ADVANCEMENTS IN THE POTENTIAL OF CARRAGEENAN FOR ENZYME IMMOBILIZATION

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ABSTRACT

Enzymes are efficient and environmentally friendly biocatalysts, which may increase the reaction rate of several industrial reactions. Enzyme applications have been a popular topic among the scientific community as they strive to improve their industrial feasibility. One such method is immobilization, which involves confining enzymes to a support system that enables reuse and enhances their overall stability. Carrageenan, a polysaccharide derived from red seaweeds, offers advantages over traditional enzyme supports, such as biocompatibility and biodegradability. Thus, this work presented a bibliometric analysis, identifying gaps and new advances in the area. The article discusses key advancements in utilizing carrageenan for enzyme immobilization, highlighting its role in enhancing enzyme stability, activity, and reusability. Several immobilization techniques were presented, such as encapsulation, cross-linking, and adsorption onto carrageenan matrices. The main immobilized enzymes of industrial interest in carrageenan-based support, such as β-galactosidase, lipase, and amylase, were also approached. Moreover, the review addresses recent research trends and prospects, emphasizing the importance of optimizing immobilization methodologies for efficient enzyme immobilization and enhanced biocatalytic processes. This research highlights the importance of developing new technologies to expand the application of biocatalysts at the industrial level.

Keywords: Enzyme Immobilization. Encapsulation. Industrial Biocatalyst. Carrageenan.

1 INTRODUCTION

Enzymes are essential biocatalysts in various applications, such as the food industry and biofuel production. These catalysts promote reactions by reducing the activation energy required for a chemical reaction. Industrial processes using enzymes have significant environmental benefits, such as reducing waste and utilizing lower temperature and pH compared to traditional chemical methods. However, using free enzymes poses challenges such as limited reusability and sensitivity to environmental conditions, hindering their industrial applications. A possible alternative is enzyme immobilization, which offers advantages in biocatalytic processes, such as stability to a wider pH and temperature range and possible reutilization of the enzymatic beads[1].

Carrageenan, a polysaccharide derived from seaweeds, has gathered the scientific community's attention due to its potential as an adsorbent material across diverse applications. These biopolymers exhibit high potential in various industrial applications such as gelling, thickening, and the production of edible films. Moreover, carrageenan exhibits biocompatibility, biodegradability, and gelation properties that are advantageous in various industrial and biomedical settings. It has the potential to form hydrogels and aerogels for adsorbing pollutants like heavy metals, dyes, and proteins, thereby being suitable for enzyme immobilization. Carrageenan is extracted from red seaweeds and classified into *kappa* (κ), *iota* (ι), and *lambda* (λ) varieties based on their sulfate group content. Among these, *kappa* and *iota* carrageenans present the potential to form hydrogels, with *kappa* carrageenan displaying stable and rigid biopolymers and *iota* carrageenan forming softer hydrogels, suitable for the release of substances[2], also for aerogel production[3].

Enzyme immobilization on carrageenan, a low-cost natural polymer, provides significant advantages in biocatalysis[1,4]. This technique enhances the enzyme's application potential, improving biocatalytic process efficiency. Carrageenan supports are widely used to immobilize enzymes such as lipases, β-galactosidase, glucose isomerase, and proteases. Immobilization overcomes limitations like reduced activity and low thermal and pH stability observed in soluble enzyme forms. It also simplifies biocatalyst separation from the reaction medium and enables reusability across multiple reaction cycles, making it adaptable to various reactor configurations. In this context, this work aims to comprehensively review the potential of carrageenan for enzyme immobilization, highlighting its advantages, applications, and contributions to advancing sustainable and efficient biocatalytic processes across various industries. Thus, it is expected to have significant impacts by providing an in-depth understanding of carrageenan's potential in enzyme immobilization, contributing to developing more sustainable and efficient biocatalytic processes with potential applications across various industries.

