Immobilization of CalB Lipase by adsorption on magnetic nanoparticles: A heterogeneous biocatalysis

Imobilização da lipase CalB por adsorção em nanopartículas magnéticas: um biocatalisador heterogêneo

DOI:10.34117/bjdv7n8-022

Recebimento dos originais: 03/07/2021
Aceitação para publicação: 03/08/2021

João Brandão Junior
Master's in Energia e Ambiente by Universidade da Integração Internacional da Lusofonia Afro-Brasileira – UNILAB.
E-mail: brandjunior@aluno.unilab.edu.br

Debora Cristina Lima Ferreira
Post graduate in Gestão de Recursos Hídricos, Ambientais e Energéticos and Master's in Energia e Ambiente by Universidade da Integração Internacional da Lusofonia Afro-Brasileira – UNILAB.
E-mail: deboracristinaferreira@gmail.com

Francisco Silvio Maia
Post graduate in Perícia Criminal and Política e Gestão em Segurança Pública; Aquiraz Security Secretary and Master's in Energia e Ambiente by Universidade da Integração Internacional da Lusofonia Afro-Brasileira – UNILAB.
E-mail: fsilviomaia@yahoo.com.br

Gabrielly Ferreira Mota
Master's in Energia e Ambiente by Universidade da Integração Internacional da Lusofonia Afro-Brasileira – UNILAB.
E-mail: gabriellyferreira0@gmail.com

Jean Gleison Andrade do Nascimento
Post graduate in Ensino de Química; Secretaria de Educação do Estado do Ceará (Seduc - CE) and Master's in Energia e Ambiente by Universidade da Integração Internacional da Lusofonia Afro-Brasileira – UNILAB.
E-mail: jandradenascimento@gmail.com

Rodolpho Ramilton de Castro Monteiro
Master in Chemical Engineering by Universidade Federal do Ceará – UFC.
E-mail: montteirorrc@gmail.com

Aluísio Marques da Fonseca
Professor Associado III at the Instituto de Ciências Exatas da Natureza of the Universidade da Integração Internacional da Lusofonia Afro-Brasileira – UNILAB.
E-mail: aluisiomf@unilab.edu.br

Maria Cristiane Martins de Souza
Professora Adjunto, Classe C, nível III at the Instituto de Engenharias e Desenvolvimento Sustentável – IEDS of the Universidade da Integração Internacional da Lusofonia Afro-Brasileira – UNILAB.
E-mail: mariacristiane@unilab.edu.br

ABSTRACT
Iron magnetic nanoparticles (Fe_3O_4) were evaluated as adsorption preparative heterofunctional support for the immobilization of lipase B from Candida antarctica (CALB). Heterogeneous magnetic catalysts are easy to recover by the magnetic field, which may optimize operational cost and enhance the purity of the products. The nanoparticles were produced by the co-precipitation method. Modifications were carried out on the nanoparticles' surfaces with aminopropyltriethoxysilane (APTS). The adsorption was evaluated for 3.0 mg protein/g of support in the presence of 5mM sodium phosphate buffer, pH 7.0, at 25 °C and 0.5h of immobilization. In a solvent-free medium, under 37 °C, the biocatalyst prepared has shown activity of 2.2 U/g for the esterification of oleic acid after 0.5h, 37 kHz, and 300 W. The results obtained with CALB adsorbed onto magnetic iron nanoparticles were compared with those of lipase B from Candida antartica adsorbed onto acrylic resin (Novozym® 435). In this regard, under the same reactional conditions, Novozym® 435 has presented activity of 2.9 U/g.

Keywords: Immobilization, Lipase (CALB), Adsorption, Magnetic Nanoparticle.

RESUMO
Foram avaliadas nanopartículas magnéticas de ferro (Fe3O4) como suporte heterofuncional preparativo de adsorção para a imobilização da lipase B de Candida antárctica (CALB). Os catalisadores magnéticos heterogéneos são fáceis de recuperar pelo campo magnético, o que pode optimizar o custo operacional e aumentar a pureza dos produtos. As nanopartículas foram produzidas pelo método da co-precipitação. Foram efectuadas modificações nas superfícies das nanopartículas com aminopropiltriethoxissilano (APTS). A adsorção foi avaliada para 3,0 mg de proteína/g de suporte na presença de tampão de fosfato de sódio 5mM, pH 7,0, a 25 °C e 0,5h de imobilização. Num meio sem solventes, a menos de 37 °C, o biocatalisador preparado mostrou uma actividade de 2,2 U/g para a esterificação do ácido oleico após 0,5h, 37 kHz, e 300 W. Os resultados obtidos com CALB adsorvido em nanopartículas de ferro magnético foram comparados com os da lipase B de Candida antartica adsorvida em resina acrílica (Novozym® 435). A este respeito, sob as mesmas condições reaccionais, Novozym® 435 apresentou uma actividade de 2,9 U/g.

