SYNTHESIS OF OCTYL OLEATE CATALYZED BY DIFFERENT IMMOBILIZED LIPASES ON MAGNETIZED POLYMERIC MATRIX

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ABSTRACT

The present study aimed to synthesize the emollient ester octyl oleate by immobilizing lipases from *Candida antarctica* (CALB), *Penicillium camembertii* (lipase G), and porcine pancreatic lipase (LPP) on a polymer support magnetized with iron ions based on styrene and triethylene glycol dimethacrylate (STY-TEGDMA-M). The copolymer synthesis occurred through suspension polymerization with the addition of magnetite, and immobilization on this support was performed using the physical adsorption technique. The quantification of catalytic activities demonstrated values between 125 - 619 U g^{-1} for the biocatalysts CALB-STY-TEGDMA-M, G-STY-TEGDMA-M, and LPP-STY-TEGDMA-M. The emollient ester was obtained discontinuously at 45°C under stirring at 150 rpm for 24 hours. After conducting the esterification reactions in a solvent-free medium, maximum conversions of 45% were observed in the synthesis employing the CALB-STY-TEGDMA-M biocatalyst, while for the others, maximum conversions of 15% and 22% were achieved for the G-STY-TEGDMA-M and LPP-STY-TEGDMA-M biocatalysts, respectively. It is concluded that, although all tested biocatalysts were capable of catalyzing the synthesis of the emollient ester, it is believed that more detailed studies on the composition of the STY-TEGDMA-M polymer matrix and the use of different enzyme immobilization techniques may improve the performance of these biocatalysts developed in the present study.

Keywords: Octyl oleate. Polymeric matrix. Emollient ester. Immobilized lipase.

1 INTRODUCTION

Lipases (triacylglycerol hydrolases, EC 3.1.1.3), enzymes belonging to the class of hydrolases, stand out among others for their ability to catalyze hydrolysis reactions of oils and fats as well as esterification and transesterification reactions, exhibiting wide industrial applications in the food, pharmaceutical, textile, cosmetic, and detergent sectors. They are effective biocatalysts due to their high substrate-specific activity, stereoselectivity, and low environmental impact [1,2,3].

Despite the advantages presented by lipase, its use in the free and soluble form is unattractive for industrial processes due to its low operational stability and high cost. Thus, new supports and immobilization methodologies are increasingly being studied and explored to expand applications and improvements in established processes, as well as to enhance stability and the possibility of enzyme recovery and reuse at the end of the process [3].

The immobilization of lipases on magnetized styrene-based copolymers has become a promising approach, as they are matrices with high hydrophobicity, suitable for immobilizing this class of enzymes, and practically do not absorb water from the reaction medium. Additionally, the recovery of the biocatalyst is facilitated by magnetic force at the end of the reaction, thus avoiding the need for filtration, column separation, and centrifugation [4,5].

The synthesis of esters catalyzed by immobilized lipases is highly attractive, as it provides purer products without coloration and odors, operates under mild pH and temperature conditions, and does not generate by-products [6]. Furthermore, conducting enzymatic synthesis in a solvent-free medium offers additional advantages over the traditional classical method, such as the absence of additional chemical reagents, which minimizes downstream steps, reduces the total process cost, and environmental impact. Enzymatic processes also enable high yields, greater selectivity, and require less energy to promote the reaction [7].

Thus, the present study evaluated the synthesis of the emollient ester octyl oleate using Candida antarctica lipase (CALB), Penicillium camembertii lipase (lipase G), and porcine pancreatic lipase (LPP) immobilized on magnetized poly(styrene-co-triethylene glycol dimethacrylate) (STY-TEGDMA-M) in a solvent-free medium.

2 MATERIAL & METHODS

The synthesis of the copolymer occurred through suspension polymerization with the addition of magnetite to the reaction medium (DE ASSIS, 2023). The immobilization process of lipases in the polymer matrix was carried out using the physical adsorption immobilization technique, as described by Bento et al. (2017) [8]. The enzymatic activities of the biocatalysts CALB-STY-TEGDMA-M, G-STY-TEGDMA-M, and LPP-STY-TEGDMA-M were quantified (Equation 1) through the methyl butyrate hydrolysis method as described by Lima et al. (2022) [9]. The synthesis of octyl oleate was conducted in batch mode on a shaker under

agitation of 150 rpm for a period of 24 hours at 45°C. The consumption of the carboxylic acid present in the reaction was monitored using Equation 2, and its conversion was calculated using Equation 3.

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

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

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

Where: V is the volume of KOH used in titrating the sample in mL, Vb is the volume of KOH used in titrating the blank in mL, M is the concentration of the aqueous KOH solution used in the titration in mol L-1, t is the reaction time in minutes, m is the mass of biocatalyst in g, MM is the molar mass of the carboxylic acid in g/mol, v is the volume of the aliquot in mL, Ci is the initial concentration of the acid in the substrate in mmol·L^{-1}, and Ct is the concentration of the acid at a given time of the esterification reaction in mmol·L^{-1}.

3 RESULTS & DISCUSSION

The enzymatic activities of the biocatalysts CALB-STY-TEGDMA-M, G-STY-TEGDMA-M, and LPP-STY-TEGDMA-M were obtained from the hydrolysis of methyl butyrate. Table 1 demonstrates the results obtained.

Table 1 Enzymatic activity of the synthesized biocatalysts.