2 MATERIAL & METHODS

The present study focuses on a bibliometric analysis of scientific productions about the application of carrageenan as support for enzyme immobilization. The literature review was performed in the Scopus database; then the found papers were exported and analyzed using VOSviewer© (version 1.6.20), applying to form maps of the published papers, most cited keywords, and significant subjects of the publications, as well as the most immobilized enzymes according to recent papers. The search was limited to document types "article" and "review," covering published articles from 2014 to 2024. The chosen keywords defined the research, resulting in 83 articles for "carrageenan enzyme immobilization" keywords.

3 RESULTS & DISCUSSION

The use of carrageenan has been investigated to enhance the efficiency of immobilized enzymes, generating significant interest in biocatalysis. Its potential to form hydrogels and aerogels aids in adsorbing pollutants such as heavy metals and dyes for environmental remediation, also providing a stable matrix for enzyme attachment, resulting in highly efficient biocatalysts. This immobilization technique, which may include cross-linking with other biopolymers, such as chitosan and alginate, usually uses glutaraldehyde or epichlorohydrin as reticulant agents, increasing the enzyme's overall stability and reusability and simplifying the separation of enzymes from reaction media. The research findings presented significant scientific developments in some countries, towards immobilizing enzymes in carrageenan support, such as Egypt, India, China, and Brazil, as presented in Figure 1. These countries are known producers of brown and red algae, such as *Rhodophyta*, *Euchema,* and *Kappaphycus*, which are predominant in tropical countries. In Brazil, specifically in the Northeast region, there is a high potential for carrageenan production due to the high cultivation of carrageenan-rich algae, such as *Soliera filiformis*, and other commonly cross-linked polymers, such as chitosan[5].

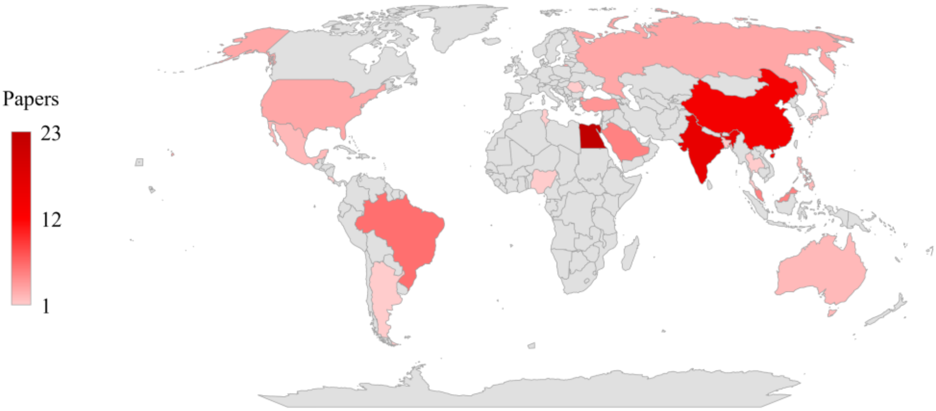

Figure 1 Countries with the most number of recent (2014-2024) published papers on the use of carrageenan as a support for enzyme immobilization.

Though the enzyme immobilization by carrageenan was presented as multidisciplinary by the finding results, as presented in Figure 2, some areas stood out, such as “Biochemistry, genetics and molecular biology,” and “Chemical Engineering,” which represents 41.21% of the published works. This is representative of the industrial applications of the immobilized enzymes and the potential of these biocatalysts. Also, it presents the potential of support in the “Pharmacology and Immunology” areas, which is representative of the studies involving the production of pharmaceuticals by enzyme and the drug delivery potential of carrageenan. Figure 2B presents the keyword cluster related to the research subject. The enzyme immobilization process by carrageenan may also involve other polymers present in the cluster map, such as chitosan[1], and the application of glutaraldehyde and alginate[4]. Also, several methods of immobilization were presented, such as adsorption, encapsulation, and covalent bonds, which may result in different strategies for immobilizing enzymes[5,6], which may differ depending on the enzyme or sought industrial reaction.

