LIPASE EVERSA® TRANSFORM 2.0 IMMOBILIZED IN A BLEND OF MARINE BIOPOLYMERS

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ABSTRACT

In this work, a protocol was developed for synthesizing a blend of marine biopolymers based on chitosan and iota-carrageenan chemically crosslinked with glutaraldehyde. Then, the material was applied as a support for the immobilization of Lipase Eversa® Transform 2.0 by adsorption. Immobilization was conducted for 1 hour by contacting the enzyme, suspended in 5 mM sodium phosphate buffer pH 7.0, with the support under gentle stirring at 25 ºC. Lipase activity was obtained by evaluating the hydrolysis of ρ-nitrophenyl butyrate (ρNPB) pH 7.0 and 25°C. The lipase immobilization in the biopolymer blend resulted in an immobilization yield of 37.3%. The heterogeneous biocatalyst stood out due to the hyperactivation of catalytic activity, with a recovered activity of 107 %, which is higher than that of the free enzyme. The synthesis protocol for a blend of iota-carrageenan and chitosan cross-linked with glutaraldehyde was established and demonstrated its potential application as a support for the immobilization of enzymes.

Keywords: Chitosan. Iota-carrageenan. Biocatalysis. Enzyme.

1 INTRODUCTION

Lipases are the enzymes most used in biocatalysis and are applied in various industrial processes, such as the food, cosmetic, detergent, and pharmaceutical industries, due to its ability to catalyze various reactions, such as the hydrolysis of ester bonds in triglycerides, in addition to esterification reactions, acidolysis, interesterification, transesterification, aminolysis, peridolysis, and a series of promiscuous reactions. Among the innovations created with enzymatic engineering tools, Eversa ® Transform 2.0 lipase appears as a genetically modified, economical, and highly active enzyme formulation in the transesterification of acylglycerols and the direct esterification of free fatty acids. The immobilization of these lipases on solid supports is a tool to overcome limitations related to activity in organic systems and low thermal and pH stability observed in enzymes used in soluble forms in processes. Furthermore, it allows easy recovery and reuse for successive reaction cycles[1,2].

Enzymes immobilized in natural polymers have been widely used due to their biodegradability, biocompatibility, and commercial availability. Some biopolymers, such as chitosan, have reactive hydroxyl and amino groups that can be cross-linked with reagents such as glutaraldehyde, forming matrices that bind to proteins. Furthermore, the protonated amino group of chitosan is capable of forming hybrid hydrogels through ionic interactions with anionic groups of some natural polymers, such as carrageenan (sulfonic groups), improving the mechanical properties of the supports[2].

Aiming to combine the development of new biocatalysts with materials produced by marine biomass biorefineries, the objective of this work is to synthesize a biopolymer blend formed by the ionic interaction between chitosan and iota-carrageenan with subsequent chemical cross-linking with glutaraldehyde, and its application for production of a biocatalyst through the immobilization of Eversa® Transform 2.0 lipase.

2 MATERIAL & METHODS

For the development of this research, chitosan with a degree of deacetylation of 90% (Exodus Científica), iota-carrageenan commercial grade type II (Sigma), Lipase produced by Aspergillus Oryzae (Eversa® Transform 2.0, Sigma), glutaraldehyde 25% (Sigma), glacial acetic acid P.A (NEON), sodium phosphate monobasic monohydrate P.A (NEON), sodium phosphate dibasic heptahydrate P.A (NEON), acetonitrile for HPLC (Sigma), ρ-nitrophenyl butyrate (Sigma), and Bradford solution (self-preparation).

