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Enzyme Immobilization: Novel Approaches

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ABSTRACT
Enzyme enzymes are widely studied and explored areas due to their abundant potential to cater wide application range which makes researchers study more of this area. The high catalytic activity enabling ease in reactions formation of multiple products and byproducts or breakage of complex substances makes it an unavoidable option. However, the cost of pure enzyme increases the economic burden on the operations obstructing its full-fledged use. This leads to making enzyme restrict or immobilize within any inert matrix which can improve the reusability and also improve the tolerance against pH and temperature that can improve the activity of the enzyme. Many different enzyme immobilization approaches have been discussed in the following article.

Keywords**:** enzyme, nanoscale, catalytic activity, surface area

I. INTRODUCTION

Enzymes have been widely used in various applications for ages in various fields such as food, pharmaceuticals, water treatment units, and many more as they increase the rate of reaction without them being getting consumed(Nguyen & Kim, 2017). However, its cost and usability hinder its wide application scope. The only one-time use of pure enzyme and its cost lets need to develop some immobilization and entrapment strategies(Feng et al., 2021).

Since ages, various enzyme immobilization strategies have been used such as covalent linking, adsorption, entrapment, cross linked enzyme aggregates, nanomaterial linked enzyme and many more techniques(Nadar et al., 2020). The immobilisation of enzyme helps in reusability and increases and improves tolerance against extreme pH and temperature shifts. Hence, the process quality is improved with improved results and reducing economic stress on process(Iype et al., 2017). Various researchers have tried various techniques for enzyme immobilization in order to overcome the drawbacks of existing techniques. The present article focuses on different enzyme immobilization strategies and its effect on enzyme activity and other operational factors such as pH and temperature affecting the process.

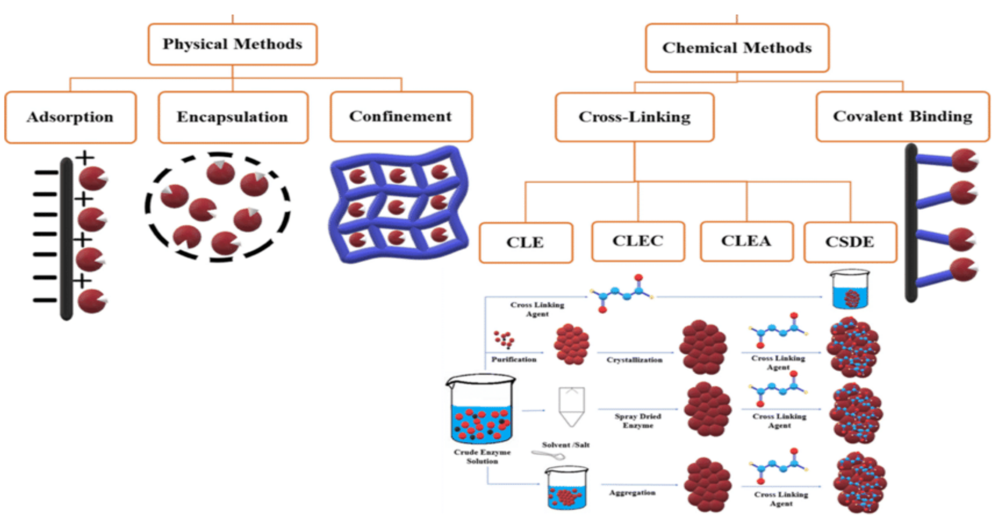

Figure 1: Enzyme immobilization approaches

A. Physical Adsorption

The physical adsorption strategy is one of the coordinate strategies of reversible immobilization that include the proteins being physically adsorbed or joined onto the bolster fabric for the most part carbon, silica base Lattice is utilized. Adsorption can happen through weak non-specific strengths such as vander Waals, hydrophobic intuitive and hydrogen bonds though in ionic holding the chemicals are bound through salt linkages. The reversibly immobilized chemicals can be evacuated from the back beneath delicate conditions, a strategy profoundly alluring. (Homaei et al., 2013).

B. Entrapment

Entrapment is characterized as an irreversible strategy of chemical immobilization where chemicals are entangled in a bolster or interior of strands, either the cross section structure of a fabric or in polymer layers that permits the substrate and items to pass through but holds the chemical. It can be utilized for raising mechanical steadiness and can be too utilized for the lessening of filtering occasions of chemicals.

Since the chemical in this prepare does not associated chemically with the polymer/fabric of the bolster fibers/lattice, it remains ensured from denaturation with time(Nadar & Rathod, 2018).

C. Chemical

Chemical Cross-linking is another irreversible strategy that does not require a bolster fabric for the connection of protein atoms. In this strategy, the particles of proteins are covalently reinforced to each other to make a lattice comprising of nearly as it were protein. The response guarantees that the authoritative location does not cover the enzyme's dynamic location, the movement of the chemical is as it were influenced by immobility

D. Covalent Bonding

The enzyme is bound covalently to an insoluble support (such as silica gel or macroporous polymer beads with epoxide groups). This approach provides the strongest enzyme/support interaction, and so the lowest protein leakage during catalysis. The activity of the enzyme being covalently bound is dependent on several factors including: shape, and size of carrier material, coupling method type, the composition.