Biocatalyst	Catalytic activity (U g^{-1})
CALB-STY-TEGDMA-M	618,47
LPP-STY-TEGDMA-M	200,85
G-STY-TEGDMA-M	125,45

In a study conducted by Silva et al. (2020) [4], employing a similar support to that used in the present study (poly(styrene-co-divinylbenzene) - STY-DVB-M) to immobilize lipase G, a value of 119 U g^{-1} was obtained. In another study where they immobilized CALB lipase on STY-DVB-M, Silva et al. (2023) [11] achieved a catalytic activity of 522 U g^{-1}. Rangel et al. (2022) [10], upon immobilizing CALB lipase on poly(styrene-co-ethylene glycol dimethacrylate) - (STY-EGDMA-M), observed catalytic activity of about 570 U g^{-1}. These results corroborate with the data obtained by the present study, further confirming the applicability of these polymeric matrices as supports for immobilization of different sources of lipases.

The maximum conversions achieved for the synthesis of octyl oleate are expressed in Figures 1a and 1b. It was possible to observe that CALB lipase showed better performance after 24 hours of reaction, reaching a maximum conversion of 45%, while the maximum conversions for the syntheses achieved for the biocatalysts employing lipases G and LPP were 15% and 22%, respectively.

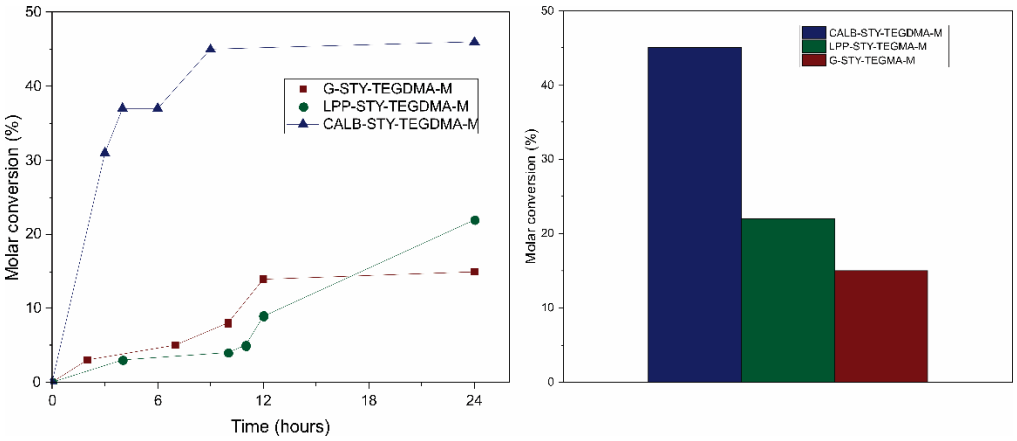

Figure 1 Maximum conversions obtained for the syntheses of octyl oleate employing the biocatalysts G-STY-TEDGMA-M, LPP-STY-TEDGMA-M, and CALB-STY-TEDGMA-M at 45°C: (a) conversion profile of the reaction; (b) maximum conversions obtained after 24 hours.

The superiority of CALB lipase observed in this study for ester production is also widely reported in the literature. In studies conducted by Rangel et al. (2022) [10] and Silva et al. (2023) [11], the high performance of this lipase in obtaining emollient esters was evident. Additionally, Arcens et al. (2020) [12], in a study employing CALB for reactions of esterification, observed that this lipase has a preference for fatty acids with longer chains. These results corroborate with those obtained in the present study, demonstrating the superior performance of CALB lipase for the synthesis of octyl oleate, in which oleic acid was employed, featuring 18 carbon atoms in its carbon chain.

Analyzing Figure 1 (a), it is noted that besides a low conversion, the consumption of the fatty acid was slower when LPP lipase was employed as the biocatalyst for the synthesis of the emollient ester. This fact may be related to the purity level of this enzyme, since other hydrolases such as esterases, amylases, and proteases are also found in its formulation, justifying its lower commercial value compared to other lipases [13].

The low performance obtained by G-STY-TEGDMA-M in the synthesis of octyl oleate may be associated with the high viscosity of the reaction medium, since the alcohol employed, octanol, has 8 carbons in its carbon chain, which may have hindered the enzyme/substrate contact and consequently reduced product formation. De Assis (2023) [14] evaluated the performance of different lipases for the synthesis of esters from fatty acids with different chain lengths and observed that lipase G demonstrated lower catalytic power for reaction media with fatty acids with longer carbon chains.

The observed results demonstrate that the enzymatic route is a promising approach for obtaining octyl oleate emollient ester. The use of different immobilization techniques for lipase, such as covalent bonding, is a possibility for improving the tested immobilized systems. Additionally, different compositions of the support employed are being explored by the Biocatalysis research group at the School of Engineering of Lorena (EEL-USP), aiming at improving the polymeric matrix for enzyme immobilization applications.

4 CONCLUSION

The results obtained in the present study demonstrated that the enzymatic route and the employed biocatalysts were able to synthesize octyl oleate ester. Through the quantification of the catalytic activity of CALB, G, and LPP lipases immobilized in STY-TEGDMA-M, using methyl butyrate hydrolysis, values between 125 and 619 U g^{-1} were obtained. The different biocatalysts showed maximum conversions ranging from 15 to 45% after 24 hours of reaction for octyl oleate synthesis, with CALB lipase demonstrating superiority over the other lipases, as it achieved the maximum conversion. It is worth noting that possible improvements in the employed polymeric matrix and different immobilization methods, such as covalent bonding, are currently being studied to enhance the performance of the biocatalysts obtained from lipase immobilization in the styrene-based copolymer and triethylene glycol dimethacrylate.

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