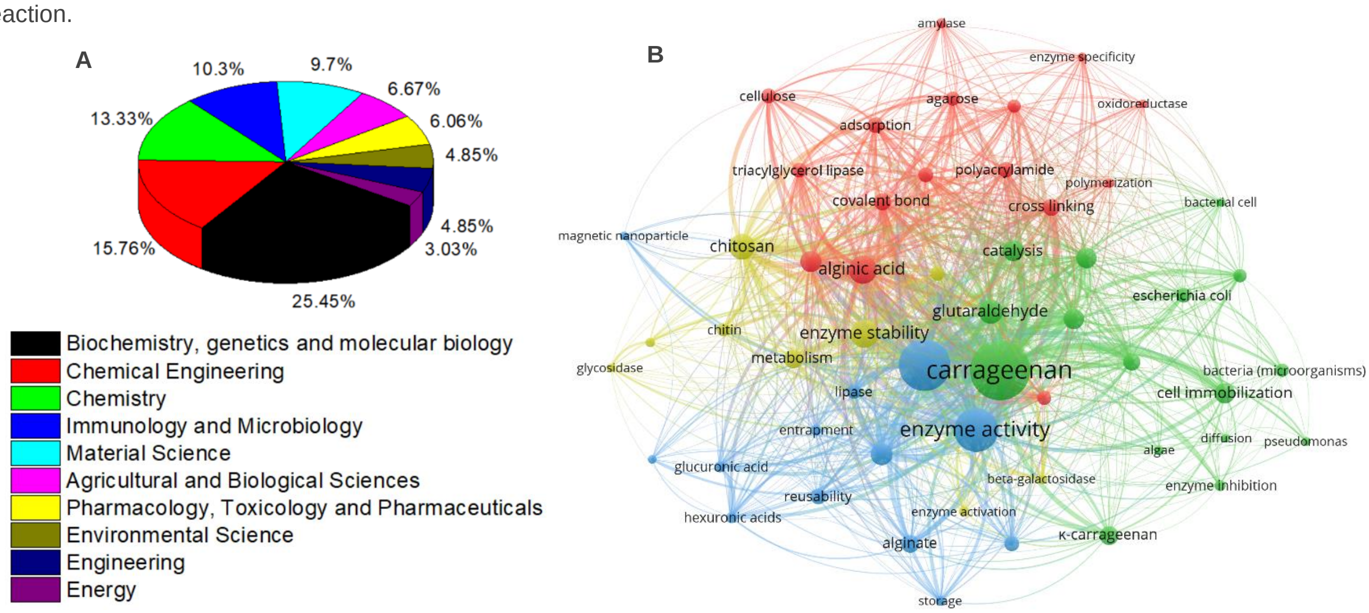

Figure 2 Bibliometric analysis of the enzyme immobilization by carrageenan support, presenting (A) the explored subject areas and (B) the keyword co-occurrence cluster map from 2014-2024.

The carrageenan support presents the potential for the immobilization of several industrial applicable enzymes, and Figure 3 presents the enzymes that are more present in the recent literature (2014-2024). Beta-galactosidase and lipase are the most studied enzymes due to their potential application in the biofuel, pharmaceutical, and food industries. These enzymes have been immobilized, especially by the encapsulation method, which results in higher stability to temperature and pH, which may increase their industrial and reusability potential compared to free enzymes, reducing operational costs.

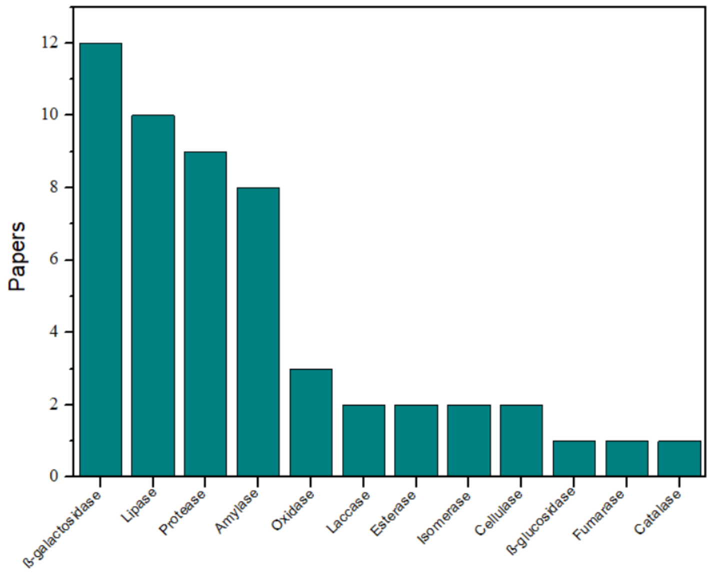

Figure 3 Number of published papers showcasing the immobilization of different enzymes by carrageenan support.

