ENZYME IMMOBILIZATION
FOR BIOPROCESSING

Enzyme immobilization has been extensively explored by chemical/biochemical/ biotechnology personnel for research and industrial uses. The ability to improve the stability and reusability of enzymes has driven this technique to be employed in a plethora of applications in these recent decades. *Enzyme Immobilization for Bioprocessing* offers up-to-date reviews on the current strategies and state of the art support systems involved in various bioprocesses. The highlights of this research book include:

- The latest enzyme immobilization methods and strategies – entrapment, encapsulation, adsorption and cross-linking.
- Mechanisms and interactions involved between enzyme and support.
- Kinetics and performance of immobilized enzyme in bench-top stirred reactor.
- Emerging support materials for effective immobilization, namely, smart polymer, silica, magnetic nanoparticles, graphene oxide and hollow fiber membrane.

Enzyme Immobilization for Bioprocessing also features the most recent applications of immobilized enzymes, including fingerprint visualizations.

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Contents

Preface

Enzymes are not a new subject in academic research and their applications in the industries. However, it has evolved tremendously in recent years, especially regarding the enzyme immobilization process and technology. The conventional enzyme immobilization technology and techniques are still relevant, but the new nanotechnology, modern bioinformatics, and molecular modelling have created a new landscape for enzyme immobilization work.

It is interesting to have an immobilized enzyme system successfully applied in the industries. However, factors such as cost, operational limitations and diffusion complexities imposed by substrates and the product are imminent. Therefore, the main focus of the researchers is to develop and improve on any enzyme immobilization processes to produce a stable, reusable, and robust system to adapt to the uncertain and harsh industrial environment. The immobilization technique and support system selection which are crucial prior to any applications have become our primary subject matter of interest in writing this book.

We are honoured to have all the authors who are directly involved in enzyme immobilization research to be on board in contributing to this book. We hope the readers will gain fruitful insights into enzyme immobilization and technology too.

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2022

CHAPTER 1

Introduction to Enzyme Immobilization

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1.1 INTRODUCTION

Recent advances in biotechnology and bioengineering have shown an increasing trend towards the development of environmentally friendly, safe and sustainable bioprocesses using enzymes. The excellent selectivity, specificity and catalytic performance have made enzymes robust biocatalysts with a wide range of applications in biomedicine, biosensing, and biocatalysis (Bilal *et al.*, 2021). Due to the low use of chemicals and the absence of hazardous metabolites/byproducts, the use of biocatalysts is expected to facilitate environmentally friendly processes. In addition, there is a recurring obstacle in the various industries where biocatalysis can be used: the application of enzyme catalysis in chemical processes is limited by the lack of stability of enzymes at high temperatures or in turbulent flow regimes, as well as in potentially toxic solvents (Chapman *et al.*, 2018).

Immobilization of enzymes on suitable supports is generally recognized as a promising approach that occupies an important place. This technology is able to stabilize or protect enzyme molecules against environmental and chemical attacks. It also means combining the selectivity, stability and kinetics of the enzyme with the physical and chemical properties of the support in a specific formulation that plays a major role in maximizing the stability of both the physical and enzymatic activity of the biocatalyst (Basso and Serban, 2019). A variety of immobilization strategies and support materials (natural/synthetic polymers or inorganic materials) have been developed and used for the immobilization of different classes of enzymes. These strategies include immobilization support, linkers, and methods such as entrapment, adsorption, and covalent and non-covalent interactions (Samak *et al.*, 2020). Figure 1.1 shows the advantages and disadvantages of enzyme immobilization.