The synthesis of the biopolymer blend considered the mass ratio between chitosan and carrageenan to be 1:3. Initially, 20 mL of 5% glacial acetic acid solution was heated to 80ºC. Then 0.61 g of iota-carrageenan was added, stirring until complete solubilization. Soon after, 0.2 g of chitosan was slowly added. The mixture was then cooled until it reached a temperature between 50 and 60ºC, and 500 µL of 25% glutaraldehyde was added. Afterwards, the agitation was stopped, and the material remained protected from light and contact with the environment for 24 hours. Finally, the biopolymer blend was vacuum filtered, washed thoroughly with distilled water, and dried at room temperature in a vacuum desiccator. Figure 1 describes the production scheme of the biopolymer blend cross-linked with glutaraldehyde.

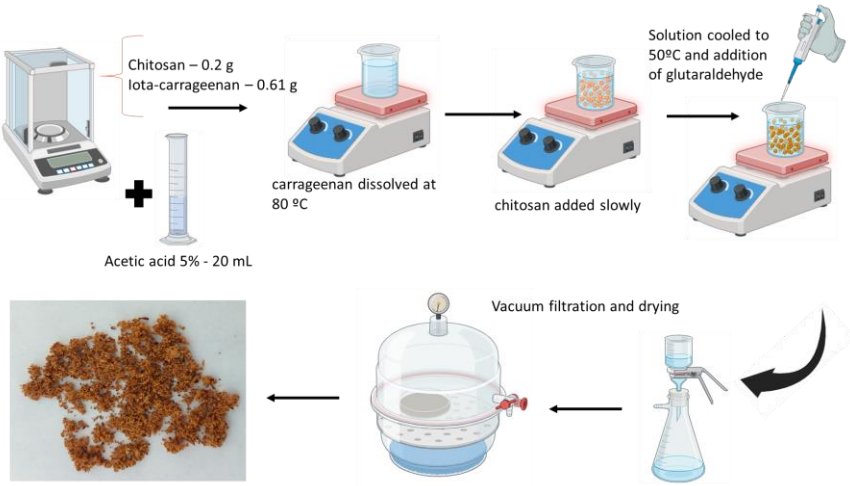

Figure 1: Scheme of the biopolymer blend production process. Source: Author.

Fourier transform infrared spectroscopy (FTIR) was applied to characterize the synthesis of the biopolymer blend. Therefore, samples of chitosan, iota-carrageenan, and the biopolymer blend (before and after immobilization) were analyzed in a Cary 630 FTIR infrared spectrophotometer (Agilent Technologies, USA). Spectra were obtained in the scan of 400–2000 cm^{-1} in transmittance mode and a resolution of 2 cm^{-1}.

The blend was applied as a support in producing a heterogeneous biocatalyst through the immobilization of Eversa® Transform 2.0 lipase. The immobilization test occurred by incubating the support with the enzyme solution in a proportion of 1 mg of protein per g of support, for 1 hour with the enzyme suspended in 5 mM sodium phosphate buffer pH 7.0 under stirring at room temperature. Immobilization was characterized by evaluating the catalytic activity of the initial and final enzyme solution, in addition to the biocatalyst resulting from immobilization.

The characterization of the enzymatic activity was obtained by evaluating the increase in absorbance caused by the release of p-nitrophenol due to the hydrolysis of p-nitrophenyl butyrate (ρNPB) resulting from its incubation with the free enzyme and the biocatalyst in 25mM sodium phosphate buffer pH 7.0, and a temperature of 25°C. To do this, we use a spectrophotometer programmed to read at a wavelength of 348nm. Activity is given in micromoles of p-nitrophenol per minute (U)[3].

3 RESULTS & DISCUSSION

FTIR spectroscopy is a tool that can be applied to characterize biopolymers, providing valuable information about the chemical structure and molecular composition of these materials. Figure 2 shows the FTIR spectra of chitosan, iota-carrageenan, and the biopolymer blend produced by the electrostatic interaction between the previously mentioned materials and their chemical cross-linking with glutaraldehyde.

