

## Review

# Immobilization technology for enhancing bio-products industry

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Immobilization is the limitation of movement of biocatalysts according to chemical or physical treatment. Immobilized molecules technique using biomaterials and nano-biotechnology is a very interesting topic that is touching almost all aspects of our life. Immobilized enzymes, molecules, and cells have been used in a variety of scientific and industrial applications. Cell immobilization biotechnology is a multidisciplinary area shown to have an important impact on many scientific sub-disciplines, including biomedicine, pharmacology, cosmetology, food and agricultural sciences. Many molecules have been immobilized and the majority of them are bio-molecules due to their biological and biomedical applications. Immobilization of enzymes has made them highly applicable to range of evolving biotechnologies. Immobilized enzymes have proven valuable for many medical applications including drug delivery systems, diagnosis and treatment of diseases, as well as in sensors for the management of weight and diabetes. Enzyme immobilization is applied in textile industry. The immobilized microorganism technology offers a multitude of advantages in wastewater treatment. The immobilized cell systems were applied for the production of many organic compounds such as organic acid and ethanol. The immobilization of tissue sections, cells and tissue components for histological and immunohistochemical staining or detection systems is applicable nowadays. Cell immobilization could potentially benefit food industry.

**Key words:** Immobilized, bio-product, immobilization.

## INTRODUCTION

Immobilized molecules technique using biomaterials and nano-biotechnology is a very interesting topic that is touching almost all aspects of our life. It uses the sciences of biology, chemistry, physics, materials engineering and computer science to develop instruments and products that are at the cutting edge of some of today's most promising scientific frontiers. In this review article, it has been focused on some of the supports for immobilization; the most important molecules to be immobilized such as cells, enzymes, molecules, etc

and their applications in medicine, food, drug, water treatment, and agriculture field. A special section will discuss what is new in the arena of supports and technologies used in enzyme immobilization and finally a recommendation for future work with a special attention to up-to-date references.

Immobilized microbial enzymes, organelles, and cells have been used in a variety of scientific and industrial applications. The economic importance of immobilization has resulted in considerable research for industrial applications. Immobilization technology has been used extensively in commercial bioreactor fermentations (Nunez and Lema, 1987). Reviews on the use of immobilized organelles (Mattiasson, 1983) and enzymes (Mosbach, 1987) have addressed the importance of

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these respective technologies. Immobilized cells have also been used in bioreactors, and production of useful compounds such as amino acids, organic acids, antibiotics, steroids and enzymes (Brodelius and Vandamme, 1987; Tanaka and Nakajima, 1990).

Various whole cell immobilized techniques (Klein and Wagner, 1983; Woodward, 1988) and the possible many applications (Crawford and Mohn, 1985; Scott, 1987) have been examined. The commercial success of these processes illustrates the value of using immobilization technology under controlled conditions. There are many different forms of cell attachment or entrapment. These different forms include flocculation, adsorption on surfaces, covalent bonding to carriers, cross-linking of cells, encapsulation in a polymer-gel and entrapment in a matrix.

### Definition

In general the term immobilization refers to the act of the limiting movement or making incapable movement or retarding the movement. Danial et al. (2010) defined immobilization as limitation of movement of biocatalysts according to chemical or physical treatment. An immobilized molecule is one whose movement in space has been restricted either completely or to a certain limit by attachment to a solid structure (Elnashar, 2010).

### HISTORY OF IMMOBILIZATION

Immobilization is a natural phenomenon existing in the universe. Microorganisms in nature are irregularly distributed and often exist in biofilms. Biofilms are surface-attached microbial communities consisting of multiple layers of cells embedded in hydrated matrices (Kierek and Karatan, 2005). Biofilms were first extensively studied during the 1940s but it was not, until the 1970s, appreciated that their formation occurs in almost all natural environments.

### THE AIM OF IMMOBILIZATION

The aim of cell immobilization or microencapsulation technology was to treat multiple diseases in cases of immune suppression. For this purpose, animal or human cells have been immobilized experimentally within carefully designed capsules that allow the long-term function of the graft. Recently, several advances have brought the whole technology to be much closer to a realistic proposal for clinical application (Orive et al., 2006).

Current advancements in biotechnology have promoted the usage of immobilized enzymes for a wide range of applications. This increase in the number of applications

of immobilized enzymes has allowed for an even wider range of research relating to the field of enzyme immobilization.

Innovative research has recently been completed relating to the invention of improved immobilization techniques, new methods for the production or break down of desired compounds using biocatalytic reactions, methods of drug delivery and tumor identification utilizing immobilized enzymes, the use of immobilized enzymes in biosensors, as well as improved reactors used to efficiently and cost effectively carry out reactions catalyzed by these enzymes (Cynthia and Shelley, 2008). Immobilization techniques were used for one or more of the following purposes;

1. Reuse of enzyme (reducing cost).
2. Easy product separation.
3. Continuous processing.
4. Facilitated process control.
5. Low residence time.
6. Optimization of product yield.
7. Stabilization by immobilization.
8. No need for enzyme isolation and purification.
9. Multi-enzyme complex reaction.
10. Cofactor regeneration in native system.
11. Using mixed cultures.

### WHAT CAN BE IMMOBILIZED?

Immobilized microbial enzymes, molecules and cells have been used in a variety of scientific and industrial applications. The economic importance of immobilization has resulted in considerable research for industrial applications. Immobilization technology has been used extensively in commercial bioreactor fermentations (Nunez and Lema, 1987). Reviews on the use of immobilized organelles and enzymes (Mosbach, 1987) have addressed the importance of these respective technologies. Immobilized cells have also been used in bioreactors, and production of useful compounds such as amino acids, organic acids, antibiotics, steroids and enzymes (Brodelius and Vandamme, 1987; Tanaka and Nakajima, 1990).

