INVESTIGATION OF DIFFERENT ADDITIVES IN THE EVERSA TRANSFORM 2.0 LIPASE IMMOBILIZATION MATRIX

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ABSTRACT

The optimization of industrial production processes in order to increase cost-benefit and the environmental responsibility inherent to them is a growing need in the most diverse sectors. Such optimization is also popular in enzymatic routes for the synthesis of products ranging from biofuels to fine chemical compounds. The use of additives such as ionic liquid (IL) and lignin (Lig), naturally available in the ionic liquid pre-treatment residue of lignocellulosic materials, during lipase immobilization may represent an alternative to provide greater stability and activity catalytic to lipase in a low-cost reaction. The present study verified the influence of using the liquor resulting from pre-treatment of sugar cane straw as well as IL and Lig in different concentrations on the immobilization yield and recovered activity of lipase immobilized by encapsulation in matrix alginate. FTIR analysis confirmed the presence of lipase in all alginate particles (beads) produced. Furthermore, beads containing lipase with IL and Lig in their formulations presented higher values of immobilization yield (>80%), recovered activity (>25%) and mechanical strength. These previous results suggest that the use of IL and Lig in alginate matrix may be a promising method for lipase immobilization by encapsulation.

Keywords: Enzyme immobilization. Encapsulation. Ionic Liquid. Lignin. Biomass pretreatment.

1 INTRODUCTION

The use of enzymes to replace conventional chemical catalysts is considered an important optimization aspect for organic synthesis reactions. Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) comprise a representative group among biocatalysts, due to their wide range of applications in the synthesis of biofuels, food products and fine chemicals[1]. The use of this enzyme in immobilized form has advantages as it may increase its activity and stability, also providing its reuse. Therefore, much research has focused on increasing the performance of lipases, through the investigation of different immobilization techniques. However, there are still few papers focused on incorporating additives into the immobilization matrix using the entrapment method, one of the most promising due to its simplicity and efficiency. Components as ionic liquids has been shown to promote increased activity and stability of enzymes. Similarly, recent investigations into lignin incorporation may exert beneficial effects such as increased catalytic activity, mechanical strength and thermal stability of enzymes[2-3].

In view of these aspects, this study proposes to investigate the effect of immobilization by encapsulation in calcium alginate accompanied by three different matrices in this formulation: ionic liquid (IL), IL + lignin and liquor resulting from sugarcane pretreatment on the Immobilization Yield, Recovered Activity and Mechanical resistance of Eversa® Transform 2.0.

2 MATERIAL & METHODS

The production of beads containing lipase was carried out with the production of solutions containing sodium alginate (2% w/w final concentration), lipase Eversa® Transform 2.0 (10% w/w) and additives: IL ([Mea][Ac]), IL + lignin and with liquor resulting from pretreatment (previously filtered through filter paper) in the proportions detailed in Table 1. The control beads (B1) containing only the enzyme encapsulated in calcium alginate (2% w/w final concentration) was also produced.

Table 1 Produced beads with their respective formulations and codes.

Beads	Concentration of additives (m/m)
B1	-
B2	0.5% IL + 0.05% Lig
B3	1.5% IL + 0.15% Lig
BL	1.5% IL + 0.15% Lig (from liquor)

The solutions were then dripped in a medium containing $CaCl_2$ solution at 200 mM using a digital peristaltic pump (Ismatec/Cole Parmer) with speed control (6.8 mL/min) kept the same for all particles produced. Lipase enzymatic activity (EA) was measured through hydrolyze of p-nitrophenyl palmitate (pNPP) into p-nitrophenyl (p-NP). The immobilization yield (ratio

between the activity of the immobilized enzyme and the initial enzymatic activity of the enzyme) and the recovered activity of the enzyme were calculated according to equations (1) and (2):

$$Imobilization\ Yield\ (\%) = \frac{EA_{free} - EA_{supernatant}}{EA_{free}} \times 100 \quad (1)$$

$$Recovered\ Activity\ (\%) = \frac{EA_{suport}}{EA_{free} - EA_{supernatant}} \times 100 \quad (2)$$

The resistance of the produced beads to compression was determined using the TA.XT.plus texture analyzer instrument (Extralab Brazil) utilizing a compressed rate of 2 mm/s up to a deformation of 80%. Measurements were conducted at room temperature (~20 °C). Furthermore, beads were characterized by Fourier-Transform Infrared (FTIR) through the Shimadzu infrared spectrophotometer (Kyoto, Japan). For each beads formulation, the spectrum of its respective blank (without enzyme) was also produced and analyzed.

