

EVALUATION OF THE EFFICIENCY OF DIFFERENT POLYMERIC MATRICES FOR THE IMMOBILIZATION OF CANDIDA ANTARCTICA LIPASE AND APPLICATION IN THE SYNTHESIS OF 2-ETHYLHEXYL OLEATE ESTER

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ABSTRACT

The search for different supports for enzyme immobilization has been increasingly explored. Therefore, the present study aimed to compare the performance of a support widely reported in the literature, styrene divinylbenzene (STY-DVB-M), with other copolymers containing different cross-linking agents, such as triethylene glycol dimethacrylate (STY-TEGDMA-M) and ethylene glycol dimethacrylate (STY-EGDMA-M), with the purpose of evaluating their performance as biocatalysts obtained by immobilizing *Candida Antarctica* lipase B (CALB) in the synthesis of the emollient ester 2-ethylhexyl oleate. It is noteworthy that these copolymers were magnetized with iron ions, aiming for easier separation from the reaction medium. The enzymatic activities of the biocatalysts obtained by the hydrolysis of methyl butyrate showed results between 522 – 810 U g⁻¹. The syntheses of 2-ethylhexyl oleate were carried out discontinuously in stirred flasks employing an equimolar mixture (1:1) for 24 hours, under constant agitation at 150 rpm and a temperature of 55°C. The results obtained demonstrated maximum conversions between 30 and 66% after the reaction period, with emphasis on the CALB-STY-DVB-M biocatalyst, which achieved 66% fatty acid consumption in 24 hours, demonstrating higher efficiency in obtaining the emollient ester. From the present study, it is concluded that although the best performance was still achieved by the divinylbenzene-based biocatalyst, it is believed that the other copolymers also have potential for use as supports for lipase immobilization, especially after more detailed studies regarding the implementation of some modifications in their composition and/or the use of other immobilization methods.

Keywords: Magnetized copolymer. *Candida antarctica*. Emollient ester. Biocatalysts.

1 INTRODUCTION

Currently, the demand for new technologies, ecological and sustainable industrial processes has led to the development of innovations employing enzymatic methods for the production of pharmaceuticals, food, cosmetics, among others. In this context, immobilized lipases have been applied in various reactions such as transesterification, interesterification, hydrolysis of oils and fats, and esterification. Special emphasis can be given to the production of esters with different properties, such as emollient esters, which possess multifunctional oleochemical characteristics, as their applicability is directly linked to the quantity of carbons present in their molecule. Emollient esters have medium-chain carbon structures (C12 to C18), which are necessary in the formulation of beauty and skincare products [1,2].

Different materials can be used as support for lipase immobilization, depending on the required properties, such as mechanical strength, physical and chemical stability, hydrophobic/hydrophilic character, enzyme adsorption capacity, and operational cost [3,4]. The use of copolymers for the preparation of biocatalysts has become a promising alternative, as they demonstrate high performance in enzymatic reactions and possess multifunctional properties [5].

Ortiz et al. (2019) [6], described that Novozym 435, a preparation of CALB lipase immobilized on a cross-linked divinylbenzene matrix, is possibly the most widely used commercial biocatalyst, both in academia and industry. However, due to the high cost of this biocatalyst, different copolymers are currently being developed for use as enzyme immobilization supports, aiming to obtain active and stable biocatalysts. Rosa (2019) [7] studied different conditions for the synthesis of magnetized copolymers obtained from styrene and other cross-linking agents for use as immobilization matrices of lipases, obtaining promising results that enable the exploration of these matrices for this purpose.

Thus, the present study evaluated the possibility of using different magnetized copolymers, poly(styrene-co-divinylbenzene), poly(styrene-co-ethylene glycol dimethacrylate), and poly(styrene-co-triethylene glycol dimethacrylate), as matrices for immobilization of the *Candida antarctica* lipase (CALB) in the synthesis of the emollient ester 2-ethylhexyl oleate.

2 MATERIAL & METHODS

The synthesis of the different copolymers occurred through suspension polymerization with the addition of Fe²⁺ and Fe³⁺, employing an organic and an aqueous phase (1:8 v/v), where the organic phase consisted of a base monomer (styrene) with varying cross-linking agents between divinylbenzene (DVB), triethylene glycol dimethacrylate (TEGDMA), and ethylene glycol dimethacrylate (EGDMA), in addition to the solvents heptane and toluene. The immobilization process of the lipase on the different synthesized supports occurred through the physical absorption technique according to the methodology proposed by Bento et al.

