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INFLUENCE OF ACID PHOSPHATASE IMMOBILIZATION TIME ON DUOLITE® A-568 RESIN

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ABSTRACT

Acid phosphatase (ACPase) is an enzyme that catalyzes the hydrolysis of phosphate bonds in organic compounds, resulting in organic phosphate conversion into a water-soluble inorganic form. However, the use of the enzyme in soluble form faces challenges due to its instability in solution. Therefore, enzyme immobilization has emerged as a strategy to circumvent these limitations. This study investigated ACPase immobilization using an ion exchange-based technique to improve the stability of commercial acid phosphatase. Using Duolite® A-568 resin, an immobilization yield of 71 %, and expressed activity of 99% were achieved after an immobilization time of 240 minutes.

Keywords: Acid Phosphatase. Enzyme Immobilization. Enzyme Stability. Duolite® A-568.

1 INTRODUCTION

Phosphorus plays a significant role in environmental growth and in manufacturing, then is an essential component of the food chain and for the agrarian economies (Parasana, Shah e Unnarkat, 2022). While phosphorus-based fertilization is crucial for maximizing crop yields, overuse can lead to phosphorus pollution in soil and water, causing harmful effects on water ecosystems due to eutrophication (Dey e Haripavan, 2023).

The limited utilization of phosphorus (P) by plants and animals arises from the fact that inorganic phosphorus, primarily, exists in insoluble mineral compounds, thus making it inaccessible to plants (Parasana, Shah e Unnarkat, 2022). Fungi such as Trichoderma and Aspergillus produce phosphatase enzymes, which convert organic phosphorus into inorganic phosphorus through hydrolysis, thereby generating ions orthophosphate (Gonçalves et. al., 2023).

The study of alternative methods for determining phosphorus (P) levels in the soil is essential to improve the management of phosphate fertilization, since soil phosphatase serves as an indicator of P. The immobilization of the enzyme aims to allow the repeated use of this catalyst. Thus, one of the immobilization methods involves mixing the insoluble support with the enzyme under appropriate conditions, followed by separation of the support containing the adsorbed enzyme from the reaction médium (Fernandes, Lima e Lopes, 2010). In this context, the current work addresses the immobilization process of the acid phosphatase enzyme by adsorption, using Duolite A-568 ion exchange resins as support.

2 MATERIAL & METHODS

2.1. Enzyme

In this study, commercially acquired ACPase-wheat enzyme from Sigma Chemical CO (Sigma-Aldrich) was utilized.

2.2. Support for Immobilization

The support employed for immobilization was Duolite® A-568 ion exchange resin (Rohm Haas), provided by Dow Chemical Company.

2.3. Activation of the Support

The Duolite® A-568 resin was activated following the manufacturer's methodology: 1 M hydrochloric acid, at a ratio of ten volumes of solution per volume of resin, for 30 minutes in a rotary incubator at 50 rpm. Subsequently, 1 M sodium hydroxide was added under the same conditions and procedures as the acid treatment. The resin was washed with distilled water between treatments. At the end of the process, the resin was washed with distilled water, vacuum-filtered for 15 minutes, and air-dried at room temperature.

2.4. Substrate

A solution of ACPase-wheat in acetate buffer pH 4.5 was used as the substrate. As a rule, the ratio of enzymatic solution to support was 10 mL of enzymatic solution per gram of support. The concentration of commercial enzyme ACPase-wheat was 5 mg/mL.

2.5. Acid Phosphatase Activity Assay

The acid phosphatase activity was measured according to Leitão et al with slight modifications. The reaction took place between the crude enzyme and the substrate 5mM p-nitrophenylphosphate hexahydrate disodium salt (p-NPP) (Sigma Aldrich) in 50 mM sodium acetate buffer (pH 5.0) at 40°C. After 15 minutes, the reaction was halted by adding 1 mL of 0.1 M NaOH. The quantity of p-nitrophenol was measured using a UV-vis spectrophotometer at 405nm. One unit (1 U) of acid phosphatase activity was defined as 1 mmol p-nitrophenol (p-NPP) formed per minute.

2.6. Immobilization Procedures

The immobilization process involved enzyme adsorption onto the Duolite A-568 ion exchange resin. A mass of 0.5 g of Duolite A-568 resin, previously activated as per section 2.3, was incubated in 10 mL of enzymatic solution with a concentration of 5 mg/mL prepared in acetate buffer at pH 5 under agitation at 100 rpm in a rotary incubator at 25 ± 1°C for 4 hours. Samples of the supernatant and suspension were taken at different times to determine the catalytic activity of the process.

Periodically, to determine activity during the process, the immobilization yield (IY) was calculated by the ratio between the activity in the supernatant and the initial enzymatic activity (Eq. (1)). The expressed activity (EA) was calculated using the final and initial activity (Eq. (2)), according to Trobo-Maseda et al., 2020.

$$IY\ (\%)\ =\ 1 - \left(\frac{Final\ supernatant\ activity}{Initial\ activity}\right) \times 100 \tag{1}$$

$$EA\ (\%)\ =\ \left(1 - \frac{Suspension\ activity}{Initial\ activity \times\ IY}\right) \times\ 100 \tag{2}$$

3 RESULTS & DISCUSSION

Immobilization was carried out using the commercial enzyme acid phosphatase from wheat (ACPase wheat) obtained from Sigma Aldrich. Figure 1 shows the ACPase activity of the immobilized enzyme (retained on the support) as a function of time at room temperature (26 ± 2 C)