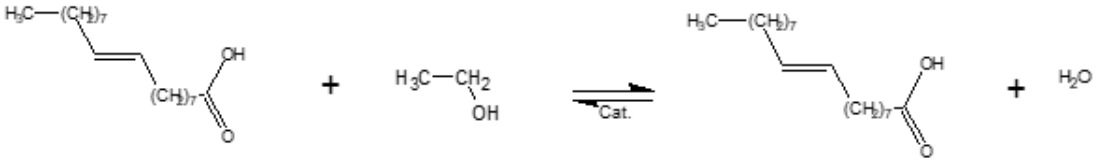
Palavras-Chave: Imobilização, Lipase (CALB), Adsorção, Nanopartícula Magnética.

1 INTRODUCTION

Several authors have widely studied enzymatic ester production, mainly using lipase B from Candida antartica immobilized onto the acrylic resin, commercially available as Novozym ® 435. The main applications of such lipases are in high value-added products (CANNILLA et al., 2018).

Esterification is a method used to eliminate free fatty acids under the action of acid catalysts, usually associated with low added value materials, such as acid sludge and waste oil frying in biodiesel production. This step usually precedes the transesterification process in which oils and fats are transformed into fatty esters by generally basic catalysts. The model reagent chosen to represent the carboxylic acids present in vegetable oils was oleic acid. The alcohol used was ethanol (EtOH) (BARBOSA et al., 2020; CANNILLA et al., 2018).

The esterification of oleic acid (OlAc) can represent biodiesel production since it is present in most oil crops. With EtOH, ethyl oleate (OleOEt) is produced, and water is the by-product, according to the following equation (VOLL et al., 2011):



oleic acid ethanol ethyl oleate water

The industrial esterification processes carried out with strong Brønsted acid catalysts are not environmentally benign (CHAI et al., 2014). They require unique treatments as neutralization involving costly catalyst separation steps from the homogeneous reaction mixtures (ZANUTTINI; PISARELLO; QUERINI, 2014). Heterogeneous catalysts could be easier separated; they show high activity and stability and could be reused, offering milder operating conditions.

An immobilization strategy is selected based on the effectiveness of enzyme utilization, cost of the immobilization procedure, toxicity of immobilization reagents, and the desired final properties of the immobilized biocatalyst (VOLL et al., 2011).

Physical adsorption (ADS) is one of the simplest methods to immobilize enzymes and does not easily alter their active site. Adsorption is the union between the enzyme molecule and inert support. Adsorption supports may be organic and inorganic. Physical forces involve only weak interactions such as hydrogen bonding, hydrophobic bonding, and van der Waals bonding (CARVALHO; LIMA; SOARES, 2014).

The molar ratio of oleic acid/ethanol was 1:5 using an ultrasonic bath (37 kHz and 300 W) at a temperature of 37°C for a period of 0.5 h. Anyhow, the biological nature of ethanol, which slightly increases the heat content and the esters cetane number, make ethyl esters (EEs) more interesting than methyl esters (MEs); moreover, EtOH is nontoxic and has better solvent properties than methanol for oil solubility, and EEs have to pour points lower than those of methyl esters(ENCINAR; GONZÁLEZ; RODRÍGUEZ-REINARES, 2007).

2 MATERIALS AND METHODS

2.1 MATERIALS

Iron magnetic nanoparticles (Fe_3O_4) were produced by the coprecipitation method (BARRETO et al., 2012). Lipase B from Candida antartica (CALB) was purchased from Codexis (Redwood, USA). Lipase B from Candida antartica immobilized onto the acrylic resin (Novozym® 435), γ-aminopropyltriethoxysilane (APTS) were purchased from. All other reagents (analytical grade) were purchased from Synth (São Paulo, Brazil) and Vetec (São Paulo, Brazil).

