EVALUATION OF CELLULOSE BEADS PERFORMANCE AS SUPPORT FOR LIPASE IMMOBILIZATION AND APPLICATION IN HYDROLYSIS REACTION

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ABSTRACT

The objective of the present study was to evaluate the performance of cellulose beads as a support for the immobilization of *Candida rugosa* lipase (CRL) and subsequent application in soybean oil hydrolysis. The cellulose beads were synthesized by dissolving sodium hydroxide in distilled water, followed by the addition of urea and cellulose. The beads were used without any pretreatment and also calcined. The lipase was immobilized on the synthesized supports using the covalent binding technique employing epichlorohydrin as the activation agent. Quantification of the hydrolytic activity of the biocatalysts showed values of 2744.90 and 2807.74 $U.g^{-1}$ for the biocatalyst obtained from natural and calcined cellulose beads, respectively, with the Tukey test revealing no statistically significant difference. Regarding the hydrolysis reaction, similar performance was obtained for both biocatalysts, achieving 84% and 88% hydrolysis with the calcined bead and in its natural state, respectively. These results highlight the good performance of the biocatalysts developed in this study, indicating that natural cellulose beads are a relevant option for lipase immobilization support, as well as being renewable.

Keywords: Biocatalysis. Cellulose beads. Immobilization. *Candida rugosa* lipase. Hydrolysis.

1 INTRODUCTION

Cellulose is the most abundant biopolymer in nature, considered inexhaustible, extracted from raw biomass materials. It possesses advantageous characteristics, such as good mechanical resistance and environmental friendliness, therefore, it is considered one of the most promising materials for the future of sustainable economy [1].

In this scenario, materials composed of cellulose are gaining prominence as supports for enzyme immobilization, due to some properties conducive to this application, such as the presence of active sites that allow efficient immobilization [1]. Among them are cellulose beads, millimeter-scale spheres that offer excellent chemical and mechanical properties, and can exhibit hydrophilic and hydrophobic characteristics through alterations and improvements made during their synthesis [2].

The immobilization of the enzyme is necessary, since in its free state, it solubilizes in certain media, besides having thermal instability and difficulty in recovery at the end of the process. The use of enzymes as biocatalysts for hydrolysis reactions allows these to occur under mild temperature and pressure conditions, as well as avoiding the formation of undesirable by-products. Lipases, in particular, have high affinity with the lipid substrate, which ensures high compatibility and selectivity, besides playing an important role in treating effluents with a high lipid content and in generating valuable by-products [3].

Among the numerous enzymes employed, *Candida rugosa* lipase is one of the most used in biotransformation reactions in industrial applications. This occurs because this enzyme belongs to the class of nonspecific lipases, which generates products similar to those derived from chemical catalysis, however, with a lower degree of thermal degradation, due to its ability to carry out reactions at milder temperatures [4].

In this context, the aim of the present study was to evaluate the performance of cellulose beads as support for the immobilization of Candida rugosa lipase (CRL) and its subsequent application in the hydrolysis reaction of soybean oil.

2 MATERIAL & METHODS

The cellulose beads were synthesized by dissolving sodium hydroxide in distilled water, followed by the addition of urea and cellulose. After complete homogenization of the reagents, the mixture was maintained at a temperature of -10°C for 40 minutes. After crystallization and homogenization, the solution was dripped using a peristaltic pump into a nitric acid solution to form uniform spheres. The spheres were then vacuum-filtered with successive washes of distilled water and sodium phosphate buffer (0.1 mol L^{-1} and pH 7), and subsequently dried in an oven until reaching a moisture content below 10%, measured on a Shimadzu moisture balance (Model MOC63u).

The *Candida rugosa* lipase was immobilized on cellulose beads in their natural state and pretreated by calcination in a tubular furnace Spencer (Model N1200) at 400°C for 1 hour under N_2 flow. The immobilization was carried out using the covalent binding technique, with epichlorohydrin as the activation agent, employed in an aqueous solution of 2.5% w/w and at a ratio of 1:10 (w/v) support:solution. The enzymatic activity of the biocatalysts was evaluated using the olive oil hydrolysis method, following the

methodology of Soares et al. (1999) [5]. Additionally, the Tukey test was performed using Minitab 21 software to statistically compare the activity values obtained by the synthesized biocatalysts.