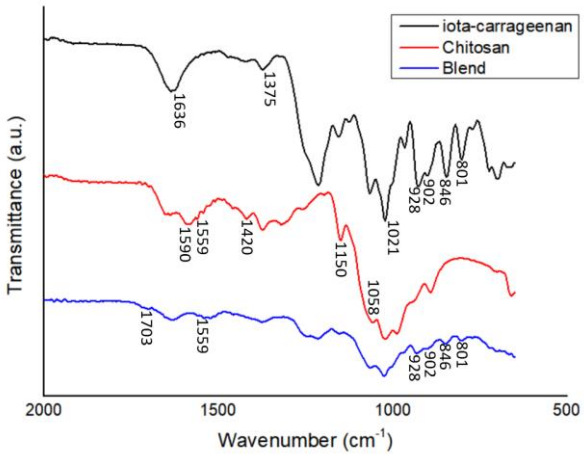

Figure 2: The 2000–500 cm^{-1} FTIR spectrum of chitosan, iota-carrageenan, and biopolymer blend.

The band at 1636 cm^{-1} was attributed to the C=O stretch of amide I from chitosan, while the band at 1375 cm^{-1} corresponds to the axial deformation of C-H in CH_3 groups, also characteristic of chitosan. These bands confirm the presence of chitosan in the blend. On the other hand, the band at 846 cm^{-1} was attributed to the stretching of the galactose-4-sulfate unit, and the bands at

902 cm^{-1} and 928 cm^{-1} were associated with the stretching of 3,6-anhydro-galactose, all characteristic of iota-carrageenan. The band at 1150 cm^{-1} was attributed to the S=O stretching of sulfate groups, further reinforcing the presence of iota-carrageenan in the material.

Additionally, the band at 1021 cm^{-1} can be attributed to C-O-C stretching, common in polysaccharides, and can also be related to the presence of chitosan. The bands at 801 cm^{-1} and 1058 cm^{-1} correspond to C-O-C stretching and out-of-plane deformation of C-H, common in polysaccharides such as iota-carrageenan. These observations corroborate that the characteristic bands of both biopolymers are present in the FTIR spectrum of the material, confirming that the blend is formed by the union of chitosan and iota-carrageenan. This analysis demonstrates the interaction between the two components and forming a new polymeric matrix.

The presence of a new band at 1559 cm^{-1} refers to the formation of the C=N bond between the blend and glutaraldehyde, indicating the occurrence of the cross-linking process. It is observed that the band referring to the amino group (1590 cm^{-1}) is not present in the cross-linked blend. The appearance of a band at 1703 cm^{-1} may be related to the presence of free aldehyde bonding (unreacted aldehyde groups).

After synthesizing the support using the biopolymer blend, the Eversa® Transform 2.0 lipase immobilization test was carried out. The results are presented in Table 1. Despite the immobilization yield remaining around 40%, the support's ability to retain enzymatic activity proved its main characteristic, allowing the lipase to be slightly more active than in its free form (107 ± 10 %). This is possibly due to the protein being positioned in a way that preserves its structure, in addition to the interaction between the lipase and the support allowing the polypeptide chain that covers its active site to remain in the open position, resulting in catalytic activity superior to of the free enzyme.

Table 1 – Characterization of enzyme adsorption and catalytic activity.

Immobilization Yield (%)	Biocatalyst activity (U/g)	Activity Recovered (%)
37 ± 3	35 ± 1	107 ± 10

4 CONCLUSION

In conclusion, the synthesis of a biopolymer blend through the ionic interaction of chitosan and iota-carrageenan, followed by chemical cross-linking with glutaraldehyde, has proven to be an effective method for creating robust support for enzyme immobilization. The FTIR analysis confirmed the successful synthesis of the blend and the occurrence of the cross-linking process. The immobilization of Eversa® Transform 2.0 lipase on this biopolymer blend resulted in a biocatalyst with high retained enzymatic activity, surpassing that of the free enzyme. Despite a moderate immobilization yield of approximately 40%, the significant recovery of enzymatic activity (107 ± 10 %) highlights the potential of this biopolymer blend as a valuable support material for biocatalytic processes.

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