2.2 SYNTHESIS OF (FE_3O_4)

Iron magnetic nanoparticles (Fe_3O_4), with a particle size of 11.0 nm, were produced by the coprecipitation method (BARRETO et al., 2012).

2.3 TREATMENT OF THE SUPPORT WITH Γ-AMINOPROPYLTRIETHOXYSILANE (APTS)

Iron magnetic nanoparticles were modified with γ-aminopropyltriethoxysilane (APTS). The reaction was initiated by adding a solution of APTS (2.0 % v/v) to the support in a liquid-solid ratio of 0.2 (mL. mg−1 of support). The solution was heated at 100 °C for 10 hours under a nitrogen atmosphere. The modified nanoparticles were washed with 100 mL of methanol and 100 mL of ethanol, separated by magnetism, and after, dried at 30 °C for 24 hours (NETTO; ANDRADE; TOMA, 2009).

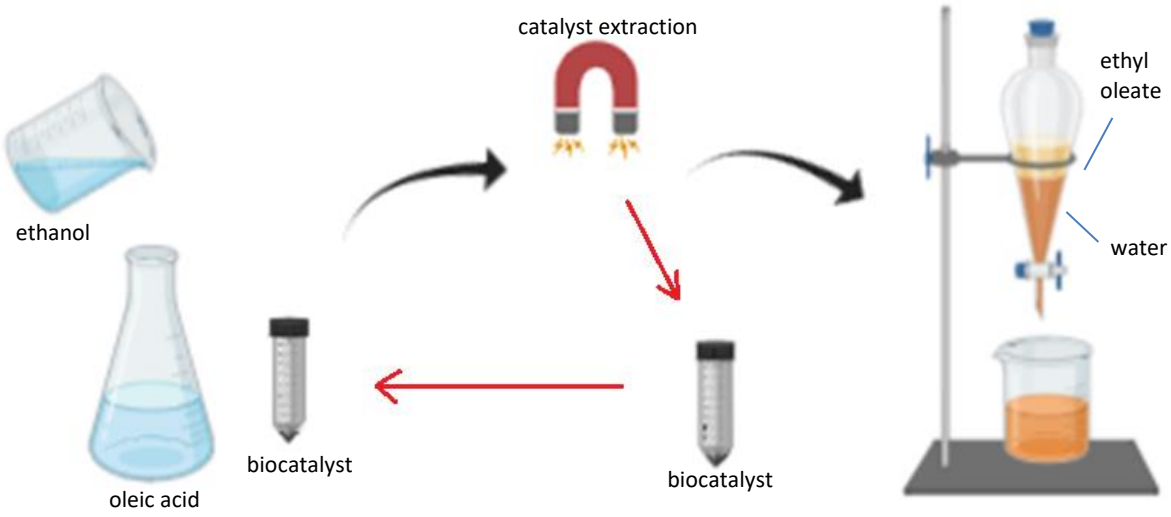
2.4. CALB IMMOBILIZATION

The immobilization of CALB in the previously functionalized support was performed in a batch at 25 ° C. 2.54 mL of the enzyme (0.51 mg) in sodium phosphate buffer (5 mM, pH = 7.0) was added. 0.1 g of APTS nanoparticles were added. Agitation

speed of 90 rpm. Contact time 0.5h. CALB adsorption immobilized, called CALB-ADNs, was removed by magnetic separation and washed with sodium phosphate buffer (5 mM, pH = 7.0) (ORTIZ et al., 2019; SOUZA, 2012).

2.5 ETHYL OLEATE SYNTHESIS

The esterification experiments were carried out in plastics tubes (2.0 mL) containing 0.6 g of oleic acid and 151 µL of ethanol (molar ratio 1:1), as well as 0.023 g of biocatalyst CALB-ADNs (1.7% of reaction mass). The reaction was conducted under orbital stirring (150 rpm) at 37°C. The acid index was evaluated by the Ca 5-40 AOCS method (MOSSOBA, M.; KRAMER, J.; DELMONT, P.; YURAWECZ, M.; RADER, 2003).



Source: authors

2.6 ENZYMATIC ACTIVITY

Enzyme activity was calculated according to Equation 1.

$$A = \frac{(V_a - V_b) \,.\, 1000 \,.\, M \,.\, V_{final}}{t \,.\, V \,.\, m}$$

Where A is the esterification activity U/g); V_a is the volume NaOH spent on titration of a sample taken at time zero (mL); V_b is the volume of NaOH spent on titration of a sample taken after time t (mL); M is the molarity of the solution of NaOH (mmol/mL); V_{final} is the final volume of reaction medium (mL); m is the enzyme mass immobilized used in the reaction (g); V is the volume of the reaction medium aliquot taken to titration (mL), and t is time (min).

