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IMMOBILIZATION OF LIPASE NS 40116 IN POLYURETHANE FOAM

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ABSTRACT

The proposal of this work was based on the possibility of immobilization of Lipase NS 40116 in polyurethane foam (PUF) support from green sources. The reaction of castor oil and glycerol, using enzymatic glycerolysis produced mono- and diacylglycerol, which were used as biopolyol for obtaining a support for immobilization of the enzyme using the entrapment technique. The characterization of immobilized enzyme was performed by Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and apparent density. A hydrolytic enzyme activity of 6.62 U/g was observed in the immobilized lipase.

1. INTRODUCTION

Enzymes are an important biocatalyst in the industry, mainly the characteristics as high catalytic activity, selectivity, specificity and high activity under very mild environmental conditions. The range of enzymes applications is extensive, being used in the agricultural, pharmaceutical, fuel and food industry. In the last years, the search for clean technologies in the industry has encouraged the study of these proteins in different applications of the usual. The lipases are triacylglycerol ester hydrolases and have gained greater prominence over the years mainly in synthetic reactions of industrial importance. The lipase can be in free and immobilized form. In the free form the enzyme consists in a liquid formulation (broad), generally stabilized by sorbitol, for prevent the denaturation. This form present the advance of easy and low-cost preparation. However, the use of the enzyme in free form present some disadvantages, as instability in reactional medium and reuse inability. Thus, the immobilization of these proteins becomes attractive from a commercial point of view (Carvalho et al., 2016).

Polyurethane is a porous polymeric matrix present a wide applicability for the enzyme immobilization. PU is a versatile polymer, enabling applications from building insulation to scaffolds.

The polymer has as monomer repeatedly unit of urethane linkages in its structure, which are non-regularly distributed, thereby differentiating it from other polymers. This polymer is obtained by reaction of a diol (OH) and diisocyanate (NCO) in a polyaddition polymerization and when employed to immobilized enzymes, entrapment method is most commonly used. In addition to low cost and easy immobilization technique this polymer has several advantages, such as the possibility to be from renewable sources, biodegradability and low cost (Adlercreutz, 2013).

The objective of the present study was use the product of enzymatic glycerolysis, between castor oil and commercial glycerol – monoacylglycerol (MAG) and diacylglycerol (DAG) – as biopolyol to obtain polyurethane foam and to immobilized the Lipase NS 40116 via entrapment method.

2. METHODS

2.1 Enzyme concentration

The free enzyme NS 40116 was purified using collagen membranes. The membranes with enzyme were submerse in phosphate buffer 0.05 M solution for 24 hours to dialysis occur. The content of the membranes was transferred to Petri dishes and subjected to freezing at -80 °C for 24 hours. After freezing, the enzyme was lyophilized (LIOTOP Lyophilizer, Model L101).

2.2 Synthesis and characterization of biopolyol

To produce the polyurethane foams, the biopolyol was obtained using enzymatic glycerolysis reaction, as previously described by Valério et al. (2010). For MAG and DAG determination the analysis was carried out according ASTM D6584-13 (2014) in a GC Shimadzu 2010 with automatic on-column injector and flame ionization detector (FID). The hydroxyl value of the polyol was measured according ASTM D 4274 (2016) using test method A.

2.3 Immobilization and characterization of lipase NS 40116 in polyurethane foam

The concentrated enzyme NS 40116 was immobilized using the entrapment technique through bulk polymerization. For this, polyurethane foam (PUF) was obtained using the polyol from enzymatic glycerolysis using immobilized CalB and methylene diphenyl diisocyanate (MDI) Papi 27 (Dow Chemistry). The reactions were carried out in room temperature with manual stirring for 1 minute, the molar ratio NCO:OH used was 1:1 and the amount of purified enzyme used was 15 wt%(w/w). The polymer obtained was characterized according to the methods described below.

Fourier transform infrared spectroscopy (FTIR) analyzes were performed on apparatus prestige-21 (Shimadzu). The morphology of the samples was verified by Scanning electron microscopy (SEM)

analysis with field emission (SEM-JEOL JSM-6390LV). The apparent density was carried out using methodology described in ASTM D 1622 (2004). The analyzes were performed on triplicate.

Enzyme activity was determined according describe by Chiou et al. (2004), when one unit (U) of enzyme activity was defined as the amount of enzyme which catalyzed the production of 1 mmol p-nitrophenol per minute under the experimental conditions. All assays were carried out in triplicate. The kinetic constants were determined for the free and immobilized NS 40116 using p-nitrophenyl palmitate (*p*-NPP) as substrate in different concentrations (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mM), enabling the determination of Michaelis–Menten constant (K_m) and maximum reaction rate (V_{max}) by Lineweaver-Burk plots.

3. RESULTS AND DISCUSSION

According to the results obtained by using CG analysis, the MAG and DAG corresponds to a fraction of 64.52±2.32% the products. The PUF was obtained using a 20% of concentrated enzyme (wt%). The FTIR analysis (Figure1a) presented a stretching in the region 2924 cm^{-1} and 2844 cm^{-1} due to the presence of asymmetric and symmetric methyl, respectively. The region at 1750–1700 cm^{-1} can be attributed to the bond linkage between enzyme and the functional group (NH) from polyurethane. Free lipase showed a typical spectrum with proteins absorption bands associated with amino group (CONH), primary, and secondary amino groups between 1580 and 1650 cm^{-1} (Stuart, 2006).

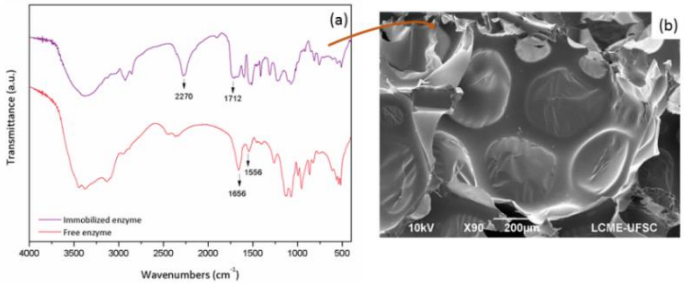

Figure 1. (a) Fourier transform infrared spectra (FTIR) of free and immobilized Lipase NS 40116; (b) SEM Micrographs of immobilized lipase NS 40116 in PUF support.

With the SEM analysis (Figure 1b) it was possible to perform the count of the mean number of cells and the morphology of the support to immobilized enzyme. The cells were observed in their large part of closed profile with medium size of 748.6 ± 0.2 µm. The apparent density of PUF support was 56.4 ± 3.1 kg/m³, indicated as characteristic being a rigid and medium density foam. The immobilized lipase presented an enzyme activity of 6.62 ± 0.36 U/g, no showing significant difference between the free and immobilized lipase. The free lipase presented a kinetic constants K_m=13.11 mM

and V_{max}=48.01 µmol/min/mg, while the immobilizated lipase showed a K_m=1.91 mM and V_{max}=12.87 µmol/min/mg. A decrease in the values when the enzyme was supported in PUF, probaby due to a conformation change of the enzyme after the immobilization, ocassionated by a linkage between the enzyme and support, because the presence of amino groups in the lipase and in the NCO groups of MDI, resulting in a lower V_{max} (Secundo, 2013).

4. CONCLUSIONS

In this study, lipase NS 40116 was immobilized in polyurethane foam using a biopolyol from enzymatic glycerolysis via entrapment technique. The high presence of MAG and DAG in this biopolyol enables the obtainment of a rigid support with medium density and with closed and uniform cells. The immobilization of the enzyme did not result in loss of activity.

5. REFERENCES

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