LIPASE EVERSA® TRANSFORM 2.0 IMMOBILIZATION ON CHITOSAN-BASED SUPPORT FUNCTIONALIZED WITH ALDEHYDES DERIVED FROM ORGANIC COMPOUNDS

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ABSTRACT

Despite several studies on the functionalization of supports to maximize the yield of enzyme immobilization, the reagents used for this process still have high toxicity and are not environmentally friendly, such as glutaraldehyde. Given this, there is an inherent concern in developing sustainable protocols for these applications. Therefore, the objective was to evaluate the immobilization of Eversa® Transform 2.0 lipase on chitosan-based support chemically cross-linked with different types of aldehydes: glutaraldehyde, octanal, and dodecanal. Immobilization by adsorption consisted of suspending the enzyme in 5mM sodium phosphate buffer pH 7.0 for 1 h under stirring at 25°C. Regarding characterization, the activity of the soluble and immobilized enzyme was determined, previously incubated in 25mM sodium phosphate buffer, pH 7.0, at 25°C, through the hydrolysis of ρ-nitrophenyl butyrate (ρNPB). The results varied depending on the use of the source of the aldehyde group since the values obtained for glutaraldehyde, dodecanal, and octanal, respectively, were 94%, 65%, and 44% for immobilization yield, and 21.7%, 16 .2% and 30.5% for recovered activity. The use of octanal for lipase immobilization showed promising results, particularly in terms of recovered activity, suggesting its potential as a replacement for the toxic reagent glutaraldehyde. This finding is significant because it can be obtained from renewable resources and presents no toxicity.

Keywords: Quitosana. Octanal. Immobilization. Lipase.Aldehyde.

1 INTRODUCTION

The immobilization of enzymes, particularly lipases, is a field of growing interest in the quest for more sustainable and efficient industrial processes. Lipases, with their catalytic versatility, can potentially improve a wide range of industrial applications. However, their direct application is often hindered by their instability under extreme operating conditions and the challenge of recovery and reuse. This makes the search for effective immobilization methods all the more crucial.
Enzyme immobilization, a widely used alternative, offers a promising solution to maximize the yield of these enzymes. This strategy enhances critical properties, such as stability, selectivity, and specificity, and simplifies the recovery and reuse of enzymes[1].
A promising strategy to overcome these challenges is the immobilization of lipases on suitable supports. Among the available immobilization methods, functionalization with short-chain aldehydes, such as octanal and dodecanal, has been an increasing focus of studies due to its effectiveness and sustainable nature. For instance, other authors found that the use of octanal for lipase immobilization led to improved enzyme stability depending on the alkyl chain selected; for instance, immobilization of CALB onto dodecyl-chitosan favored stabilization while for h butyl-chitosan was the best support for RML. This underscores the current relevance and importance of this topic in the field of biotechnology and industrial processes.[2]
Functionalization with octanal and dodecanal, derived from natural sources, offers a more ecological alternative to the widely used glutaraldehyde in lipase immobilization. This method not only addresses concerns about toxicity and environmental impact but also provides additional benefits. These aldehydes form stable bonds with the functional groups of lipases and supports, leading to effective and long-lasting immobilization, thereby enhancing the sustainability and efficiency of industrial processes.

2 MATERIAL & METHODS

The chitosan-based immobilization support was prepared by using 2,5% (w/v) solution of chitosan powder in 1% acetic acid. The solution was added dropwise to a 3% sodium tripolyphosphate solution using hypodermic needles. The method employed involved the preparation of a 2.5% chitosan gel, in which 1.25 grams of chitosan were dissolved in 50 ml of 1% acetic acid solution, stirred for 30 minutes, and then sonicated for 10 minutes to remove bubbles. Chitosan particles were then prepared by dripping 25 ml of the gel into 250 ml of TPP alginate solution under agitation for 5 minutes, followed by resting for 1 hour and 30 minutes. Subsequently, the spheres were vacuum-filtered and washed three times with 100 ml of distilled water, followed by a wash with 100 ml of acetone. Finally, the particles were stored in 50 ml falcon tubes and refrigerated.The chitosan beads were functionalized with glutaraldehyde in the following steps: 1g of the support was added to 10 ml of a 5% glutaraldehyde solution. The support was then left in contact with the solution at room temperature for 1 hour. After the reaction period, the functionalized support was thoroughly washed with water or a 5 mM sodium phosphate buffer solution to remove excess reagent. Finally, the support was stored at 4 °C, maintaining its integrity for future use.

