Immobilized Enzymes: A Review on Immobilization Techniques and Impact of Enzyme Immobilization Technology in Industrial and Pharmaceutical Applications

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Abstract— ***With increased demands of world of biotechnology industries, there is need to enhance the productivity, reaction stability, reusability and shelf life of enzymes. Immobilization of enzymes is a novel technique which can answer most of the challenges facing the economical industrial application of enzymes. The immobilized enzymes are the enzymes physically confined or localized in a certain defined region of space with retention of retention of their catalytic activities which can be used repeatedly and continuously. The enzymes can be attached to the support by interactions ranging from reversible physical adsorption and ionic linkages to stable covalent bonds. The heterogeneity of immobilized enzyme systems allows and easy recovery of both enzymes and products, multiple reuse of enzymes, continuous operation of enzymatic processes, rapid termination of reaction, and greater variety of bioreactor designs. The industrial applications of immobilized enzymes are progressively increasing. The use of immobilized enzymes is now a routine process for the manufacture many industrial products in the pharmaceutical, chemical, food industries and many more sectors. One of the best uses of enzymes in modern life is their application diagnose and treatment of many diseases especially when used in drug delivery system or when used in nanoform. This review summarizes various immobilization techniques and applications of immobilized enzymes in various industries.***

Indexed Terms— ***Immobilization methods, Industrial applications, pharmaceutical applications, Nanoimmobilization.***

I. INTRODUCTION

Enzymes are biological catalysts with high selectivity and substrate specificity. Enzymes are comprised of protein component (biopolymer) and cofactors or prosthetic groups most of the times [1]. These biological catalysts speed up the chemical reaction, at which equilibrium is achieved without altering its position, and undergoing no significant chemical change in itself [2]. In recent years the application of enzymes in different industries is continuously increasing. The industrial applications of enzymes include food (baking, dairy products, starch conversion) and beverage processing (beer, wine, fruit and vegetable juices), animal feed, textiles, pulp and paper, detergents, biosensors, cosmetics, healthcare and nutrition, wastewater treatment, pharmaceuticals and chemical manufacturing and bio fuels such as biodiesel and bio-ethanol [3].

However, the applications and desired traits of enzymes are often hampered by their sensitivity to process conditions, low stability, their cumbersome recovery and reuse and tendency to be inhibited by high concentration of reaction components [4]. To make the use of enzymes in industrial and biotechnological processes more convenient different methods have been employed to reduce cost [5]. Immobilization is one of these methods. The main aim of immobilization is associating enzyme with an insoluble matrix so that it will separated easily and reused under stabilized conditions [6]. So, the immobilized enzyme is more convenient than the free one. By using immobilized enzyme, low-cost operation can be done in industrial processing.

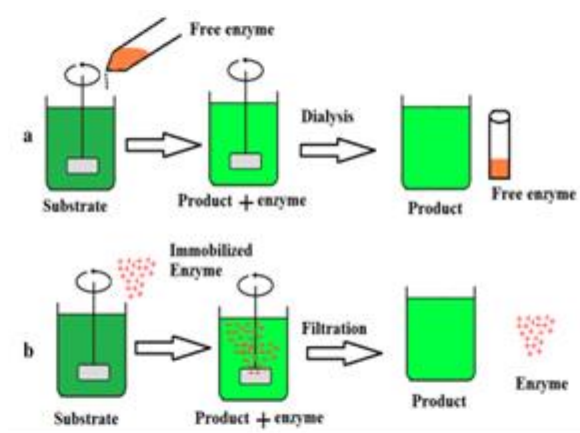

Figure 1: Schematic diagram of free and immobilized enzyme reactions: (a) Reaction of free enzyme with substrate and formation of product, which has to be separated via dialysis; (b) reaction of immobilized enzyme with substrate and formation of product, which can be separated via filtration.

II. ADVANTAGES AND DISADVANTAGES OF IMMOBILIZED ENZYMES [7]

A. Potential Advantages

- Reusability of enzyme or catalyst
- Easier product recovery
- Easier reactor operation
- Wider choice of reactors

B. Potential Disadvantages

- Loss in activity
- Reduced activity per unit volume
- Diffusion limitation
- Additional cost

III. CLASSIFICATION OF IMMOBILIZATION TECHNIQUES

There are different approaches for classification of immobilization techniques. In one approach immobilization methods are classified into two broad type: irreversible and reversible methods (Fig. 2). According to other approach immobilization methods are also classified into two broad types on the basis of type of chemical reaction used for binding: support binding and entrapment methods (Fig. 3) [8].