(2017) [5]. The enzymatic activity of the synthesized biocatalysts was carried out using the methodology of methyl butyrate hydrolysis as described by de Lima et al (2022) [8] and quantified employing Equation 1. The synthesis of 2-ethylhexyl oleate ester was conducted discontinuously at a pre-established temperature of 55°C under constant agitation at 150 rpm for 24 hours. The consumption of the present fatty acid was monitored through collected aliquots and analyzed via titration with a 0.04 mol L⁻¹ KOH solution and phenolphthalein as an indicator. The quantification of the fatty acid consumption in the reaction medium was performed using Equation 2, and the conversion of the acid into percentage was obtained through Equation 3.

$$\text{Activity (U g}^{-1}\text{)} = \frac{(V-V_b) \cdot M \cdot 1000}{t \cdot m} \quad (1)$$

$$\text{Carboxylic Acid (g.L}^{-1}\text{)} = \frac{V \cdot M \cdot MM}{v} \quad (2)$$

$$\text{Conversion (\%)} = \frac{(C_i - C_t)}{C_i} \times 100 \quad (3)$$

Where: V is the volume of KOH in mL, M is the concentration of KOH in mol.L⁻¹, MM is the molar mass of the carboxylic acid in g.mol⁻¹, v is the volume of the aliquot in mL, C_i is the initial concentration of the acid in the substrate in mmol.L⁻¹, and C_t is the concentration of the acid at a determined time of the esterification reaction in mmol.L⁻¹.

3 RESULTS & DISCUSSION

The catalytic activities of *Candida antarctica* lipase immobilized on STY-DVB-M, STY-EGDMA-M, and STY-TEGDMA-M were assessed through the hydrolysis of methyl butyrate, yielding results in the range of 522 – 810 U.g⁻¹ for the synthesized biocatalysts. Figure 1 (a) and (b) show the conversion profile of emollient ester syntheses and the maximum conversions achieved at 55°C for 24 hours, respectively.

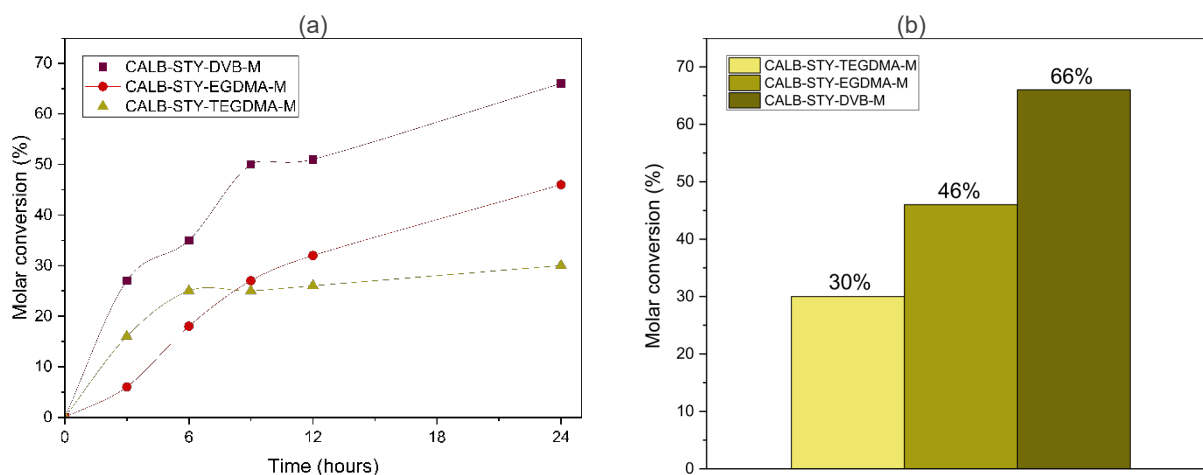


Figure 1 Conversions of 2-ethylhexyl oleate ester syntheses from esterification reactions employing the biocatalysts: CALB-STY-TEGDMA-M; CALB-STY-EGDMA-M; CALB-STY-DVB-M at 55°C: (a) conversion profile of the reaction; (b) maximum conversions obtained.

Upon analyzing the conducted syntheses, the high efficiency of the biocatalyst obtained by immobilized lipase on the support employing divinylbenzene as the cross-linking agent was noted when compared to the other synthesized supports, reaching maximums of 66% after the reaction time. Silva et al. (2020) [1], in a study utilizing CALB-STY-DVB-M in the synthesis of 2-ethylhexyl oleate at 50°C, observed a maximum conversion of 60%, which corroborates with the results obtained in the present study.

When the CALB-STY-EGDMA-M biocatalyst was employed, maximum conversions of 46% were achieved for the conducted synthesis. In a study conducted by Rangel et al. (2022) [9], where they explored the same biocatalyst in obtaining octyl oleate ester at 55°C, conversions of around 60% for oleic acid were observed. The result obtained by the present study may be related to the polarity of the reaction medium, as the use of octanol makes the medium less polar compared to 2-ethylhexyl, demonstrating a possible preference of these lipases for alcohols of intermediate polarity.

In a study conducted by Rosa (2019) [7], which conducted exploratory studies regarding these supports, it was observed from thermogravimetric analyses (TGA) that the STY-DVB-M copolymer is more thermally resistant when compared to the other analyzed supports, with thermal degradation onset at 406.66°C, followed by STY-EGDMA-M with a temperature of 369.3°C, and lastly STY-TEGDMA-M, which presents a decomposition onset temperature at 328.24°C.