3 RESULTS AND DISCUSSIONS

3.1 CALB IMMOBILIZATION

The enzyme load may be explained from the analysis of interactions between the enzyme and the support. The increase in enzyme load allows more protein-protein interaction. Thus, an optimum amount of enzyme is sought. Increasing the enzyme load also leads to the diffusional limitations, a barrier to products and substrates diffusion, and steric hindrance, which can also explain a greater difficulty in adsorption processes when related to processes involving covalent bonding; thus, the same enzymatic charge of the commercial biocatalyst was used for the adsorption process.

The contact time was investigated as short as possible, 0.5h, due to rapid protein adsorption to the support, unlike for covalently bonded supports. This condition is necessary because the adsorption immobilization conditions favor the desorption of enzymes, a milder condition when the reaction medium with a higher affinity for lipases, such as fatty acids, is used.

This work aims to obtain the conversion rate of the oleic acid esterification reaction with ethanol using the heterogeneous CALB immobilized catalyst in magnetic nanoparticles.

3.2 CATALYTIC ESTERIFICATION

Oleic acid was esterified. In a solvent-free medium, under 37 °C, the biocatalyst prepared has shown activity of 2.2 U/g for the esterification of oleic acid after 0.5h, 37 kHz, and 300 W. The results obtained with CALB adsorbed onto magnetic iron nanoparticles were compared with those of lipase B from Candida antartica (covalent immobilization) onto acrylic resin (Novozym® 435). In this regard, under the same reactional conditions, Novozym® 435 has presented activity of 2.9 U/g.

The type of agitation was analyzed in which the interference of agitation can be directly related to the shear rate, which initially promotes more interactions between enzyme and support (SOUZA et al., 2017). The ultrasonic bath allowed a higher substrate enzyme interaction in a shorter reaction time, 0.5h for both catalysts, Novozym® 435 and CALB-ADNs. The residue was baked at 30 °C until complete drying. This process was completed after 32h and repeated two more times.

The results of our study are in agreement with those obtained for covalent commercial support. Enzyme stability is maximized through the use of nanoscale

supports, modulating catalytic specificity, low resistance to mass transfer, thereby improving diffusion and reducing operating cost (SOUZA et al., 2017).

The adsorption conditions of ethanol at the step of oleic acid reactions contribute to reducing the steric energy where the reaction medium was ultrasonically bathed at a temperature of 37 °C and for a period of 0.5h.

Therefore, by differentiating the reactions of esterification and transesterification in obtaining esters, it can be understood that catalysts in these reactions produce a change in the catalytic medium (PANG et al., 2016). By comparing the samples, it can be analyzed that the reaction occurred, and there was ester production (CEA et al., 2019).

Temperature is one of the factors that can influence the speed of reaction catalyzed by two mechanisms: the chemical mechanism, in which the temperature increase and the consequent increase of the kinetic energy of the reagents result in the process acceleration, and the enzyme denaturation mechanism by the rise of temperature (SOUZA et al., 2017). Thus, the temperature of 37 °C, as shown in the Souza et. al. works, is ideal for performing esterification reactions (NETTO; ANDRADE; TOMA, 2009).

The effect of pH on the adsorption of enzymatic activity has immobilization efficiency at pH 7.0 for this reaction (CEA et al., 2019).

The conversion rate for biodiesel production attracts considerable attention because of its profitability, degradability, low toxicity, high safety, and renewability obtained through esterification and catalytic transesterification of free fatty acids. In the chemically catalyzed transesterification process for biodiesel production, chemical catalysts are used. Enzymatic immobilization in materials as heterogeneous catalysts for esterification is used with complementary environment contributions (PANG et al., 2016).