II. NOVEL APPROACHES

Using combined approaches of Entrapment and Covalent linking various new methods are been emerging such as Nanoflowers, Metal Organic Frameworks, Carbon based Nanoflowers and frameworks, magnetic based Nanoflowers and frameworks and many more.

1. Metal Organic Frameworks

These are poros extended versions made with metal ions and organic linkers mostly covalently linked to allow enzyme to entrap within these structures. Various researchers have tried various techniques using different linkers and metal ions such as Zinc, copper, along with organic linkers such as immidazole. Iron based metal frameworks imparts additional magnetic properties. The immobilization strategies for metal organic frameworks again can be divided based on synthesis technique(Salgaonkar et al., 2019).

- Physical adsorption: The metal organic frameworks using metal ions and organic linkers are prepared and externally enzyme is immobilized onto the surface of structure by various interactions.

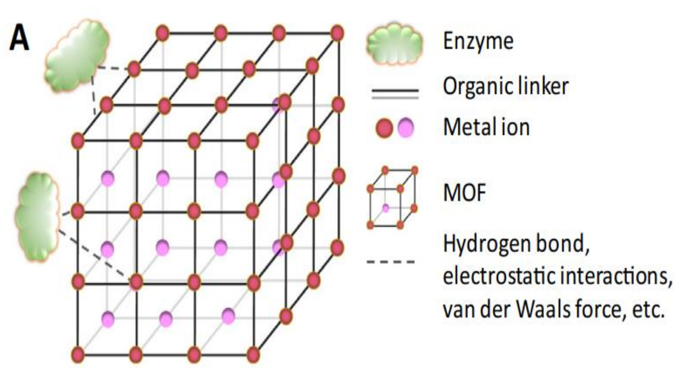

Figure 2: Enzyme Immobilization by physical adsorption

- Covalent grafting: Same as above, the structure of metal organic framework is prepared and enzyme is externally linked using a mediator chemical bond providing covalent linkage.

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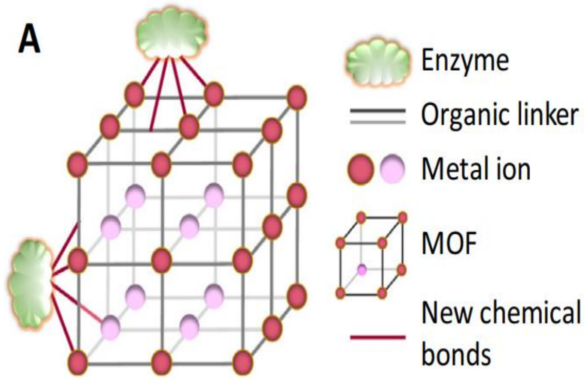

Figure 3: Enzyme immobilization by covalent grafting

- Infiltration: As above process, metal organic frameworks are prepared and enzyme are made to entrap within the cage of metal ions and organic linkers.

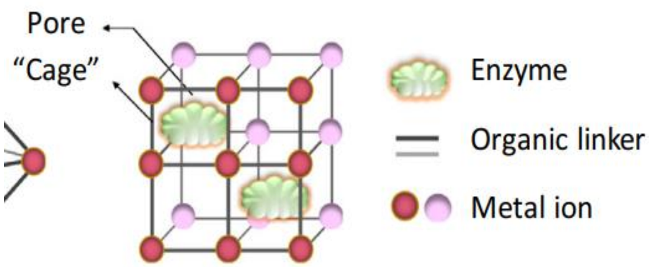

Figure 4: Enzyme immobilization by Infiltration

- One pot synthesis: The enzyme is added during the synthesis of Metal Organic Frameworks only and enzyme gets entrapped within the cage.

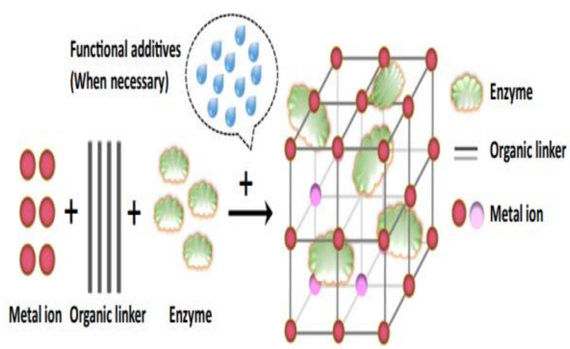

Figure 5: Enzyme Immobilization by One-pot synthesis

2. Nanoflowers

Nanoflowers are an emerging strategy for enhancing stability of enzymes within harsh reaction conditions, such as pH change, temparature change, etc. These Nanoflowers are hybrid structures of organic and inorganic particles that gain flower like structure at nanoscale. They provide high surface area, easily modifiable structures and easy functionalities. For preparation of such structures polyatomic metal ions such as copper, cobalt and zinc are used whereas, enzyme that is to be immobilized serves as organic linker. The synthesis conditions play a pivotal role in nanoflower synthesis(Fotiadou et al., 2019)(Lee et al., 2020).

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