IMMOBILIZATION OF FUNGAL LIPASE IN CALCIUM ALGINATE FOR BIODIESEL PRODUCTION USING WASTE COOKING OIL

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ABSTRACT

Biodiesel is a renewable fuel which could be an alternative to replace diesel of fossil origin. However, some challenges still must be overcome regarding its production, such as the cost of raw materials and the use of an environmentally friendly industrial process. In this study we used the biocatalytic transesterification of waste cooking oil and ethanol to produce biodiesel. A lipase produced by an Amazonian endophytic fungus, immobilized in calcium alginate was used as biocatalyst. The enzymatic extract presented 11262 U/mL of lipolytic activity before immobilization. Calcium alginate was used to immobilize the lipolytic extract. The immobilization method presented 89.7% yield and was able to retain 67% of the lipase activity. The transesterification reaction was carried out using waste cooking oil and ethanol (4:1 ratio) at 40°C for 360 minutes, and the immobilized enzyme proved to be reusable for up to 3 cycles. A maximum of 93.9% biodiesel yield was observed after the first reaction cycle. Our results show that the immobilized lipolytic extract can be applied in the synthesis of biodiesel using waste cooking oil, as well as can be recycled.

Keywords: Endophytic fungus. Biocatalysis. Enzyme. Residue. Biofuel.

1 INTRODUCTION

Biodiesel, a renewable fuel, has gained prominence as an environmentally attractive alternative to fossil diesel. Its biodegradable, sustainable, and clean-burning nature makes it a growing focus of interest [1,2]. Chemically, biodiesel is an alkyl ester, mainly synthesized from renewable lipid feedstock such as vegetable oils and animal fats [2,3]. In Brazil, biodiesel is often used in blends with fossil diesel, with the addition of 10% biodiesel to diesel sold in 2018[4]. The use of non-edible oils and of oily residues has been explored in biodiesel production, to reduce costs and to address concerns that involve food security [5,6].

Transesterification, or alkaline catalysis, is the main method for biodiesel production, but it faces challenges such as alkaline effluent generation and high energy demand [7,8]. Biocatalysis, using lipases, offers a promising alternative, eliminating the need of complex purification steps and allowing enzyme recycling[9] (AL-SUHAIR, 2007). However, practical challenges such as the high cost of commercial enzymes and product contamination have led to enzyme immobilization, which allows their reuse, as well as improve their properties [10,11].

Biodiesel production is of growing interest due to its relevance as a renewable and sustainable energy source. In this context, the use of biocatalysis with immobilized lipase presents itself as a promising approach to advance in this field. Therefore, the investigation of new methodologies that can optimize and make biodiesel production more efficient is justified. Thus, this study aims to produce biodiesel using an immobilized fungal lipase as a biocatalyst. In this sense, we evaluated the feasibility of the biocatalytic transesterification, considering its effectiveness and potential benefits in terms of yield and biocatalyst reuse.

2 MATERIAL & METHODS

The waste cooking oil used in this study was provided by a restaurant located in the city of Manaus. The residue was collected in sufficient volume for all experiments, filtered, and stored in barrels. The lipase-rich enzymatic extract used in this study was obtained through the cultivation of the endophytic fungus *Endomelaconipsis endophytica*, isolated from the Amazonian species *Aniba canelilla* [12]. The extract was produced as described previously [13] and had an activity of 11262 U/mL. For the immobilization process of the enzymatic extract produced by *E. endophytica*, alginate spheres were used as support. To trap the lipase, the microencapsulation technique was employed with a concentration of 5% (w/v) of sodium alginate. The preparation of calcium alginate occurred through ionic gelation, using 0.1M calcium chloride as a crosslinking agent in a gel-forming solution. The sodium alginate solution was then combined with the enzymatic extract, which had an enzymatic. After complete homogenization, this solution was directed to the sphere production system [14].The immobilization yield (η) was calculated based on the enzymatic activity obtained and the residual enzymatic activity present in the reaction medium after the immobilization process, as shown in Equation 1, where U_0 is the activity obtained at the beginning of the immobilization (U/g) and U_f is the residual activity present in the supernatant after immobilization (U/g) [14].

$$\eta(\%) = \frac{U_0 - U_f}{U_0} x100 \quad (1)$$

The enzymatic transesterification reaction of the waste cooking oil (biodiesel production) was carried out with the lipase-rich enzymatic extract and with the immobilized lipolytic extract. The reactions were conducted in a bench reactor, with constant heating and stirring, for 360 minutes at 40°C. Ethanol was used as the short chain alcohol, in an alcohol:oil ratio of 3:1, with

approximately 5% of the biocatalyst (w/w) [15,16]. The biodiesel yield was calculated as the mass of biodiesel weighed after the reaction divided by the mass of waste cooking oil used in the transesterification reaction (Equation 2 and Equation 3) [17].

$$Yield\ (g/g) = \frac{M_{biodiesel}}{M_{óleo}} \quad (2)$$

$$Yield\ (\%) = \frac{M_{biodiesel}}{M_{óleo}} * 100 \quad (3)$$

The reuse of the biocatalyst was evaluated through consecutive biodiesel synthesis reactions, reusing the immobilized lipase. After each reaction cycle, the immobilized lipase was washed with phosphate buffer and reused in another batch. At the end of each cycle, the enzymatic activity was measured according to the methodology described by Winkler and Stuckmann (1979).[18].

