STUDY OF THE THERMAL STABILITY AND CHARACTERIZATION OF IMOBILIZED *C. viswanathii* LIPASE

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ABSTRACT

Lipase is an important enzyme in the hydrolysis of lipids in fatty acids and glycerol, widely applied in industry, but limited by its instability. Enzymatic immobilization emerges as a promising strategy to overcome this challenge. This work evaluates the thermal stability of *Candida viswanathii* lipase in green coconut biochar by covalent bond and performs its physicochemical characterization. The microorganism was cultivated in submerged medium for lipase production and immobilized in a biochar functionalized with glutaraldehyde. The enzymatic activity was determined by measuring the p-nitrophenol released. The thermal stability of the immobilized biocatalysts (T2, T4, PC) was evaluated at temperatures from 30 C to 60 C for 0-180 minutes. The biocatalyst T4 showed the best thermal stability, maintaining high relative activity, especially at 40 C and 50 C. The morphological analysis by the method of Brunauer, Emmett and Teller indicated a significant increase in surface area (1.9193 m^2/g) and in the volume of the support (0.001372 cm^3/g) after immobilization. The characterization by infrared spectroscopy identified bands associated with amide structures I and II, confirming the immobilization. This study proposes an efficient and sustainable methodology for immobilization of enzymes, expanding the use of biocatalysts in industrial processes under adverse temperature conditions.

Keywords: Biocatalyst. Covalent bond. Residues.

1 INTRODUCTION

Lipases belong to the family of hydrolytic enzymes (α/β-Hidrolases) and have the ability to catalyze the hydrolysis of triglycerides in fatty acids and glycerol, perform transesterification and esterification of esters[1]. It has a wide application in several sectors that comprise the food, pharmaceutical, detergents, leather, textiles, cosmetics, paper industries, among others[2]. Immobilization methods, such as covalent bond, appear as promising alternatives to improve enzymatic activity, facilitate the recovery of the biocatalyst, protect it against degradation and deactivation, and ensure greater stability during the industrial process[3]. In addition to the immobilization technique, the choice of support material has significant impacts on the effectiveness of enzyme immobilization. The biochar is "a heterogeneous substance rich in aromatic carbon and minerals", obtained through biomass pyrolysis. Agroindustrial residues have been shown to be a viable source to produce high quality biochar, useful in the removal of organic matter and other compounds present. In the Legal Amazon, plant extractivism generates a significant amount of waste after the processing of biomass, so this region is promising for the production of biochar sustainable application in the immobilization of enzymes[4]. The objective of this work is to evaluate the thermal stability of *C. viswanathii* in green coconut biochar by covalent bond, besides performing its physicochemical characterization.

2 MATERIAL & METHODS

2.1 Microorganism: The strain *C. viswanathii* CCR8137 belongs to the strain bank of the Research Group Microbial Enzymes and Bioprocesses (GPEMB). **2.2 Submerged cultivation:** The culture medium used was adapted from Dalmau with pH 6.0[5]. The experiment was conducted in triplicate in Erlenmeyer flasks. The inoculum was prepared from a cell suspension at a concentration of 10 cel/mL, was added to the medium in the proportion of 1 mL per vial. The cultures were incubated at 28 C and 180 rpm for 72 h. Later, centrifuged obtaining the crude extract. and stored in freezer for future experiments. **2.3 Support:** The green coconut shell biochar was obtained in partnership with the Postgraduate Program in Food Science and Technology of the Federal University of Tocantins (UFT). The functionalization of the support was performed with glutaraldehyde. The support was dispersed in 100 mL of glutaraldehyde solution at 2.5%, pH 7.0, washed with distilled water and 0.1M buffer HAc-NaAc pH 5.0. **2.4 Immobilization:** The specific conditions of the samples were: T2-40 ºC with 0.5 mL, T4-40 ºC with 1.5 mL, and CP-30 ºC with 1.0 mL in 100 mg of support. 2.5 Enzymatic activity: Hydrolysis of p-NPP was determined by measuring p-Nitrophenyl released (pnp) at 40 ºC in a spectrophotometer at 410 nm[6]. Lipase activity was calculated using a standard curve of p-nitrophenol, with controls without enzyme. An enzymatic unit (U) was defined as the amount of enzyme that releases 1 µmol of p-nitrophenol per milliliter, every minute of reaction. **2.6 Thermal stability:** The thermal stability was determined by incubating the biocatalyst in McIlvaine buffer pH 4.0, at temperatures of 30 ºC, 40 ºC, 50 ºC and 60 ºC, in a time interval of 0-180 min. **2.7 Physico-chemical analysis:** The surface area was calculated using the method of Brunauer, Emmett and Teller (B.E.T.). Pore volume, diameter (Å) and area distributions were evaluated by the B.E.T. apparatus software (Gemini VII Area and Porosity Analyzer from Micromeritics). The activated support and immobilized biocatalyst were characterized by infrared spectroscopy (FT-IR), using the Agilent Cary 630 FTIR Spectrometer, in the range 4000 cm-1 and 800 cm-1 using Fourier transform.