For functionalization with other aldehydes, firstly, the aldehyde was diluted in ethanol at a specific ratio of 28ml of ethanol per gram of aldehyde, taking into account the purity and density of the aldehyde. Octanal and dodecanal were used, with respective densities of 0.82 g/ml and 0.83 g/ml. Subsequently, 1g of chitosan particles was added to 10 ml of the aldehyde solution. The functionalization reaction was conducted under agitation at 25 °C and protected from light for 30 minutes. After this time, the

reducing agent, sodium borohydride (NaBH4), was slowly added at a ratio of 17mg per ml of aldehyde solution, and the mixture was stirred at room temperature for one hour. The particles were then washed with ethanol and water, filtered, and stored at 4 °C. After functionalization, the chitosan particles were used as immobilization support for Eversa® Transform 2.0 lipase. For this immobilization, a method based on literature with modifications[3] was employed. 0.1 g of support was suspended in 1 mL of enzyme solution (enzyme suspended in 5mM sodium phosphate buffer at pH 7.0). The assay lasted for 1 hour and was carried out with agitation at room temperature.

The enzyme activity was assessed by measuring the hydrolysis of ρ-nitrophenyl butyrate (ρNPB) using the method described in the literature (GARCIA-GALAN et al., 2014). This was done with both soluble and immobilized enzymes in a 25mM sodium phosphate buffer at pH 7.0, with agitation at 25°C.

The immobilization parameters were calculated based on the literature[4]. The immobilization yield (IY) is the difference between initial and final enzyme activity divided by initial activity. The recovered activity (At_R) represents the percentage of active enzymes after immobilization, defined as the ratio of immobilized enzyme activity to theoretical activity (AtT), which is the activity that would theoretically be obtained if all enzymes immobilized remained active.

3 RESULTS & DISCUSSION

Chitosan beads were prepared as described before, and Figure 1 shows the aspects of unmodified and modified particles before immobilization. The results of lipase immobilization in chitosan functionalized with different sources of aldehyde groups are summarized in Table 1.

(a) (b)

Figure 1 – Images of unmodified (a) and modified (b) 1chitosan beads

Table 1 The effect of support functionalization on the immobilization parameters of Eversa® Transform 2.0 lipase. The immobilization parameters were calculated according to previously described methods: Immobilization Yield (IY), Activity of the immobilized enzyme (At_D), and Recovered Activity (At_R).

	IY (%)	At_D (U/g)	At_R (%)
Glutaraldehyde	94% ± 0,5	32.0 ± 0,2	21,7%± 0,5
Octanal	41% ± 1,8	22.4 ± 2,4	30,5% ± 1
Dodecanal	65% ± 1,5	18.7 ± 1,8	16,2% ± 0,3

From the immobilization results presented in Table 1, it is evident that when dealing with octanal, despite the immobilization yield being approximately 41%, this yield was sufficient for the recovered activity to reach a satisfactory percentage of 30.5% (higher than that of glutaraldehyde). Furthermore, the recovered activity in enzymatic immobilization refers to the enzymes' ability to maintain their catalytic function after the immobilization process. In other words, it measures how effectively the immobilized enzymes continue to catalyze chemical reactions after being removed from the immobilization system. The higher the recovered activity, the more efficient the immobilization process, indicating that the enzyme retains its capacity to catalyze reactions even after immobilization. Therefore, for a process where the variable of interest is the recovered activity, octanal, besides being a sustainable option, will be more efficient than glutaraldehyde. Moreover, since octanal achieved a 41% immobilization yield, the enzyme solution used could be reused in a future immobilization, thereby reducing process costs. Nevertheless, more studies are needed to improve this yield. When comparing the activity of the immobilized enzyme (AtD), glutaraldehyde-chitosan also outperformed the others as a support, probably due to its higher capacity to retain enzymes on its surface.

4 CONCLUSION

Based on the results of the immobilization experiment, it is evident that octanal shows promise as a viable alternative to glutaraldehyde in certain processes. The achieved recovered activity of approximately 30.5% demonstrates the effectiveness of the immobilization process, highlighting its potential benefits in terms of enzymatic activity. These findings open opportunities for more sustainable and efficient applications in biotechnology and enzymatic catalysis in the future.

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