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Development of a heterogeneous biocatalyst by the immobilization of Eversa® Transform 2.0 lipase on mesoporous silica to applications of industrial interest

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Abstract

Enzyme immobilization has been studied as an alternative to the difficulty of enzyme recovery and reuse, in addition to providing protection to enzymes from adverse environmental conditions (high temperatures, extreme pH values, etc.). Physical adsorption is one of the strategies that can be used involving the direct binding of the enzyme to the support through ionic/hydrophobic interactions. This approach is cost-effective and easy to apply. Therefore, the main objective of the present work is to study the immobilization by adsorption of the lipase Eversa® Transform 2.0 (ET) on mesoporous silica of the Santa Barbara Amorphous type - 15 (SBA-15), exploring the specific interfacial activation mechanism of lipases. This unique property of lipases occurs in the presence of hydrophobic surfaces. SBA-15 is a partially hydrophobic support and has important technical and environmental characteristics that provide interactions with Eversa lipase. The heterogeneous biocatalyst produced (SBA15-ET) showed (27.1 ± 0.6) U/g of derivative activity, high immobilization yield (≈100%) and recovered activity (292.6 ± 7.6) %. The preliminary results were promising, indicating the feasibility of advancing studies to characterize the heterogeneous biocatalyst.

Keywords: Heterogeneous biocatalyst; lipase *Eversa® Transform 2.0*; SBA-15

1. Introduction

The use of immobilized enzymes has become a common practice in the production of various industrial products, in the pharmaceutical, chemical, food, detergent, animal feed and biofuel industries [1].

Enzyme immobilization emerged to overcome the problems of enzyme recovery, as these catalysts are extremely expensive. Thus, the single use of an enzyme followed by its disposal make the process economically unviable [2,3].

Currently, the prices of enzymes, and specifically lipases (such as Eversa), have decreased. Some authors have even suggested the use of liquid enzymes for just one use, producing a product with relatively low added value, such as biodiesel [3,4]. However, a properly designed immobilization process can provide better process control, the design of efficient continuous processes, as well as improving some enzymatic properties: activity, selectivity and stability [5].

Immobilization using hydrophobic supports is an interesting strategy studied to immobilize lipases, allowing the stabilization of the open form of this enzyme [6]. The Santa Bárbara Amorfo support (SBA-15) is a support with a hydrophobic character. According to Rios et al. (2018), hydrophobic interactions are likely the driving force between lipases and the mesoporous support (SBA-15) during the immobilization process.

SBA-15 is a well-ordered material with short channels, which facilitates mass transfer. The literature reports that not only lipase binding, but also easy accessibility to substrate molecules should be favored by the internal morphology of the porous support, in order to minimize diffusional limitations [7]. Furthermore, SBA-15 features high surface area, large pore volume, parallel channels and the combination of micro and mesoporosity [8], high thermal, mechanical and chemical resistance [9] and its mold (P123) is relatively cheap, non-toxic and biodegradable.

2. Methodology

The assays were performed in duplicate and the results are presented as the mean of these values, with a standard deviation below 10%.

2.1 Synthesis of SBA-15

SBA-15 with expanded pores was synthesized by hydrothermal route following the methodology applied by dos Santos et al. (2013) [8], and adapted by Rios et al. (2018) [10].

2.2 Characterization of SBA-15

The specific surface area (SBET), total pore volume (VT) and mean pore diameter (dp) of the solid samples were determined from nitrogen adsorption-desorption isotherms at 77 K, as reported by Rios et al. (2018) [10].

2.3 Immobilization of Eversa® Transform 2.0 lipase in SBA-15

Free Eversa was immobilized on SBA-15 by hydrophobic interaction. 1g of the support was added to 20 ml of sodium phosphate buffer (5 mM, pH 7) containing the enzyme. Immobilization was carried out in a batch reactor, under stirring, at 25 ºC for 40 min. Then, the biocatalysts were washed with excess sodium phosphate buffer (25 mM, pH 7) and filtered.