Table 1 provides examples of immobilized enzymes using carrageenan support. Notably, the immobilized enzymes showed higher relative activity than the free enzyme, indicating an efficient immobilization process that does not result in enzyme deactivation. This showcases the high efficiency of carrageenan as a support. The immobilized enzymes also exhibit high pH and temperature stability, which is highly valued in the industry, resulting in a stable industrial process. Therefore, the potential of carrageenan in improving these biocatalysts for several reactions is highly valued for the industrial feasibility of biocatalysts. It was noted that immobilizing various enzymes in carrageenan can provide stability across a wide range of pH levels (from 3.0 to 9.0) and thermal stability at temperatures of up to 80°C for glucoamylase for example. This is due to the covalent bonds between the amine groups of the enzymes and the surface of the gel, increasing the stability over pH and temperature changes[6].

Table 1 Immobilized enzymes supported by carrageenan, the microorganism of the enzyme's origin, the relative activity compared to the free enzyme, and the improved parameters, such as pH and temperature stability range.

Enzyme	Microrganism of origin	Relative Activity (%)	pH stability range	Temperature stability range (º C)	Reference
β-D-galactosidase	*Aspergillus oryzae*	95-100	4.0-5.0	50-60	4
Glucoamylase	-	100	4.0-5.5	60-80	6
Glucose isomerase	*Streptomyces rochei*	90-100	6.0-7.5	65-75	7
Laccase	*Trametes versicolor*	100	3.0-6.5	30-55	8
Lipase	*Tsukamurella tyrosinosolvents*	100	6.0-8.0	30-40	9
Lipase	*Burkholderia cepacia*	100	5.0-9.0	25-45	10

4 CONCLUSION

Carrageenan, a seaweed polysaccharide, presented a high potential for enzyme immobilization. It provides a stable matrix for enzyme attachment, applying to the immobilization of several industrial biocatalysts, such as β-galactosidase, lipase, and amylase. This technique offers increased stability, reusability, and simplified separation of enzymes from reaction media. Thus, this review presented Carrageenan as a tool for the synthesis of efficient biocatalysts. Its potential for support of enzymes may advance green chemistry practices across diverse industries, presenting innovation for enhancing biocatalyst industrial reactions.

REFERENCES

1 DE SOUSA FONSECA, T. *et al.* 2021. Bioprocess Biosyst. Eng. 44. 785–792
2 MONCADA, D. *et al.* 2023. Int. J. Biol. Macromol. 235. 1-9.
3 ABDELLATIF, M. M., SOLIMAN, S. M. A., EL-SAYED, N. H. & ABDELLATIF, F. H. H. J. 2020. Porous Mater. 27. 277-284.
4 WAHBA, M. I., HASSAN, M. E. & ALI, K. A. 2021. Biocatal. Biotransformation. 38. 138-151.
5 CARNEIRO, E. A. *et al.* 2020. Quim. Nova. 43. 1234-1239.
6 HASSAN, M. E., YANG, Q. & XIAO, Z. 2019. Bull. Natl. Res. Cent. 43. 1-11
7 HASSAN, M. E. *et al.* 2023. Biotechnol. Lett. 45. 175-189.
8 MAKAS, Y. G., KALKAN, N. A., AKSOY, S., ALTINOK, H. & HASIRCI NESRIN, N. 2010. J. Biotechnol. 148. 216-220.
9 HUANG, J., YAN, R., HE, J. YAO & WANG, P. 2016. Appl. Biochem. Biotechnol. 180. 609-622.
10 JEGANNATHAN, K. R., SENG, C. E. & RAVINDRA, P. 2009. J. Environ. Res. Dev. 4. 431-439.

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