3 RESULTS & DISCUSSION

Approximately 520 calcium alginate spheres were obtained, with an average weight of 30 mg and a diameter of 6 mm. After 24 hours of sphere production, it was found that the immobilization efficiency reached 89.7%. The immobilized lipase reteined 67% of its initial activity. When analyzing other studies that investigated different concentrations of sodium alginate for the immobilization of *Candida rugosa* lipase[19], our results prove to be promising. Other studies have shown that higher concentrations of alginate resulted in higher immobilization efficiencies; however, retained activities did not exceed 60% for such concentrations[19]. In another similar study on the immobilization of precipitated lipase obtained from *Bacillus* sp. in a 2.5% sodium alginate solution, an efficiency of 69.5% and a retained activity of 9% were achieved [20]. Therefore, this study demonstrates the feasibility of the adopted methodology for lipase immobilization and highlights its potential compared to previous studies.

The lipolytic extract immobilized in calcium alginate was employed in the biodiesel production as biocatalyst in order to evaluate its reuse. Three transesterification cycles were conducted, and at the end of each cycle, the biodiesel yield and the enzymatic activity of the immobilized lipolytic extract were calculated. The results are shown in Table 1.

Table 1 Biodiesel yield and relative activity of lipase immobilized in alginate calcium after 3 cycles of transesterification using waste cooking oil and ethanol. The reactions were carried out at 40 ºC, for 360 min, with an ethanol:residue ratio of 3:1.

Reaction Cycle	Biodiesel Yield (g/g)	Biodiesel Yield (%)	Retained Enzymatic Activity (%)
0[a]	0.89	89.5	100
1	0.94	93.9	95
2	0.83	82.9	77
3	0.75	75.1	11

* a - Cycle carried out with the non-immobilized enzymatic extract.

As indicated in Table 1, the biodiesel yield remained above 75% during three reaction cycles, despite the reduction in its efficiency of around 20% compared to the first cycle. However, it is crucial to note that the enzyme activity decreased by approximately 90% in the third reaction cycle, resulting in the interruption of the next batch. Additionally, from this table, it is observed that the yield of the immobilized enzyme in the first cycle exceeded that one obtained with the non-immobilized enzyme, represented by cycle zero. This outcome is noteworthy and suggests that the enzyme properties may have been enhanced by the immobilization process, enabling a higher biodiesel yield [21,22].

A study conducted by Knevic *et al*. [19] demonstrated the feasibility of using immobilized lipase in calcium alginate for six cycles in biodiesel production, with an 83.3% reduction in activity; however, in the first three cycles, the reduction was only of 10%[19]. Another study, conducted by Bhushan *et al* [23], revealed that the immobilization of the lipase produced by *Arthrobacter* sp. in a 1.2% calcium alginate solution allowed its reuse for 10 cycles in the hydrolysis of triacyl glycerides. These authors indicated an increase in thermal stability, pH tolerance, and storage of the immobilized biocatalyst, when compared to the free enzyme [23]. In a more recent study, Zhang *et al*.[24] showed excellent recyclability of the enzyme trapped in alginate, with residual activity exceeding 80% in the tenth reaction cycle . Therefore, it is possible to observe that the recycling of the immobilized enzyme we described here did not surpass the cited studies. However, it is important to highlight that this study used crude liolytic extract for immobilization, which contains impurities and other molecules, a variable that possibly influenced the results, especially when compared to other studies that used purified enzymes. These findings point to the potential application of this methodology for the production of biodiesel.

4 CONCLUSION

In this study, the immobilization of fungal lipase in calcium alginate showed promising results for biodiesel production using waste cooking oil. Despite the reduction in efficiency over the reaction cycles, the biodisel yield remained above 75% until the third reaction cycle. The significant decrease in enzymatic activity after the third cycle indicates the need for optimization of the immobilization process and/or the choice of biocatalyst. Future experiments could explore different strategies to improve the stability and the reuse of the immobilized biocatalyst, aiming to contribute to more efficient and sustainable biodiesel production.

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