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PRODUCTION OF HETEROFUNCTIONAL SUPPORT FOR LIPASE IMMOBILIZATION USING STEAM EXPLOSION PRE-TREATED AND SODIUM PERIODATE-MODIFIED GREEN COCONUT FIBER

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ABSTRACT

The following study aims to investigate the reuse of a widely abundant raw material in Brazil, the green coconut fiber, for the production of a heterofunctional support for the immobilization of *Thermomyces lanuginosus* lipase. Tests were conducted by activating the steam explosion pre-treated coconut fiber at different concentrations of sodium periodate (500 – 6000 µmol/g). Immobilizations were performed by adsorption and covalent binding, and the biocatalysts were evaluated based on immobilization parameters. The highest immobilization yield at pH 7.0 was 62.7% when the biomass was activated with 1000 µmol/g, while the recovered activity was 27.8%. This indicates that the immobilization procedure was successful. After immobilization at pH 7.0, the enzyme was incubated at pH 10.2 for the formation of covalent bonds. The results showed a decrease in catalytic activity, with a retained activity of approximately 77.3%, after 18 hours of incubation. In the thermal stability tests, the immobilized biocatalysts showed excellent results, with the best performance observed using the biomass activated with 1000 µmol/g, which exhibited a t1/2 30.31 times higher than the soluble enzyme.

Keywords: *Thermomyces lanuginosus* Lipase. coconut fiber. Immobilization.

1 INTRODUCTION

Enzymes represent a significant cost for the industry because they are produced through fermentative processes, and when in soluble form, their reuse is hindered[1]. However, when enzymes are immobilized on supports, they present several advantages, such as, easy recovery and reuse through simple filtration, thus reducing costs during the process. In addition, the immobilized enzyme can have improved properties, such as improved stability and activity, among others.

Due to the high cost of commercial supports, studies have been conducted on enzyme immobilization using cheaper and biodegradable materials. In the study Brígida et al. (2007)[2], it was found that one suitable raw material for immobilization matrix, the coconut fiber. The main objective of this study was to analyze the pre-treated green coconut fiber as a heterofunctional support for the immobilization of *Thermomyces lanuginosus* lipase (TLL), where the fiber was activated with sodium peroxide to form aldehyde groups. The immobilization procedure was carried out by adsorption at pH 7.0 and, after incubation at pH 10.2, covalent bonding can be formed[3].

2 MATERIAL & METHODS

The coconut fiber was pre-treated by steam explosion. Under the conditions of 210°C, 20 bar for 10 minutes in a pilot-scale reactor to remove hemicellulose, according to related by Ribeiro et al (2022)[4].

The biomass was activated with different concentrations of sodium periodate (500 – 6000 µmol/g) at pH 5.0, and, if necessary, pH adjustment of the solution was made using 10% (v/v) acetic acid. The biomass in contact with the sodium periodate solution was kept under agitation at 24 rpm for 5 hours at room temperature in the absence of light. Quantification was performed using a spectrophotometer, by adding 10 µL of the sodium periodate solution before oxidation with 1 mL of saturated sodium bicarbonate solution 1:1 (v/v) and 1 mL of 10% (w/v) potassium iodide solution. The aldehyde groups present were calculated by the following equation:

$$CCHO\ (\mu mol/g) = 2 * ClO4 - o\ (\mu mol/g) * (1 - sample\ abs\ /\ standard\ abs)$$

Where CCHO is the concentration of aldehyde groups generated per g of biomass, ClO4-o is the initial concentration of sodium periodate solution per gram of biomass.

For the immobilization process, the TLL enzymatic solution (0.1 mg/mL) in sodium phosphate buffer (5 mM, pH 7.0) was added to the biomass (Ratio 1:20 – m/v), and stirred for 24 hours at 24 rpm (enzyme loading 2 mg/g). Afterward, the test tube was centrifuged to separate the supernatant from the immobilized enzyme (iTLL). The iTLL was washed with sodium phosphate buffer (5 mM, pH 7.0) and vacuum filtered. The Bradford method by Bradford, M.M. (1976)[5] was used to monitor and quantify the proteins in the solutions before and after the immobilization process. The substrate p-nitrophenyl butyrate (pNPB) was used to measure enzymatic activities after immobilization according to Rios et al. (2019)[3]. The immobilization parameters were calculated following the method of Silva et al. (2012)[6]. After immobilization, iTLL was incubated for 18 hours in sodium carbonate-bicarbonate buffer (50 mM, pH 10.2), producing the biocatalyst iTLL+inc

