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INFLUENCE OF POLYETHYLENE GLYCOL ON LIPASE PHYSICAL ADSORPTION TO BE USED IN PURIFICATION PROCESSES

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ABSTRACT

Enzymes are proteins of great importance for all living organisms, since they can catalyze a wide range of reactions. These molecules are also valuable for different industries, such as food and pharmaceutical ones, among others, in which lipases stand out as a versatile and efficient biocatalyst. Despite the advantages associated with the use of enzymes, they represent an economic challenge, since their commercial prices are highly affected by the high cost of purification steps. Considering this, this study evaluates the possible optimization effects of adding polyethylene glycol (PEG) in different molecular weights (MW) to the immobilization process by physical adsorption on different supports. The results obtained demonstrate the potential of applying PEG-added physical adsorption in novel simple and low-cost lipase purification processes through enzyme immobilization.

Keywords: Lipases. Immobilization. Physical adsorption.

1 INTRODUCTION

Enzymes are protein molecules that catalyze most of the reactions essential to living organisms. They have high substrate specificity, are biodegradable, nontoxic and require middle conditions. However, despite the benefits of using biocatalysts in comparison with chemical catalysts, these proteins present a high commercial value and require large production investments. Since the enzyme market is mostly from microbial sources, the downstream processes for these proteins account for up to 80% of the global production cost, thus, it is necessary to search for simple and low-cost purification methods.[1,2,3]

Currently, enzymes are crucial to many industrial sectors, such as food, cosmetical, pharmaceutical and biofuels. Among industrial enzymes, lipases (triacylglycerol ester acylhydrolases, EC 3.1.1.3) stand out as the third most globally commercialized enzyme. As an alternative to microbial lipases purifications high-cost methods, the use of the immobilization technique by physical adsorption may be applied as a single low-cost purification step, also able to improve enzymatic performance. [4,5,6]

In order to improve the immobilization yield, it is common to add a spacer molecule ("tether") or a stabilization agent, such as polyethylene glycol (PEG), which allegedly also improves the stabilization of the biomolecule at the water/oil interface, increases its bioactivity when immobilized and reduces the adsorption of non-specific proteins. The polymer in question is amphiphilic and has no toxicity, being a good option for food and pharmaceutical industries.[7,8,9,10]

In this sense, here we have evaluated the influence of different molecular weights of polyethylene glycol in the application of lipases from *Rhizopus oryzae* immobilization to increase the process efficiency for further application as purification tool.

2 MATERIAL & METHODS

Immobilization by physical adsorption of *Rhizopus oryzae* commercial lipase (Sigma-Aldrich®) was carried out using 20 mL of 0.1 mol L^{-1} phosphate buffer pH 7.0, and 0.25 g of enzyme/1 gram of support. The supports used were MB400 resin (Purolite ®), HP-20 resin (Diaion ®) and corncobs, an agro-industrial residue.

Also, PEG was added to the immobilization system by pipetting 500 µL/gram of support of 1 mg mL^{-1} PEG 400 MW (Sigma-Aldrich ®), 1500 MW (Sigma-Aldrich ®) and 20000 MW (Sigma-Aldrich®) solutions into the buffer. To determine the enzymatic activity the olive oil hydrolysis method was applied, and the equation 1 applied according to dos Santos *et al* (2021).[11]

$$Activity\ \left(\frac{U}{g}\right) = \frac{\left(V_{blank\ (mL)} - V_{sample\ (mL)}\right) \times\ M_{HCl}\ \left(\frac{mol}{L}\right).10^3}{time(min) \times\ amount\ of\ enzyme(g)} \quad (1)$$

Immobilization yields (N) were calculated according to equation 2.

$$N\ (\%) = \frac{U\ .100}{U_0} \quad (2)$$

Where U_0 equals the hydrolytic activity units offered for immobilization; while U corresponds to the total hydrolytic activity units present in the immobilized derivative.

3 RESULTS & DISCUSSION

The influence of different PEG molecular weights in the hydrolytic activity of *Rhyzopus oryzae* lipase immobilized in different materials are showed in Table 1. It is possible to notice that the type of PEG have influenced the adsorption process in differente ways according to the support used. A higher molecular weight PEG have contributed to 151% increase in the activity of lipase immobilized in Diaion® HP-20, while did not positively affected the Corncobs immobilized derivative. This is probably related to the fact that Diaion® HP-20 is a hyghly hydrophobic styrene-divinylbenzene polymer, which have hindered the action of the lower molecular weights molecules of PEG. Since PEG 20000 presents a higher hydrophobicity it was able to interact in the immobilization microenvironemt.

Table 1 Enzymatic activities of lipase immobilized on different supports with and without PEG.

Support	Average enzymatic activity without PEG (U/g)	Average enzymatic activity with PEG 400 (U/g)	Average enzymatic activity with PEG 1500 (U/g)	Average enzymatic activity with PEG 20000 (U/g)
Corncobs	286.71 ± 28,20	479.60 ± 28,07	362.67 ± 19,86	282.66 ± 20,38
MB-400	347.80 ± 8,18	729.25 ± 13,55	709.61 ± 9,74	495.47 ± 19,49
HP-20	198.90 ± 12,37	108.80 ± 10,63	220.80 ± 15,28	301.34 ± 12,39

The immobilization yield values were calculated and presented in **Fig. 1**. The results showed an increase from 18.67 to 29.52% in the immobilization yield when PEG 400 was used in the adsorption on Purolite ® MB-400, which corresponded to the highest hydrolytic activity (729.25 U mg^{-1}). These results indicated that the addition of PEG may positively influence the physical adsorption of lipases, however the polymer molecular weight must be analyzed according to each support material since they present different physicochemical characteristics, as hydrophobicity.

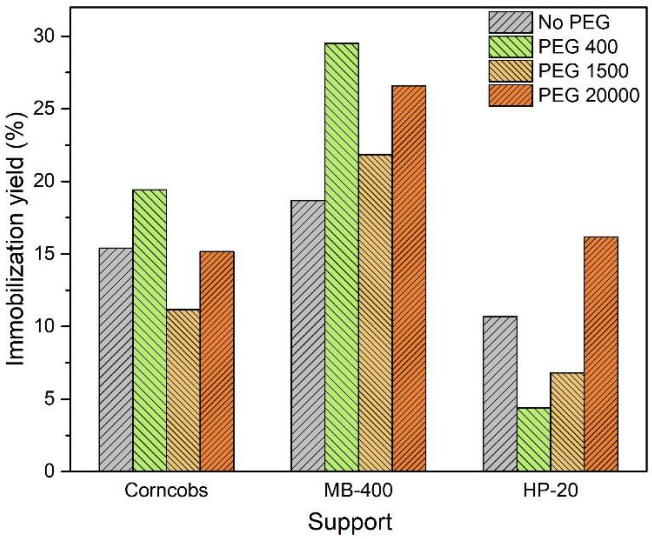

Figure 1 Immobilization yields of lipases immobilized on different supports with and without PEG..

4 CONCLUSION

This study has showed that Polyethylene Glycol in different molecular weights may influence the physical adsorption of lipases and demonstrate potential to be used as a tool in enzymatic purification processes. Highly hydrophobic materials as Diaion® HP-20 did not showed positively influence when lower molecular weights of PEG were used (MW 400 and MW 1500) but have indicated satisfactory improving results when a large MW was used (MW 20000). The results have indicated the use of PEG may be an efficient alternative in order to enhance immobilization processes by physical adsorption and may contribute to the development of new biotechnological purification methods.

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