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Immobilization of peroxidase enzyme in NaY zeolite by sonochemical method

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Resumo-Abstract

Kaolin mineral extraction has a high potential for generating waste, with approximately 70% being discarded into the environment, which requires care to prevent negative impacts on soil, water and reservoirs [1]. Studies indicate that this extraction can generate between 80% and 90% of waste [2]. Kaolin waste is a raw material for the production of zeolites that are useful in biotechnological applications due to their high surface area, harmonized hydrophilic and hydrophobic properties, Bronsted acidity by controlling the Si/Al ratio, as well as thermal, mechanical, product storage and pH stability, ensuring increased catalytic activity of the enzyme in some cases, and the union of the structures guarantees promoting effects in chemoenzymatic reactions [3,4]. The synthesis of NaY zeolite was carried out using kaolin waste previously dried in an oven at 100°C for 4 h, followed by calcination at 700°C for 2 h. Approximately 2.1000 g of the calcined waste, 4.56 g of $Na_2SiO_3.5H_2O$, 19 mL of 6M NaOH solution and 40 mL of distilled water were used, placed in a PTFE cup in an autoclave at 100°C for 20 h. The product presented an octahedral bipyramidal shape, intense peaks in the º2θ reflections: 6.09º; 10.02; 12.43º; 21.58º; 28.01º and 33.28º and a BET specific surface area of 284.57 m²/g. The protein concentrate was extracted from 0.996 kg of peach palm pulp (*Bactris gasipaes*), initially obtaining starch extraction by centrifugation at 4ºC for 10,000 g/5 min in the form of pellets and subsequently, 250 mL of the supernatant was transferred to a centrifuge tube with the addition of 200 mL of a methanol:chloroform mixture (1:2) and the set was centrifuged at 4ºC, 10,000 g/7 min. The protein concentrate containing the enzymes was separated by filtration through a plastic sieve and stored between 4 and 10°C in contact with a pH 4.5 buffer solution. Thus, a 20 mg/mL enzyme solution was formed whose activity for the peroxidase enzyme presented a value of 1.35 U/L in the reaction with 50 mM guaiacol solution and 10 mM H_2O_2. Immobilization was performed by sonication at 40 W and 32ºC of 150 mg of support in contact with 600 µL of 0.04 g/mL enzyme solution for 3 h. The absorbance of the control at 600 nm was read using Bradford reagent, obtaining 0.840 u of absorbance. Collection of the supernatant after 3 h of sonication generated 0.708 u of absorbance, representing a reduction of 15.71% in relation to the control, showing effective protein immobilization on the support. Hydrophobic interactions, Van der Waals forces, hydrogen bonding and ionic interactions occurred between the enzyme and the support, thus, immobilization by adsorption on the external surface of the material.

Keywords: zeolite Y, Peroxidase, Immobilization

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