The performance of the biocatalysts was evaluated in batch hydrolysis reactions of soybean oil, conducted in jacketed glass reactors (approximately 100 mL volume), using 33% soybean oil, 3% arabic gum relative to the mass of the pH 7 buffer, totaling 110 g of reaction medium, and 5% by mass of the dried immobilized derivative relative to the total substrate mass. The reactions were conducted for a period of 24 hours, with the temperature of the thermostatic bath maintained at 45°C and constant magnetic stirring at 100 rpm. Aliquots were withdrawn at predetermined time intervals for quantification of the free fatty acids present by titration, with the percentage of hydrolysis calculated using Equation 1.

$$\text{Hidrolysis}(\%) = \frac{V_{KOH} * M_{KOH} * MW}{W * f} * 100 \quad (1)$$

Where: VKOH is the volume of potassium hydroxide (KOH) solution required for titration; M_{KOH} is the concentration of KOH (0.04 $mol.L^{-1}$); M_W is the average molecular weight of the fatty acids present in the oil ($g.mol^{-1}$); W is the weight of the sample, and "f" is the fraction of oil at the beginning of the reaction.

3 RESULTS & DISCUSSION

The results of the hydrolytic activities of the biocatalysts obtained by immobilizing CRL on the beads in their natural and calcined states were very similar, showing 2744.90 ± 76.3 $U.g^{-1}$ and 2807.74 ± 12.89 $U.g^{-1}$, respectively. From the Tukey test, with 95% confidence, it was found that there was no statistically significant difference between the values of catalytic activities obtained by both evaluated biocatalysts. Table 1 presents the values obtained for the catalytic activities and Tukey test.

Table 1 Enzymatic activity of the synthesized biocatalysts and results of the Tukey test

Biocatalyst	Hydrolytic activity ($U.g^{-1}$)
LCR – Bead in their natural state	2744.90 ± 76.3 A
LCR – Bead calcined	2807.74 ± 12.89 A

In a study conducted by Silva and Silva (2014) [6], where they immobilized CRL through covalent binding on a synthetic support consisting of silica-PVA, they obtained a hydrolytic activity of 1389 $U.g^{-1}$. Simões et al. (2011) [7], when immobilizing the same lipase on SiO_2-chitosan particles previously activated by epichlorohydrin, found a hydrolytic activity value of 668 $U.g^{-1}$. Domingues et al. (2020) [8] achieved activities ranging from 115.18 to 167.27 $U.g^{-1}$ with immobilization on a magnetized support, using immobilization techniques by adsorption and covalent binding, observing that the highest activities were obtained for the biocatalyst resulting from covalent binding immobilization.

Therefore, the potential presented by the cellulose beads synthesized in this study as a viable alternative for use as enzyme immobilization support is evident.

The performance of both biocatalysts was evaluated in the hydrolysis of soybean oil, with the results presented in Figure 1. It can be observed in Figure 1 that the biocatalyst obtained from the calcined bead initially exhibited a higher reaction rate profile compared to the untreated bead. However, it is noted that after 6 hours of reaction, the hydrolysis profile of both biocatalysts was very similar, reaching approximately 84% hydrolysis for the biocatalyst obtained from the calcined bead and 88% for the one obtained from the bead in its natural state.

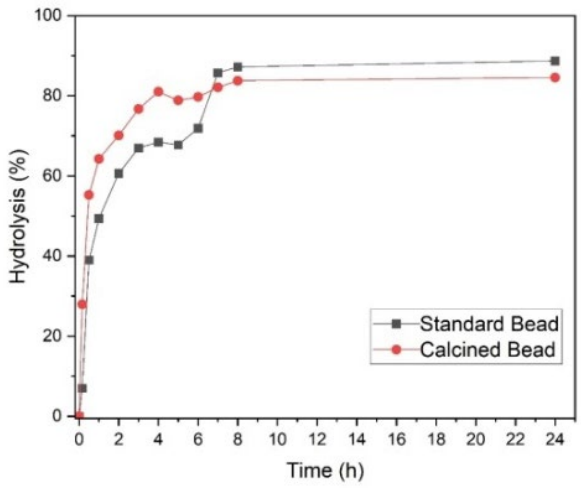

Figure 1 Conversion profile of the free fatty acids formed in the hydrolysis of soybean oil

In a study conducted by Comchowdhury et al. (2013) [9], they achieved a 92% hydrolysis of residual kitchen soybean oil employing free *Candida rugosa* lipase. Domingues et al. (2022) [10] immobilized CRL on magnetized nanoparticles via covalent binding and obtained a 96% hydrolysis of residual frying oil. In a recent study conducted by Correia et al. (2024) [11], the percentage of soybean oil hydrolysis was 57.91% for a combination of *Burkholderia cepacia* lipase (BCL) and immobilized *porcine pancreas* lipase (PPL) on niobium oxide (Nb_2O_5), and 61.11% for the derivative immobilized on Polyhydroxybutyrate (PHB).