3 RESULTS & DISCUSSION

According to NYPELÖ et al., (2021)[7], the oxidation process of cellulose with sodium periodate introduces two aldehyde groups into a monomeric carbohydrate unit. Thus, it can be added up to 12000 µmol of aldehyde groups per gram of biomass if the activation yield was 100%. However, the yield showed a decay as the concentration of sodium periodate increased. It can be observed that the stabilization of aldehyde groups occurs practically from an initial concentration of 2500 µmol/g of sodium periodate. According to Sirvio et al. (2011)[8], sodium periodate oxidizes the vicinal hydroxyl groups at carbon atoms 2 and 3 in an anhydroglucose unit of cellulose. Therefore, this stabilization in the amount of aldehyde groups formed in the biomass indicates that all available hydroxyl groups were modified when concentrations higher than 2500 µmol/g were used.

Table 1: Concentration of aldehyde groups present and immobilization parameters at pH 7.0 of TLL on coconut fiber activated with different concentrations of sodium periodate.

Initial concentrations used (µmol/g)	Amount of aldehyde groups (µmol/g)	Ypro(%)	Acti(U/g)	Actt(U/g)	Actr(%)
500	825,18 ± 29,92	21,00 ± 3,39	6,14 ± 0,35	14,33 ± 1,67	41,67 ± 8,48
1000	987,02 ± 54,77	62,73 ± 10,87	10,63 ± 0,76	38,24 ± 5,13	27,80 ± 6,39
2500	1929,89 ± 65,22	44,49 ± 0,08	9,10 ± 2,96	30,07 ± 1,54	34,46 ± 4,35
5000	2150,13 ± 89,53	37,92 ± 1,87	6,97 ± 1,07	42,38 ± 1,33	15,75 ± 2,79
6000	2214,91± 149,61	40,38 ± 0,87	7,32 ± 0,60	33,57 ± 0,73	21,82 ± 1,47

*Immobilization protein yield (Ypro), Immobilized enzyme activity (Acti), Theoretical activity (Actt), Recovered activity (Actr).

The biomass that yielded the best results was activated with 1000 µmol/g of sodium periodate, achieving a yield of approximately 62% with a standard deviation of ±10%. This indicates that the majority of the enzyme was immobilized, with 27.80% ± 6.39% of the immobilized enzyme remaining active after immobilization. However, the interactions involved in this process are likely physical interactions, such as electrostatic and hydrophobic interactions. This is because the immobilization pH was performed at 7.0 when the lysine residues of the enzymes are not active to enable a Schiff base between the enzyme and support. Therefore, to achieve better results in altering the enzyme properties through immobilization, the immobilized enzyme was incubated at pH 10.2 to facilitate the formation of covalent bonds.

When analyzing Figure 1, we can observe that the enzymes immobilized at pH 7.0 exhibit half-life times close to that of the soluble enzyme. In this case, iTLL immobilized at pH 7.0 with 1000 µmol/g of sodium periodate shows a half-life time (t1/2) 2.63 times higher than that of soluble TLL. This indicates the presence of physical interactions between the enzyme and the support. However, when the biomass activated with 1000 µmol/g, 2500 µmol/g, and 5000 µmol/g, is immobilized and incubated at pH 10.2, the thermal stability of the biocatalysts increases by 30.31 times, 14.69 times, and 16.97 times, respectively, compared to the soluble enzyme at 70°C. This is the case of iTLL immobilized at pH 7.0 and incubated at pH 10.2, which lasted over 11 hours in a water bath at 70°C until it reached its half-life time. This can be explained by the incubation process of iTLL, which activates the lysine residues of the enzyme, enabling the formation of Schiff base covalent bonds, and making the thermally incubated biocatalysts more resistant.

