed at different temperatures (10 °C, 20 °C, 30 °C, 40 °C and 50 °C). To assess the effect of initial protein concentration 5 mL aliquots of lipase solution at different concentrations (2000 mg.L^{-1}, 3000 mg.L^{-1}, 4000 mg·L^{-1}, 5000 mg·L^{-1} and 6000 mg·L^{-1}) and optimal pH. The tubes were kept under constant stirring (20 rpm) on an orbital shaker at temperatures established in the previous section. Once reached the contact time (preliminary determination), the tubes were removed and centrifuged.

2.5. Protein determination after immobilization

The supernatant obtained after centrifugation was filtered using a hydrophilic PTFE syringe filter (0.45 pore size and 25 mm in diameter, Analytical). The protein concentration was determined by the Bradford