3 RESULTS & DISCUSSION

As can be seen in Figure 1 (a), except for the control bead B1 (without additives), all beads produced obtained an immobilization yield above 83%, which indicates that the use of IL and IL + lignin favored the encapsulation of the enzyme in the alginate matrix. Toscano et al.[4] obtained 44.16% immobilization yield for *Trichoderma harzianum* lipase immobilized in calcium alginate, a value significantly lower than the minimum obtained in this work (78.81% ± 0.7%). The recovered activity generally reflects the success of the total immobilization process, that is, through activity recovery; the immobilized activity is compared with the total initial activity of the free enzyme[5].The maximum recovered activity (81.74% ± 6.35%) was verified in B2, which contains 0.5% IL + 0.05% lignin. Almeida et al.[6], obtained a maximum value of 28.26% ± 1.19% for activity recovered in particles formed by dripping in their study on immobilization of Eversa® Transform 2.0 in calcium alginate. BL bead presented a lower recovered activity than the others (9.27 ± 2.87%), however the highest immobilization yield (94.25% ± 1.98%). Moreover, because the environmental and cost-benefit nature that involves the reuse of a by-product of sugarcane pre-treatment for its formulation may it would require additional characterizations to comparatively analyze its potential in relation to the others. Mechanical strength represents an important aspect, especially when considering that the biocatalyst in question may be applied in reactors that will require high resistance from the beads, so that it can be reused in several reaction cycles. Concerning this, B3 beads presented the highest resistance value (11.7± 0.39 N) as can be seen from Figure 1(b). This fact may be explained by the higher concentration of lignin present in the formulation of these beads, as it is known that lignin is capable of providing greater structural rigidity[7].

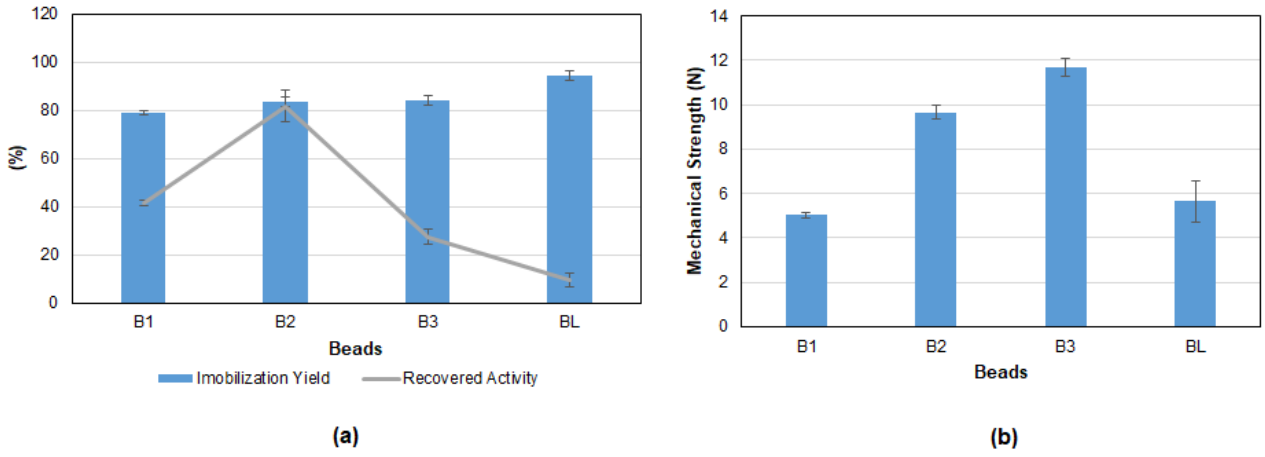

Figure 1 Immobilization Yield and Recovered Activity (a) and Mechanical Strength of the beads (b).

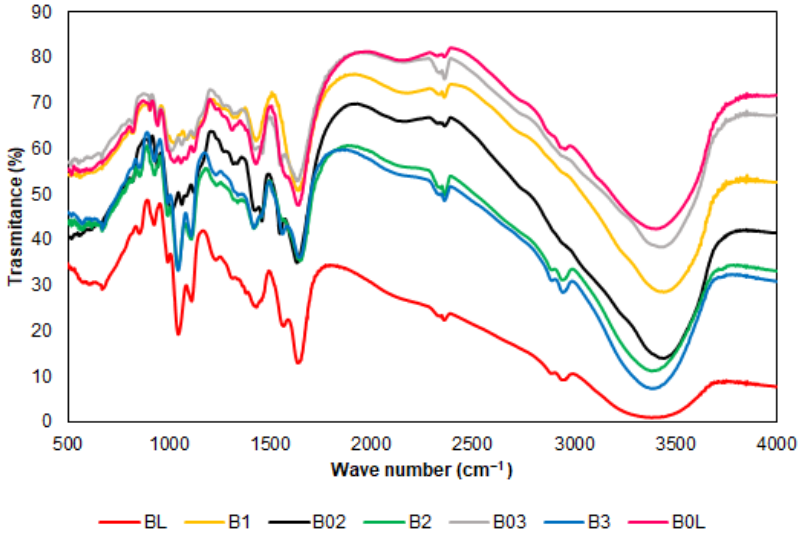

Figure 2 FTIR spectra of beads (codes containing "0" represent the blanks- beads without lipase of each formulation).

As can be seen in Figure 2 the FTIR spectra of the beads B2, B3 and BL containing lipase in its formulations, revealed the presence of peaks at 3400–3200 cm^{-1} (attributed to O–H stretching and asymmetric stretching of the primary amide NH_2). Also, is perceived the accentuation of the peak at 1400–1200 cm^{-1} related to the amide III. These are indications that the enzyme was successfully entrapped in the alginate support in all the formulations mentioned above[6,8].

4 CONCLUSION

All immobilized lipases formulations showed great immobilization yield, also beads B2 and B3 (with amounts of ionic liquid and lignin in their immobilization matrix) presented high-recovered activity and mechanical strength, indicating that they are the most promising for future characterizations. Moreover, the FTIR of particles with or without lipase, revealed the presence of the characteristic enzyme peaks. Hence, the presence of IL + lignin in the alginate matrix presented beneficial effects on the parameters evaluated in this study.

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