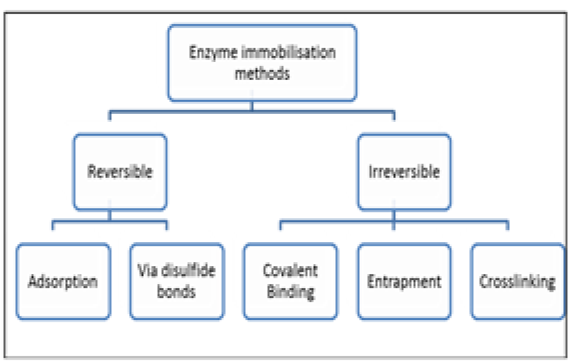

Figure 2: Classification of immobilization techniques into irreversible and reversible methods.

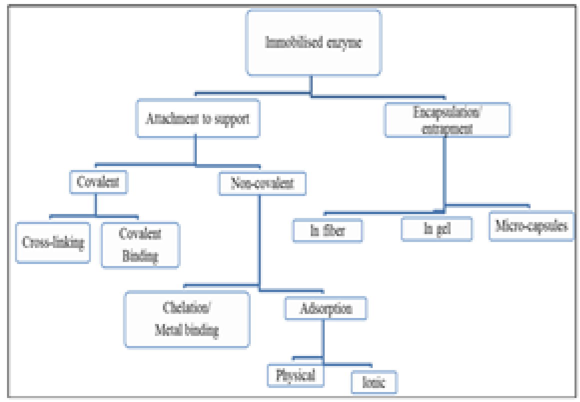

Figure 3: Classification of immobilization techniques into support binding and entrapment methods.

Different methods of immobilization methods falling under both approaches are briefly reviewed below (Fig. 4).

A. Irreversible Enzyme Immobilization Methods

In irreversible enzyme immobilization the biocatalyst bound to the support can not be detached without affecting either the biological activity of enzyme or the support. The most widely used irreversible enzyme immobilization methods are mentioned below.

(i) Immobilization via formation of covalent bonds: It is the most widely used immobilization technique. This method depends on the formation of a stable covalent bond between functional groups found in enzyme and the functional groups found on the surface of the support material such as amino group, carboxylic group, hydroxyl group and sulfhydryl group.

(ii) Cross linking: This method of immobilization involves the formation of covalent bonds between the enzyme or active molecules by means of bi- or multifunctional reagents.

(iii) Entrapment: In this method, the enzyme is physically restricted inside the network of support material. Entrapment of enzyme can improve the stability of the enzyme with no loss of activity as there is no chemical interaction between enzyme and support material.

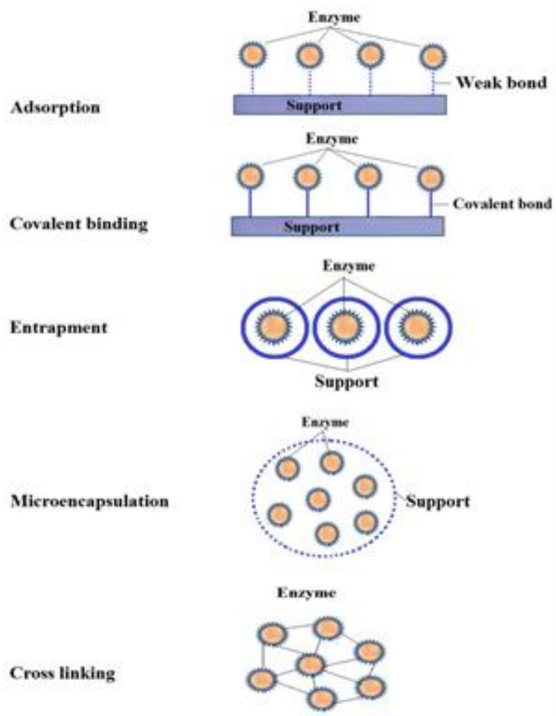

Figure 4: Immobilization methods

B. Reversible Enzyme Immobilization Methods

These methods involve weak monovalent interactions between the enzyme and the supporting material. The common methods of reversible enzyme immobilization methods are mentioned below.

(i) Adsorption: Physical adsorption method is one of the simplest immobilization methods; in this method the enzyme adsorbed on the surface of the matrix that may be an organic or inorganic matrix [9]. The enzyme is bound to bound support by means of non-covalent linkages like ionic, hydrophobic interactions, hydrogen bonding depending on the nature of amino acids available at the surface of enzyme and chemical nature of the support.

(ii) Ionic binding: Immobilization via ionic binding is based, mainly, on ionic binding of enzyme molecules of active molecule to solid support containing ionic charges. The strength of interactions in ionic binding are much stronger compared to adsorption but weaker compared to covalent binding.\

(iii) Immobilization via disulphide linkage: This method involves formation of disulphide bonds (-S-S-) between non-essential thiol (SH) group of enzyme and reactive disulphides or disulphide oxides of support material.