3 RESULTS & DISCUSSION

3.1 Thermal stability

Thermal stability is an important parameter that characterizes biocatalysts in industrial applications, where they occur under adverse temperature conditions, indicating efficiency and durability in demanding industrial environments[7]. Figure 1 shows the results of thermal stability, with immobilized biocatalysts incubated in McILVAINE buffer pH 4.0 for 0 to 180 min, at temperatures of 30, 40, 50 and 60 C.

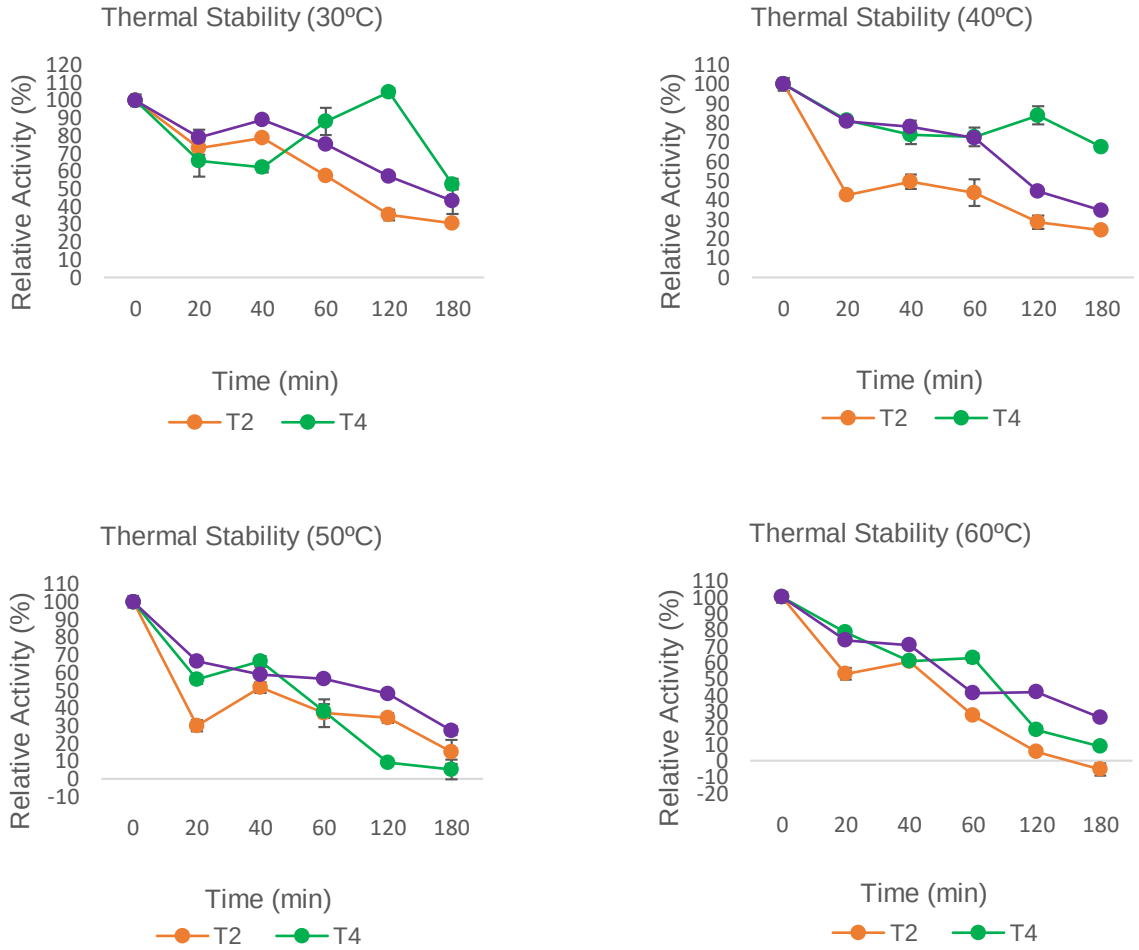


Figure 1 Thermal stability of the biocatalyst immobilized by covalent bonding with glutaraldehyde at temperatures of 30 °C, 40 °C, 50 °C and 60 °C.