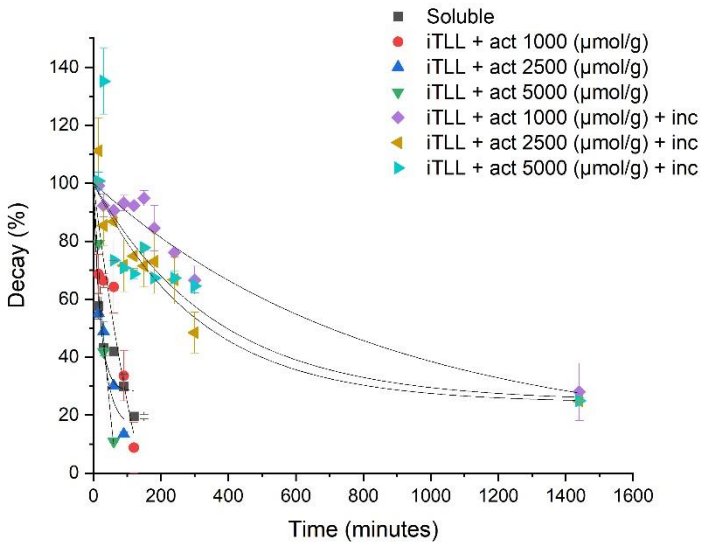

Figure 1: Thermal stability of soluble and immobilized TLL. Deactivation in the presence of sodium phosphate buffer (5 mM, pH 7.0) at 70°C

4 CONCLUSION

Coconut fiber activated with sodium periodate has proven to be a promising matrix, producing an heterofunctional support for immobilization processes. Additionally, the fiber is sustainable, low-cost, and a highly abundant raw material in Brazil.

The concentration of sodium periodate solution for activation of the support that showed the most promise was 1000 µmol/g, which yielded the best immobilization yield (around 65%) and significant recovered activity after immobilization (> 20%).

A significant increase in the half-life time of the sample incubated at pH 10.2 was observed. This increase may be attributed to the probable formation of covalent bonds that emerged after incubation. To confirm the presence of these bonds, electrophoresis and desorption analyses will be conducted to investigate the nature of these interactions.

REFERENCES

[1] Pinheiro, B. B.; dos Santos, K. P.; Rios, N. S.; de Macedo, A. C.; dos Santos, J. C.S; and Gonçalves L. R.B; (2019) Enzymatic Reactions and Biocatalytic Processes. In: Reedijk, J. (Ed.) Elsevier Reference Module in Chemistry, Molecular Sciences and Chemical Engineering. Waltham, MA: Elsevier. 29-Nov-19 doi: 10.1016/B978-0-12-409547-2.14571-8.

[2] BRÍGIDA, A. I. S.; PINHEIRO, A. D. T.; FERREIRA, A. L. O.; PINTO, G. A. S.; GONÇALVES, L. R. B., Immobilization of Candida antarctica Lipase B by Covalent Attachment to Green Coconut Fiber. Fortaleza, CE: Applied Biochemistry and Biotechnology, 2007. 67-80 p. v. 136. ACKNOWLEDGEMENTS.

[3] RIOS, N. S.; MENDEZ-SANCHEZ, C.; ARANA-PEÑA, S.; RUEDA, N.; ORTIZ, C.; GONÇALVES, L. R.B.; FERNANDEZ-LAFUENTE, R.; Immobilization of lipase from Pseudomonas fluorescens on glyoxyl-octyl-agarose beads: Improved stability and reusability. BIOCHIMICA ET BIOPHYSICA ACTA-PROTEINS AND PROTEOMICS, v. 1867, p. 741-747, 2019.

[4] RIBEIRO, V. T.; CAMPOLINA, A. C.; COSTA, W. A.; PADILHA, C. E. A.; FILHO, J. D. B. da C.; LEITÃO, A. L. O. de S.; ROCHA, J. da C.; SANTOS, E. S. dos. Ethanol Production from Green Coconut Fiber Using Sequential Steam Explosion and Alkaline Pretreatment. Biomass Conversion and Biorefinery, v. 14, p. 8579–8589, 2024.

[5] BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. 2. ed. Georgia: Analytical Biochemistry, 1976. 248-254 p. v. 72

[6] SILVA, J. A.; MACEDO, G. P.; RODRIGUES, D. S.; GIORDANO, R. L. C.; GONÇALVES, L. R. B. Immobilization of Candida antarctica lipase B by covalent attachment on chitosan-based hydrogels using different support activation strategies. Biochemical Engineering Journal, v. 60, p. 16–24, 2012

[7] NYPELÖ, T.; BERKE, B.; SPIRK, S.; SIRVIÖ, J. A.; Review: periodate oxidation of wood polysaccharides: modulation of hierarchies. Carbohydrate Polymers, [S.L.], v. 252, p. 117105, jan. 2021. Elsevier BV. http://dx.doi.org/10.1016/j.carbpol.2020.117105.

[8] SIRVIO, J.; HYVAKKO, U.; LIIMATAINEN, H.; NIINIMAKI, J. Periodate oxidation of cellulose at elevated temperatures using metal salts as cellulose activators. Carbohydrate Polymers, Volume 83, Issue 3, p. 1293-1297, 30 January 2011.

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