(iv) Affinity binding: This technique exploits specificity of enzyme to its support under different physiological conditions. It can be accomplished by two ways – either the matrix is pre-coupled to an affinity ligand for target enzyme or the enzyme is conjugated to an entity that develops affinity towards the matrix. The advantages of this method are the enzyme is not exposed to any harsh chemicals conditions, conformational changes during immobilization are minimal, and the retention of high activity by immobilized bio molecule [10].

Criterion for Choosing Immobilization Method

It is very important to choose the suitable method of immobilization to avoid deactivation of the active site found on the surface of the enzyme. Choice of a suitable method of immobilization is based on many factors such as stability, enzyme catalytic activity and also cost factor (Fig. 5).

IV. INDUSTRIAL AND PHARMACEUTICAL APPLICATIONS OF IMMOBILIZED ENZYMES AND CELLS

Continuous research is going on for the improvement enzyme activity, reproducibility efficiency and also stability during industrial processes. Enantioselective and regioselective compounds have been produced for pharmaceutical applications by immobilization technology. These applications are summarized in Fig. 6.

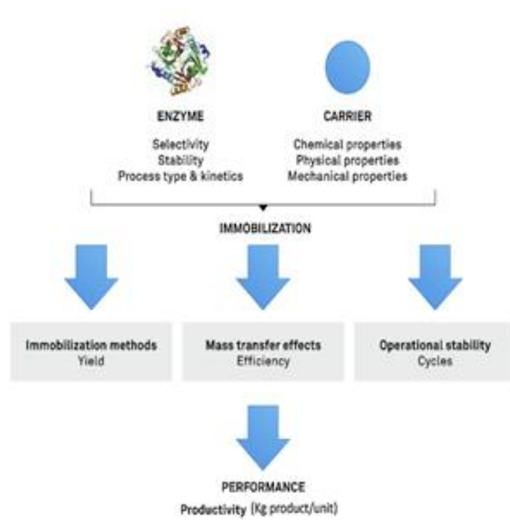

Figure 5: Factors affecting choice of enzyme immobilization.

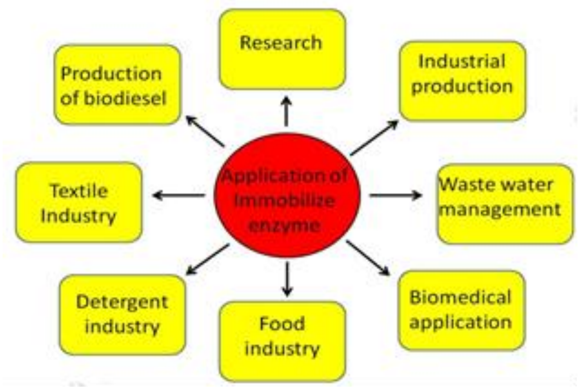

Figure 6: Applications of immobile enzymes

A. Industrial Applications of Immobilized Enzymes

Many efforts have been made by researchers to reduce the cost of products by using the immobilized enzymes in various industrial applications. Some of those applications are briefly reviewed here.

(i) Food industry: The applications of immobilized enzymes in food industry are continuously increasing [11]. The main applications of immobilized enzymes in the food industry are as follows:

- Anti-microbial packaging material of foods
- In the clarification of juices
- Production of lactose free milk (Fig. 7)
- In cheese ripening
- In wine processing
- In the production of flavor esters
- In the production of syrups/sweeteners
- In the analysis of food samples as biosensors

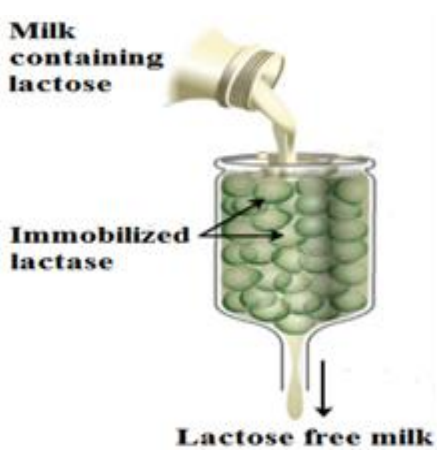

Figure 7: The immobilized enzyme converts lactose into glucose and galactose as the milk flows through.