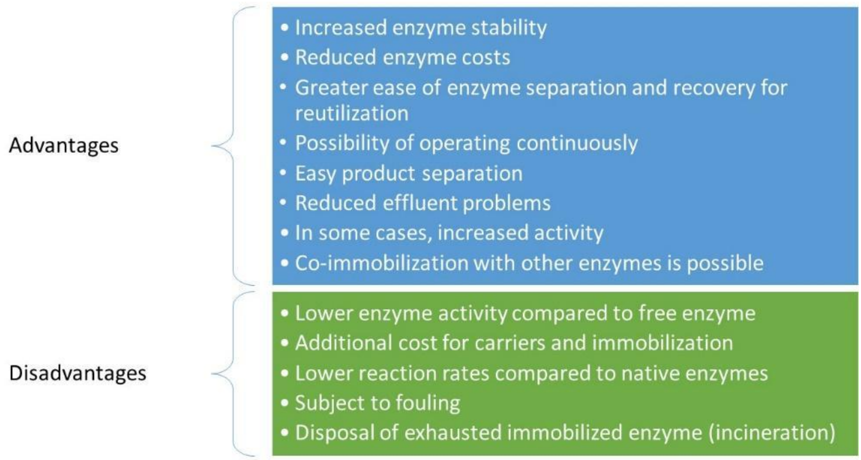

Figure 1.1 Advantages and disadvantages of immobilized enzymes

1.2 TECHNIQUES OF ENZYME IMMOBILIZATION

In an enzymatic reaction, an enzyme acts as a biological catalyst that promotes the reaction rate and does not wear out during the reactions. Therefore, the enzyme can be used repeatedly as long as it remains active. To date, a variety of immobilization methods have been developed to immobilize enzymes on solid surfaces. The different enzyme immobilization methods are grouped as shown in Figure 1.2.

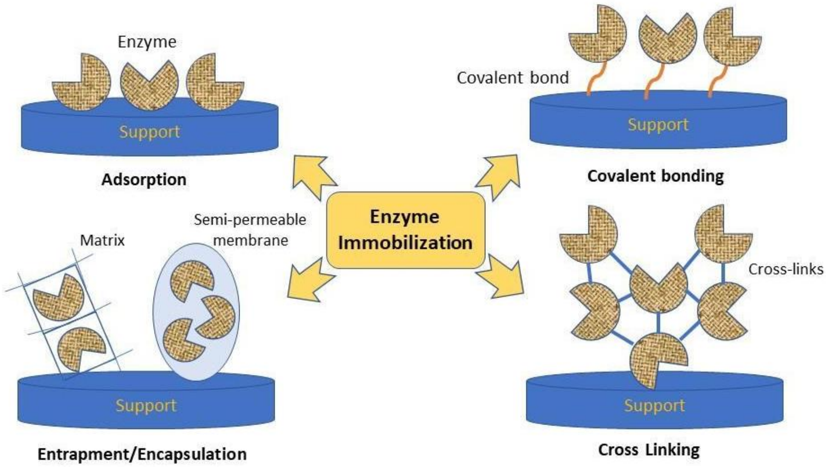

Figure 1.2 Immobilizing enzymes by different methods

The types of immobilization techniques are also classified according to chemical reactions that are used for binding (Table 1.1). Sometimes a combination of several immobilization methods must be used to immobilize an enzyme. As an example, enzymes can be immobilized on a bead by adsorption, affinity, or covalent bonds prior to encapsulation.

In addition to the benefits and drawbacks of each immobilization technique (Table 1.1), the technique that is best suited to the enzyme is determined by its biochemical and kinetic properties as well as the carrier properties (chemical and mechanical). Thus, enzymes interact with certain supports to yield biocatalysts with biochemical and physicochemical properties that are tailored for specific applications.

Table 1.1 Benefits and drawbacks of immobilization methods for enzymes

Binding Techniques and Types	**Benefits**	**Drawbacks**
Adsorption Weak binding: hydrophobic, van der Waals, or ionic interactions.	Simple and cheap Little conformational change of the enzyme	Desorption Non-specific adsorption (spelling)
Affinity Bond forms between two affinity partners	Simple and oriented immobilization Remarkable selectivity	High cost
Covalent bonding Occurs between functional groups of the carrier and the enzyme	No leakage of the enzyme Potential to stabilize the enzyme	It is not possible to regenerate matrixes and enzymes Severe activity loss
Entrapment Entrapment in a polymer network	Broad applicability	Restricts mass transfer Leakage of enzymes
Cross-linking A functional reagent cross-links enzyme molecule	Stabilization of the biocatalyst	Packed beds are less suitable for cross-linked biocatalysts Limited mass transfer Enzyme deactivation

1.3 CARRIER SELECTION

Various materials of different origins can be used to immobilize enzymes. There are three types of materials: organic, inorganic, and hybrid or composite. Attachment of enzymes to insoluble support allows not only their reusability but also additional stabilization through covalent binding at multiple points or binding of multiple subunits of enzymes on solid supports (Aggarwal and Pundir, 2016).