4 CONCLUSION

The selection of an immobilization strategy is based on the efficiency of the enzyme use, the cost of the immobilization procedure, the toxicity of the immobilization reagents, and the desired final properties of the immobilized biocatalyst. The use of the ultrasonic bath, besides the enzymatic extract, was an indispensable factor in obtaining the comparative value with covalent commercial support. It is noteworthy that the enzymatic adsorption process compared to other methods used for immobilization has potential with a preparative immobilization. Thus the high enzymatic load can be used quickly and stabilized after a second immobilization from covalent connections

(heterofunctional biocatalysts). The utilization and conversion are efficient for the studied reaction medium. Finally, it was found that the esterification of oleic acid with ethyl alcohol and enzymatic combination of the enzyme CALB immobilized onto magnetic nanoparticles by adsorption achieved a favorable conversion rate and proper stoichiometry and may even serve as a parameter for prospecting new catalyst combinations.

ACKNOWLEDGMENTS

We gratefully recognize the financial support from the CNPq project: 409058/2016-5/Universal 01/2016, FUNCAP, CAPES, and UNILAB.

REFERENCES

BARBOSA, A. DOS S. et al. Hydrophobic immobilization of Burkholderia cepacia lipase onto octyl-silica for synthesis of flavors esters. **Brazilian Journal of Development**, v. 6, n. 5, p. 27145–27170, 2020.

BARRETO, A. C. H. et al. Novel ferrofluids coated with a renewable material obtained from cashew nut shell liquid. **Microfluidics and Nanofluidics**, v. 12, n. 5, p. 677–686, mar. 2012.

CANNILLA, C. et al. Biofuels production by esterification of oleic acid with ethanol using a membrane assisted reactor in vapour permeation configuration. **Applied Catalysis A: General**, v. 566, p. 121–129, set. 2018.

CARVALHO, N. B.; LIMA, Á. S.; SOARES, C. M. F. USO DE SÍLICAS MODIFICADAS PARA IMOBILIZAÇÃO DE LIPASES. **Quim. Nova**, v. 00, p. 1–15, 2014.

CEA, M. et al. Enzymatic esterification of oleic acid by Candida rugosa lipase immobilized onto biochar. **Journal of Environmental Management**, v. 242, p. 171–177, 15 jul. 2019.

CHAI, M. et al. Esterification pretreatment of free fatty acid in biodiesel production, from laboratory to industry. **Fuel Processing Technology**, v. 125, p. 106–113, 2014.

ENCINAR, J. M.; GONZÁLEZ, J. F.; RODRÍGUEZ-REINARES, A. Ethanolysis of used frying oil. Biodiesel preparation and characterization. **Fuel Processing Technology**, v. 88, n. 5, p. 513–522, maio 2007.

MOSSOBA, M.; KRAMER, J.; DELMONT, P.; YURAWECZ, M.; RADER, J. Official methods for the determination of trans fat. [S.1.]. **AOCS Pres Champaingn**, 2003.

NETTO, C. .; ANDRADE, L. .; TOMA, H. Enantioselective transesterification catalysis by Candida antartica lipase immobilized on superparamagnetic nanoparticles. **Tetrahedron: Asymmetry, Elsevier**, v. 20, p. 2299–2304, 2009.

ORTIZ, C. et al. Catalysis Science & Technology MINI REVIEW Novozym 435: the "perfect" lipase immobilized biocatalyst? **Cite this: Catal. Sci. Technol**, v. 9, p. 2380, 2019.

PANG, J. et al. Esterification of oleic acid with methanol by immobilized lipase on wrinkled silica nanoparticles with highly ordered, radially oriented mesochannels. **Materials Science and Engineering C**, v. 59, p. 35–42, 1 fev. 2016.

SOUZA, M. C. M. DE. **Imobilização de lipase de Candida antartica do tipo B em nanopartículas magnéticas visando a aplicação na síntese de ésteres.** [s.l.] Universidade Ferderal do Ceará, 2012.

SOUZA, M. C. M. DE et al. Production of flavor esters catalyzed by Lipase B from Candida antarctica immobilized on magnetic nanoparticles. **Brazilian Journal of**

Chemical Engineering, v. 34, n. 3, p. 681–690, 1 jul. 2017.

VOLL, F. A. P. et al. Thermodynamic analysis of fatty acid esterification for fatty acid alkyl esters production. **Biomass and Bioenergy**, v. 35, n. 2, p. 781–788, fev. 2011.

ZANUTTINI, M. S.; PISARELLO, M. L.; QUERINI, C. A. Butia Yatay coconut oil: Process development for biodiesel production and kinetics of esterification with ethanol. **Energy Conversion and Management**, v. 85, p. 407–416, 2014.