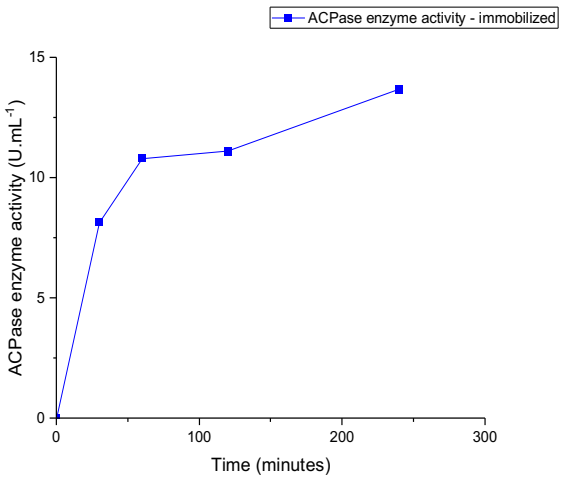

Figure 1 Kinetics of acid phosphatase activity (retained on the support) for wheat enzyme (ACPase wheat)

For the enzyme immobilized at 15 minutes, an activity of approximately 8 U/mL was achieved (Figure 1). At pH 5, the immobilized enzyme performed well in the initial hours, maintaining increasing enzymatic activity throughout the analyzed period.
The ionic interaction of the enzyme with support Duolite® A-568 was tested for immobilization by carrying out a control experiment conducted with the same amount of enzyme, in the same reaction environment, and with the same buffer solution. The results of this control experiment showed that the enzymatic activity was maintained constant over time.
The calculations were based on the enzyme activity present in the solid, which represents the amount of enzyme that was effectively immobilized on the support, and the enzyme activity in the supernatant, which represents the amount of enzyme that remained in the solution (not adsorbed by the support) after the immobilization process. This value was used to calculate the immobilization efficiency by comparing the initial activity of the enzyme in solution with the activity remaining in the supernatant.

The acid phosphatase from the wheat germ (ACPase) immobilization yield was 71%, and an expressed activity was 99% for this study, using Duolite® A-568 as the support and operating at pH 5. These results indicate that although little enzyme was immobilized, the activity of the immobilized enzyme retained was very well preserved. This result suggests that Duolite® A-568 at pH 5 provides a more favorable environment for maintaining enzymatic activity, even though the immobilization efficiency is not the highest. The immobilization of this support is promising due to the exposure of reactive groups on its external surface, which facilitates rapid interaction and adhesion.

Lima et al. (2023) used various ion exchange supports to immobilize acid phosphatase derived from wheat germ. The results showed that Toyopearl DEAE 650s at pH 7 for 1 hour (only immobilized) achieved an immobilization yield of 99.37% and an expressed activity of 96.98%; Toyopearl DEAE 650s treated with NaIO4 and NaBH4 achieved an immobilization yield of 97.34% and an expressed activity of 73.31%; DEAE Sepharose 6BC at pH 7 overnight achieved an immobilization yield of 99.15% and an expressed activity of 2.76%; and MANAE Agarose 6BC at pH 7 overnight achieved an immobilization yield of 98.08% and an expressed activity of 3.21%. These results show significant variation in the values of yield and expressed activity, indicating high efficiency in immobilization for all supports used. However, the expressed activity varies significantly, suggesting that additional factors, such as unfavorable interactions with the support or conformational changes in the enzyme, may drastically reduce the activity of the immobilized enzyme.

4 CONCLUSION

The immobilization of (ACPase) from wheat germ in ion exchange resin demonstrated strong interference from the immobilization time. The immobilization method for acid phosphatase using the Duolite® A-568 resin was efficient, showing a yield of 71%, an expressed activity of 99% for the biocatalyst, and an immobilization time of 240 minutes for 0.5 g of resin.

REFERENCES

[1] SILVA, A. F. C., FERREIRA, B. CASTRO, C. T. 2023. Lat. Am. J. Biochem. Process. 27 (1). 429-440.
[2] GREEN, T. PARKER, R. 1999. Interpreting landscapes. *In:* Evolution of Bioinformatics. Dawson, L. (ed). 2nd ed. Pearson, Los Angeles. 180-205.
[3] DEY, S. E HARIPAVAN, N. 2023. Materials and biomedical devices, 1 (1), 122-145.
[4] FERNANDES, K. F., LIMA, C. S., & LOPES, F. M. 2010. Chemical Processes Magazine, 4 (7), 53-58.
[5] GONÇALVES, E. V.; MARTINS, M. D.; SANTOS, S. X.; BORGES, L. L.; CARAMORI, S. S. 2023. Environmental Challenges. V. 11.100693.
[6] L. TROBO-MASEDA, A. H. ORREGO, J. M. GUISAN and J. ROCHA-MARTIN, Int. J. Biol. Macromol., 157 (2020).
[7] PARASANA, N., SHAH, M., & UNNARKAT, A. 2022. Environmental Science and Pollution Research, 29(26), 38985-39016.
[8] SHELDON, R. A., & VAN PELT, S. (2013). Chem. Soc. Rev., 42(15), 6223–6235.doi:10.1039/c3cs60075k.
[9] TUTU, K.; AMBASHT, P. K. 2019. Journal of Proteins and Proteomics. V. 10. Pages 291-297.

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