There are some limitations in this area, as the matrix should not interfere with the enzyme or negatively affect its structure beyond what is necessary to ensure stable enzyme-matrix interactions. Further, the functional groups of the two materials need to be compatible in order for the enzyme to effectively bind to the support and form enzyme–matrix interactions. Such an affinity is significant when covalently immobilizing enzymes (Vijayalakshmi *et al.*, 2020). The support should expose the catalyst's active sites so that molecules from substrates can easily attach to the catalyst, reducing diffusion barriers between substrates and products (Mandari and Devarai, 2021). As summarized in Figure 1.3, support materials have several important properties for efficient enzyme immobilization.

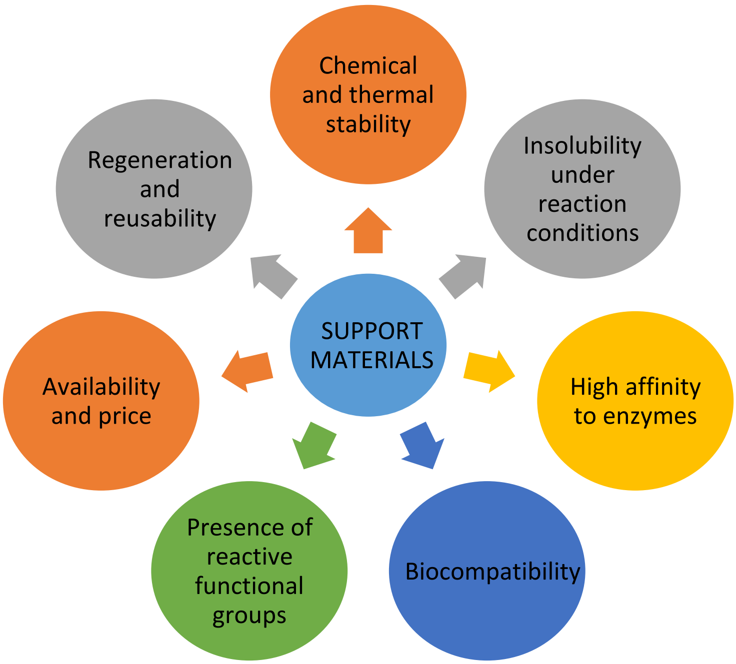

Figure 1.3 Main features of support materials used for immobilizing enzymes

As immobilized enzymes become more widely available, they can be used in many practical applications. It has recently become extremely important to find materials tailored to specific enzymes that have the desired properties. Both organic and inorganic materials derived from these sources exhibit outstanding thermal and chemical stability as well as excellent mechanical properties. In addition, these support materials can be shaped in various morphologies with particle sizes that can be controlled, often at the nanoscale, which facilitates their use as enzyme carriers. Further, these materials contain a variety of functional groups that correspond to protein chemical groups and enhance enzyme binding and surface modification (Liao *et al.*, 2019). However, in the past decade, the scientific community has turned its attention to hybrid and composite materials, combining the advantages of both types of composite precursors (Navrotskaya *et al.*, 2020). As a result of using these enzyme carriers (see Figure 1.4) for the technological process, the immobilized enzymes display a greater catalytic efficiency, and the reaction product quality and purity improve.