The graph shows the thermal stability of biocatalysts (T2, T4, PC) immobilized by covalent bonding with glutaraldehyde at different temperatures (30 C, 40 C, 50 C, 60 C). At all temperatures, the biocatalyst T4 demonstrated the best thermal stability, maintaining a higher and constant relative activity over time. This biocatalyst stood out as the most thermally stable, especially at higher temperatures (40ºC and 50ºC), proving to be more resistant. Oliveira evaluated the thermal stability for lipase of *C. viswanathii* immobilized in magnetic chitosan nanoparticles functionalized with glutaraldehyde[8]. The results indicated that the immobilized biocatalyst maintained activities above 50% in 50-60 C and 30-40 C after 150 minutes. In addition, the author highlights the importance of enzymatic immobilization by covalent bond to increase the stability of the enzyme, since the covalent bond technique is strong and can keep the enzyme stable for longer, even in adverse conditions[8].

3.2 Morphological Analysis (B.E.T)

The investigation of the morphological properties of porous solid materials is commonly conducted by gas nitrogen adsorption, using volumetric measurements to determine the amount of adsorbed gas[2]. The results of the B.E.T analysis are presented in Table 1, where the results for the functionalized supports and the immobilized biocatalysts are displayed in terms of surface area (m2/g), pore volume (cm3/g) and pore diameter (Å).

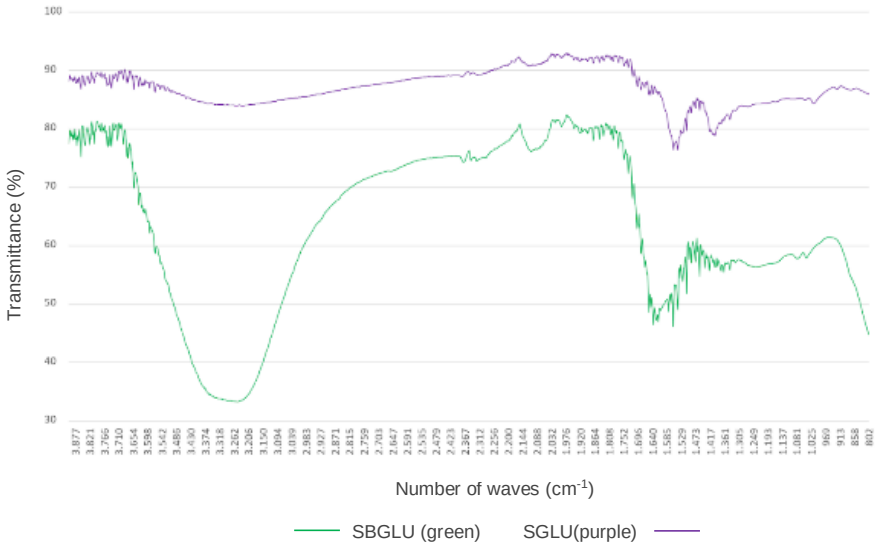
Table 1 B.E.T. of the biochar support of coconut fiber activated with glutaraldehyde (SGLU) and the biocatalizer immobilized in biochar activated with glutaraldehyde (BSGLU).

Sample	Surface area (m^2/g)	Pore volume (cm^3/g)	Pore diameter (Å)
SGLU	1,2644	0,000096	20,608
BSGLU	1,9193	0,001372	20,592

3.3 Analysis of FTIR

The analysis by Fourier Transform Infrared Spectrophotometry (FTIR) were performed to qualitatively identify the chemical composition of the functionalized support and the immobilized biocatalyst. The principle of the technique is based on the specific

vibration frequencies of the chemical bonds, corresponding to the vibrational levels[9]. Figure 2 shows the FTIR spectra obtained for functionalized biochar and covalently immobilized biocatalysts. It is possible to identify discrete peaks present in 1,038 cm-1 to 900 cm-1, characteristic of vibrations related to CO-C bonds and vibrations associated with the C-H bond in aromatic compounds. In the bands 1,300 cm-1 and 1,370 cm-1, angular deformations of the C-H bond are observed[10]. Elongation of O-H bonds is observed at the peak of 3,600 cm-1[11]. In the spectra of the immobilized biocatalysts, bands at 1,635 cm-1 were observed that are associated with amide I and amide II, indicating that the bands around are attributed to the presence of the structure of the ß sheet of the biocatalyst[11,2].



4 CONCLUSION

The immobilization of *C. viswanathii* lipase in green coconut biochar by covalent bond and the subsequent physicochemical characterization present interesting results. The thermal stability analysis showed that the biocatalyst T4 had the best stability at all temperatures tested, especially at 40 ºC and 50 ºC, maintaining a high and constant relative activity. The morphological analysis by the method of Brunauer, Emmett and Teller revealed that the surface area and the volume of the functionalized support increased significantly after immobilization. These results promote a sustainable approach to the immobilization of enzymes, using abundant residues in the Amazon region.

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