(ii) Biomedical applications: Medical applications of immobilized enzymes have been extensively studied and developed [12]. Given below are some examples of applications of immobilized enzymes in biomedical industry:

- Immobilized enzymes in biosensors
- Enzymes in medical devices

(iii) Immobilized enzymes in biodiesel production: Biodiesel is monocrystalline esters of long chain fatty acids. It is produced through a chemical combination of alcohol with triglycerides in the presence lipase enzyme as catalyst (Fig. 8). This enzyme can catalyze the esterification reaction with fewer requirements of energy and mild reaction conditions [13]. Also, it produces fewer by-products or wastes.

(iv) Immobilized enzymes as bioremideis: Bioremediation is a technique the involves the use of enzyme and biological organisms to remove pollutants from a contaminated site. Biosorbents such as immobilized algae are used to remove heavy metals pollution from waste water [14] (Fig.9). Immobilized peroxidase on alginate-starch beads is used to remove dye from textile [15]. Immobilized peroxidase is also used for continuous palm oil mill effluent (POME) treatment.

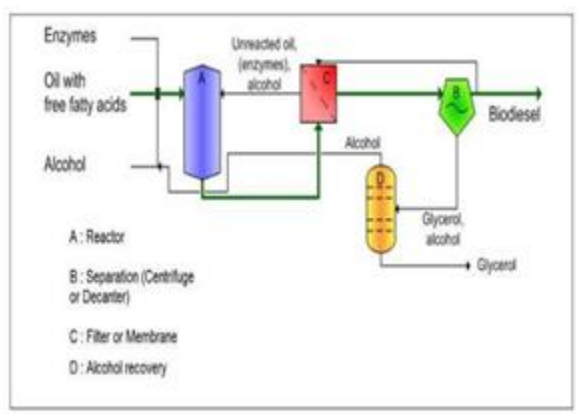

Figure 8: Flow chart showing biodiesel production

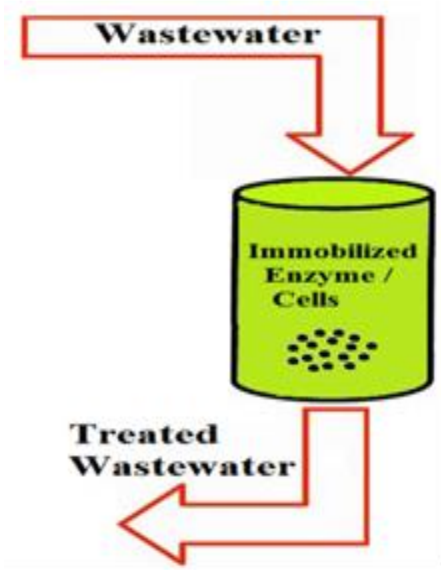

Figure 9: Waste water treatment

(v) Immobilized enzymes in textile industry: It is traditional industry in many countries that has large proportion of the economy. Microbial enzymes are of great interest in this industry. These enzymes such as amylase, cellulose, laccase, cutinase, pectinase etc. are used in various textile applications such as bio-polishing, as scouring, denim finish and wool processing design [16].

B. Pharmaceutical Applications of Immobilized Enzymes

Manufacturing or processing of enzymes to be used as a drug is an essential secondary aspect of the pharmaceutical industry today. Attempts were made to utilize the benefits of enzymes as medicines in almost all scientific centers of pharmacy in the world. Some of the examples of immobilized enzymes used in the pharmaceutical industry are listed below:

(i) Immobilized enzymes in clinical medicine: Several enzymes have been evaluated with their clinical use [17]. Immobilized streptokinase is used to treat clotting coagulation. Immobilized glucose oxidase is used to deal with diabetics.

(ii) Antibiotic production: One of the most important antibiotic group, historically and medically, is β-lactum group, especially penicillin and cephalosporin [18]. Immobilized enzymes like penicillin G acylase is used in the production of manyantibiotics.

(iii) Drug delivery systems: Many enzymes are used as part of the drug delivery systems.

(iv) Immobilized enzymes on nanoparticles: Nanoparticles materials are one of the most efficient support material used in enzyme immobilization. Nano-immobilized enzymes are used as part of the drug delivery systems to treat diseases like diabetes, cancer treatment, bones and tendons regeneration

V. CONCLUSION

Enzyme immobilization is a technology widely used in various fields and industries such as bioremediation, environmental monitoring, biotransformation, food industry, textile industry, detergent industry, pharmaceutical industry, diagnostics etc. This technology has economic and technical advantages. Vast number of enzymes have been used in the immobilized form in various processes. Immobilization of enzymes can lower the cost and also provide operational stability for the enzymes. Recently there are many methods of immobilization that are used. But unfortunately, there is no universal material or method that can be used as support material and also immobilization method may differ from enzyme to enzyme and also according to application. Future investigation should endeavor at adopting the development of enzyme analgesia and provide new prospects for the pharmaceutical and industrial sector.

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