Rangel (2023) [9], when employing the CALB-TEGDMA-M biocatalyst in the synthesis of the same emollient ester, obtained a maximum conversion of 46% at a temperature of 45°C. This result, when compared to the one obtained in the present study, where a maximum conversion of 30% was achieved at a temperature of 55°C, corroborates with what Rosa (2019) [7] detected through thermal analyses, showing that the TEGDMA support is less thermally stable at higher temperatures, proving to be less efficient in catalyzing the synthesis of 2-ethylhexyl oleate.

The better thermal resistance exhibited by the copolymer composed of DVB is likely related to the packing of the aromatic rings present in divinylbenzene, providing greater resistance, both chemically and thermally, to these particles, as discussed by Theodoro (2018) [11]. This statement is consistent with the data obtained in the current study, as at 55°C, the best performance in synthesis was obtained by the CALB-STY-DVB-M biocatalyst (66% conversion).

Regarding the support constituted by TEGDMA, Rosa (2019) [7] mentions that this cross-linker has a longer chain and a greater amount of ester groups in its structure, which may result in lower thermal resistance of STY-TEGDMA-M particles compared to the other copolymers evaluated as supports, which was confirmed by TGA and also observed in the present study, since the biocatalyst constituted by this cross-linking agent was the one that showed the lowest efficiency in catalyzing the synthesis of the emollient ester (30% conversion).

The studies conducted so far have revealed that the polymeric matrices, in addition to those employing divinylbenzene as the cross-linking agent, have shown promising results for use as lipase immobilization supports. It is worth mentioning that tests are already being conducted to improve the polymeric matrix, through changes in their compositions, as well as the exploration of different immobilization methods, such as covalent bonding.

4 CONCLUSION

From the results obtained, it was concluded that the magnetized copolymers demonstrated good potential for use as supports for the immobilization of *Candida antarctica* lipase. The obtained biocatalysts were applied to catalyze the synthesis of 2-ethylhexyl oleate, achieving maximum conversions in the range of 30 - 66% of the carboxylic acid at the end of the reaction. It is noteworthy that, although the CALB-STY-DVB-M biocatalyst showed the best performance in the synthesis of the emollient ester, it is believed that the use of these polymeric matrices constituted by other cross-linking agents, alternatives to DVB, may be promising for use as supports for lipase immobilization, after more detailed/in-depth studies.

REFERENCES

- ¹ SILVA, M.V.C.; ROSA, C.M.R.; AGUIAR, L.G.; OLIVEIRA, P.C.; DE CASTRO, H.F.; FREITAS, L. 2020. *Chemical Engineering and Technology*, v. s/v, p. 1-15.
- ² SANTOS, J. S. 2015. Thesis (PhD in Process Engineering) - Tiradentes University, Aracajú, 2015.
- ³ BILAL, M.; RASHEED, T.; ZHAO, Y.; IQBAL, H. M. N.; CUI, J. 2018. *International journal of biological macromolecules*, v. 119, p. 278-290.
- ⁴ RODRIGUES, R. C.; VIRGEN-ORTÍZ, J. J.; DOS SANTOS, J. C. S.; BERENQUER-MURCIA, Á.; ALCANTARA, A. R.; BARBOSA, O.; ORTIZ, C.; FERNANDEZ-LAFUENTE, R. 2019. *Biotechnology Advances*, v. 37, n. 5, p. 746-770.
- ⁵ BENTO, H. B. S.; DE CASTRO, H. F.; OLIVEIRA, P. C.; FREITAS, L. 2017. *Journal of Magnetism and Magnetic Materials*, v. 426, p. 95-101.
- ⁶ ORTIZ, C.; FERREIRA, M. L.; BARBOSA, O.; DOS SANTOS, J. C. S.; RODRIGUES, R. C.; BERENQUER-MURCIA, A.; BRIAND, L. E.; FERNANDEZ-LAFUENTE, R. 2019. *Catalysis Science & Technology*, V. 9, P. 2380-2420.
- ⁷ ROSA, C. M. R. 2019. Dissertation (Master of Science) - Lorena School of Engineering, University of São Paulo, Lorena. 130p.
- ⁸ DE LIMA, R., BENTO, H. B., REIS, C. E., BÔAS, R. N. V., DE FREITAS, L., CARVALHO, A. K., DE CASTRO, H. F. 2022. *Catalysis Letters*, 152(2), 547-558.
- ⁹ RANGEL, AMANDA B. S.; SILVA, MATEUS V. C.; DE ASSIS, GABRIELLE P.; ROSA, CÍNTIA M. R.; DOS SANTOS, JÚLIO C.; DE FREITAS, LARISSA. 2022. *Catalysis Letters*, v. 1, p. 1.
- ¹⁰ RANGEL, A. B. S. 2023. Dissertation (Master of Science) - Lorena School of Engineering, University of São Paulo, Lorena. 97 p.
- ¹¹ THEODORO, R. T. 2018. Dissertation (Master of Science) - Lorena School of Engineering, University of São Paulo, Lorena. 76 p.

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