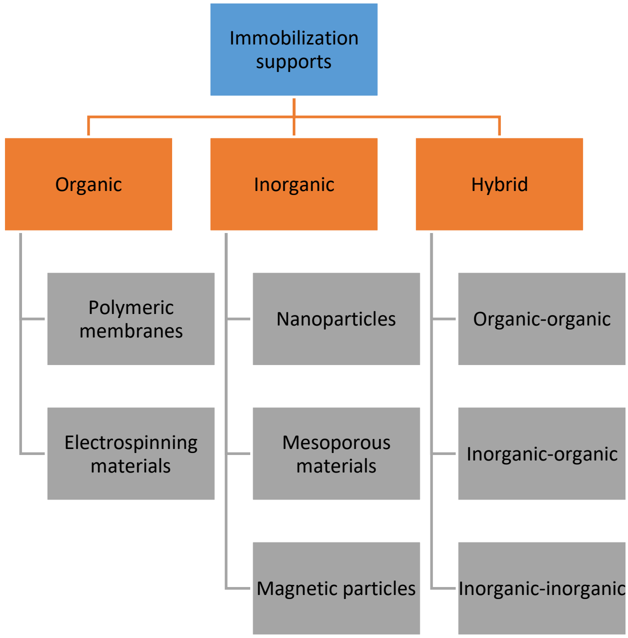

Figure 1.4 A selection of immobilization supports from organic, inorganic and hybrid sources

1.4 INDUSTRIAL APPLICATIONS

Immobilized enzymes have shown exceptional stability and reusability compared to the free enzyme form, resulting in robust biocatalytic systems suitable for various applications of industrial interest (Chatzikonstantinou *et al.*, 2018). It offers the possibility of repeated flow processing; easy recovery and low-cost operation can also be performed on a large scale (Hassan *et al.*, 2019). Some immobilized biocatalysts are also used in the food, pharmaceutical, and biotechnology industries due to their natural properties (Kuribayashi *et al.*, 2021). Processes for immobilization of mesoporous silica for nutraceuticals and pharmaceutical compounds have a significant impact on various fields of biomedicine and biotechnology (Cipolatti *et al.*, 2021) (Figure 1.5).

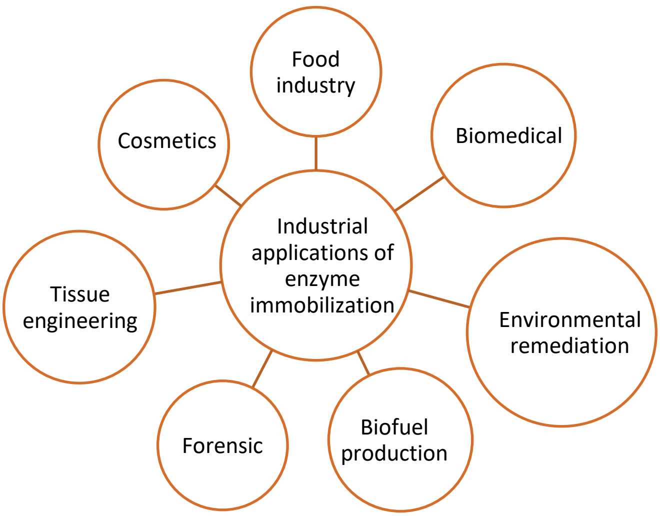

Figure 1.5 A chart showing a number of industrial applications of enzyme immobilization indicating their importance in various fields

1.5 CONCLUSION

Recent advances in biotechnology, especially in enzyme immobilization, have demonstrated good stability, increased enzyme activity, reusability, and cost-effective techniques. Different types of techniques and supports for immobilization have been investigated with different advantages and disadvantages. Even though immobilized enzymes demonstrated positive results, they cannot be generalized. It is important to consider and optimize several different conditions when developing a biocatalyst. It is common for enzyme activity to be drastically reduced or even lost completely after immobilization as a result of structural changes in the enzyme. Different methods exist for binding enzymes to support materials in this context, including single-point and multi-point binding. Additionally, a suitable carrier material should exhibit various desirable qualities, including stability, biocompatibility, nontoxicity, resistance to microbial invasion, minimal diffusion limitations, commercial accessibility, and cost-effectiveness. Due to the immobilization process, the kinetics of the immobilized system have shown a higher enzyme-substrate affinity and catalytic conversion rate, which is due to changes in the enzyme structure. These techniques and supports are therefore likely to be used in many industrial applications in the future.

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Index

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