2.4 Enzyme activity

The enzymatic activities of free and immobilized Eversa were determined by measuring the increase in absorbance, according to the methodology described in the literature Arana-Peña et al. (2020) [11].

2.5 Immobilization parameters

Immobilization parameters were calculated according to the literature Silva et al. (2012) [12], in order to evaluate the efficiency of the biocatalyst produced.

3. Results and discussion

3.1 Characterization of SBA-15

The characterization in terms of physical properties, such as surface area (S_{BET}) and mean pore diameter (d_p) of the SBA-15 synthesized for this work was determined [13] and is presented in Table 1. The material (SBA-15) was synthesized with ammonium fluoride and presented a larger pore diameter than SBA-15 obtained by Ojeda-López et al. (2015) [14], produced in the absence of ammonium fluoride (NH_4F). The addition of ammonium fluoride increases the total pore volume and the average pore diameter, indicating that these materials have a pore diameter that allows the adsorption of the enzyme within the pore of the material [10]. Therefore, SBA-15 has an average pore diameter larger than the diameter of Eversa (8.8 nm × 5.6 nm × 4.8 nm) [15].

Tabela 1: Textural parameters of SBA-15 synthesized in this work and data from the literature.

Materials	Specific surface area (m^2/g)	Total pore volume (cm^3/g)	Average pore diameter (nm)
SBA-15 synthesized with NH_4F [13]	476	2.4	20.2
SBA-15 synthesized without NH_4F [14]	486	0.6	6.6

According to the data presented in Table 1, SBA-15 synthesized with ammonium fluoride presents a considerable safety margin in pore diameter when compared to the material synthesized without NH_4F. It is important to highlight that the adsorption processes that occur within the support pores tend to produce more stable biocatalysts, as they protect the enzyme from changes in conditions [16].

3.2 Eversa's immobilization on SBA-15

Surface-active molecules, such as P123 ($PEO_{20}PPO_{70}PEO_{20}$) are used to synthesize mesoporous silicates. These molecules act as a structure-directing agent, as they are responsible for the ordered pores and well-defined channels of the

material. This occurs after the last step of the synthesis process, the calcination step, where this surfactant is removed [17]. Therefore, as adsorption can occur inside the pores of the support, the immobilization of lipase on SBA-15 was evaluated and the results obtained are presented in Table 2. The immobilization time was evaluated and pre-defined at 40 min and load of enzyme used was $1mg_{enzyme}/g_{support}$.

Table 2: Immobilization parameters of Eversa Transform 2.0 lipase on SBA-15. Yield Immobilization (IY), Immobilized enzyme activity (At_d) and activity recovered (At_R).

Biocatalyst	IY (%)	At_d (U/g)	At_R (%)
SBA15-ET	99.4 ± 0.5	27.1 ± 0.6	292.6 ± 7.6

As shown in Table 2, the immobilization yield was (99.4 ± 0.5) % and the immobilized enzyme activity was (27.1 ± 0.6) (U/g) (Table 2). Other authors also show that the immobilization of lipases on hydrophobic supports is fast and efficient [18]. The recovered activity exceeds 100%, which may be related to immobilization via interfacial activation of the enzyme, so that the lid covering the active site of the lipase moves and exposes it to the reaction medium, leading to the open form of the adsorbed lipase [13].

Ojeda-López *et al*. (2015) [14] states that the materials obtained after calcination are mesoporous solids with high thermal and mechanical stability. Therefore, a more compact matrix is produced. Given this, the thermal stability of biocatalysts will also be evaluated later.

4. Conclusion

An adapted support was produced for the immobilization of Eversa, as synthesis with ammonium fluoride allows the immobilization of the enzyme within the pores of the support. The immobilization of Eversa lipase on SBA-15 nanostructured mesoporous silica was successful. Hydrophobic interactions were likely the main driving forces in the adsorption. Therefore, based on these results, it was demonstrated that it is possible to improve the efficiency of enzyme immobilization by adapting the design of a mesoporous support such as SBA-15.

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