These results highlight the good performance of the biocatalysts developed in the present study and indicate potential for the utilization of cellulose beads as an alternative and renewable support for lipase immobilization.

4 CONCLUSION

In the present study, cellulose beads were evaluated for their performance in immobilizing Candida rugosa lipase through covalent binding, using both the support in its natural state and calcined state, and subsequently applied in the hydrolysis reaction of soybean oil. The hydrolytic activity results were similar for both biocatalysts, with 2744.90 ± 76.3 $U.g^{-1}$ for the bead in its natural state and 2807.74 ± 12.89 $U.g^{-1}$ for the calcined one. Likewise, the percentages of soybean oil hydrolysis were similar, ranging between 84% and 88%, after 24 hours of reaction. Therefore, it can be concluded that the cellulose bead in its natural state is the most advantageous option for use as a support for immobilizing LCR lipase, as the calcination process involves additional steps and incurs high energy costs, in addition to low yield (33%) compared to the bead in its natural state. Thus, the cellulose bead has demonstrated to be an alternative and renewable support option for lipase immobilization and has potential for application in hydrolysis reactions.

REFERENCES

1 Chen, X., Chen, J., You, T., Wang, K., &Xu, F. 2015. Effectsofpolymorphsondissolutionofcellulose in NaOH/ureaaqueoussolution. Carbohydratepolymers, 125, 85-91.

2 Gericke, M., Trygg, J., &Fardim, P.2013.Functionalcellulosebeads: preparation, characterization, andapplications. Chemical reviews, 113(7), 4812-4836.

3 das Neves, A. M., Visioli, L. J., Enzweiler, H., & Paulino, A. T. 2024. Lipase fromCandida rugosa incorporated in pectinhydrogel via immobilization for hydrolysisoflipids in dairyeffluentsandproductionoffattyacids. JournalofWaterProcessEngineering, 58, 104821.

4 Vetrano, A., Gabriele, F., Germani, R., &Spreti, N. (2022). Characterizationof lipase fromCandida rugosa entrapped in alginatebeadstoenhance its thermalstabilityandrecyclability. New JournalofChemistry, 46(21), 10037-10047.

5 Soares, C.M.F., Castro, H.F., Moraes, F.F., Zanin, G.M., 1999. Characterization andutilization of Candida rugosa lipase immobilized on controlled pore silica. Appl.Biochem. Biotechnol. 77 (9), 745–757.

6 Silva, A. F., Silva, E. M. 2014. Caracterização físico-química de lipase de Candida rugosa e avaliação da imobilização por ligação covalente em POS-PVA.10ºSeminário de Iniciação científica da UFT, 2014, Palmas, TO.

7 Simões, A. S., Mori, R. Y., Faria, R., Castro, H. F. D., & Mendes, A. A. 2011. Desempenho da matrizhíbrida SiO2-quitosana naimobilização da lipase microbiana de Candida rugosa. Química nova, 34, 33-38.

8 Domingues, O., dos Santos, L. K., Herculano, R. D., Flumignan, D. L., & de Paula, A. V. 2020. Evaluation of magnetic carriers employed for immobilization of lipase from candida rugosa. The Journal of Engineering and Exact Sciences, 6(4), 0498-0504.

9 Chowdhury, A., Mitra, D., & Biswas, D. 2013. Biolubricant synthesis from waste cooking oil via enzymatic hydrolysis followed by chemical esterification. Journal of Chemical Technology & Biotechnology, 88(1), 139-144.

10 Domingues, O., Remonatto, D., Dos Santos, L. K., Galán, J. P. M., Flumignan, D. L., & de Paula, A. V. 2022. Evaluation of Candida rugosa lipase immobilized on magnetic nanoparticles in enzymatic/chemical hydroesterification for biodiesel production. Applied Biochemistry and Biotechnology, 194(11), 5419-5442.

11 Correia, T. B. D. A., Sousa, G. P. R. D., Reis, W. D. S. M., Theodoro, J. M., Hidalgo, V. B., Santos, J. H. V., ... & Pereira, E. B. 2024. Evaluation of Different Heating Systems for the Hydrolysis of Residual Frying Oil Catalyzed by Free and Immobilized Lipase. Brazilian Archives of Biology and Technology, 67, e24220625.

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