Various whole cell techniques (Klein and Wagner, 1983) and the many possible applications (Doaa and Wafaa, 2009) have been examined. The commercial success of these processes illustrated the value of using immobilization technology under controlled conditions.

Immobilization of biocatalysts helps in their economic reuse and in the development of continuous bioprocesses. Biocatalysts can be immobilized either using the isolated enzymes, cellular organelles or the whole cells. The advantages of various methods of cell immobilization for bioreactor systems have been addressed in several reviews (Kolot, 1988; Woodward, 1988; Dervakos and Webb, 1991).

One method that has emerged as successful in the laboratory and useful in commercial applications is the encapsulation of cells in a polymer gel-matrix. Results from bioreactor studies have demonstrated that encapsulated cells have advantages over free cells under numerous conditions. Increased metabolic activity and metabolite production (Scherer et al., 1981; Gadkari, 1990), protection from toxic substances (Dwyer et al., 1986; Keweloh et al., 1989) and increased plasmid stability of encapsulated cells compared to free cells, have all been observed. Immobilization of whole cells has been shown to be a better alternative to immobilization of isolated enzymes.

### IMMOBILIZATION OF CELLS

Cell immobilization biotechnology is a multidisciplinary area shown to have an important impact on many scientific sub-disciplines, including biomedicine, pharmacology, cosmetology, food and agricultural sciences, beverage production, industrial waste treatment, and analytical applications. There are different kinds of cells that can be immobilized and applied in various areas (Doaa and Wafaa, 2009) such as: animal cells, insect cells, plant cells, algal cells, microbial cells.

#### Cell immobilization categories

Commercial and experimental cell/tissue immobilization systems normally fall into one of three categories:

1. Cells entrapment in polymer gels or porous supports.
2. Adhesion on micro carrier surface.
3. Capture behind membrane.

Sometimes the distinction between these different categories may not be very clear, depending on the particular cell immobilization system employed.

#### Requirements of an ideal cell culture support

The ideal support for immobilized cell culture should fulfill as many as possible the following requirements:

1. High cell mass-loading capacity.
2. Easy access to nutrient media.
3. Simple nontoxic immobilization procedure.
4. Optimum diffusion distance from flowing media to center of support.
5. Sterilization.
6. Mechanical stability.
7. Reusable.
8. Easy separation of cells and carrier from media.
9. Suitable for conventional reactor systems.

10. Suitable for suspension as well as anchorage dependent cells.

### IMMOBILIZATION OF MOLECULES

Many molecules have been immobilized and the majority of them are biomolecules due to their biological and biomedical applications. Proteins are examples of some of these molecules. Enzymes, antibodies, antigens, cell adhesion molecules and blocking proteins are examples of the protein molecules (Doaa and Wafaa, 2009).

### IMMOBILIZATION OF ENZYMES

Many technologies have been affected or could be affected in the near future by the immobilization of enzymes. The ability of enzymes to catalyze reactions has made them indispensable to science for decades (Ngo, 1979).

The immobilization of enzymes has proven particularly valuable, because it has allowed enzymes to be easily reused multiple times for the same reaction with longer half-lives and less degradation and has provided a straightforward method of controlling reaction rate as well as reaction start and stop time. It has also helped to prevent the contamination of the substrate with enzyme/protein or other compounds, which decreases purification costs. These benefits of immobilized enzymes have made them highly applicable to a range of evolving biotechnologies (Barabino et al., 1978).

Several methods have been used to immobilize soluble and insoluble enzymes; however every method has its advantages and drawbacks. The methods of cross linking, physical adsorption, ionic binding, metal binding, covalent binding and entrapment methods like gel entrapment, fiber entrapment, and micro encapsulation can be used for insoluble enzymes. Ultra filtration membranes and hollow fiber devices are used for soluble enzymes.

The major components of an immobilized enzyme are:

1. The enzyme,
2. The matrix,
3. Mode of interaction of the enzyme with the carrier.

A matrix chosen can enhance the operational stability of the immobilized enzyme system. Although it is recognized that there is no universal carrier, there are numbers of characteristics which should be common to any carrier material considered for immobilization (Doaa and Wafaa, 2009).

### METHODS OF IMMOBILIZATION

Enzymes can be immobilized by different techniques, but

they are categorized as follows (Figure 2):

1. Adsorption onto an inert carrier.
2. Entrapment within the lattice of a polymerized gel (synthetic and non-synthetic)
3. Cross linking of the protein.
4. Covalent bonding to a reactive insoluble support.
5. Ion-exchange.
6. Copolymerization.

### Adsorption onto an inert carrier

Physical adsorption of an enzyme onto a solid support is probably the simplest way of preparing immobilized enzymes. The method relies on non-specific physical interaction between the enzyme protein and the surface of the matrix brought about by mixing a concentrated solution of enzyme with the solid. Among the advantages of adsorption as a general method of immobilization of insoluble enzyme is that no reagents are needed and only minimum steps of activation are required. Therefore, adsorption is cheap, easily carried out, and tends to be less disruptive to the enzyme than using chemical means of immobilization.

Immobilization by adsorption is the simplest method and involves reversible surface interactions between enzyme and support material as shown in Figure 3. The binding occurs mainly by hydrogen bonds, multiple salt linkages and Van der Waal's force. In this respect, the method bears the greatest similarity to the situation found in biological membrane *in vivo* and has been used to model such systems (Ciaron, 2003). Because of the weak bonds formation among enzyme and the carrier, the enzyme can be leaked out due to changes in temperatures, pH, ionic strength or even the mere presence of substrate (Dariush, 2003).

Disadvantage of this method is that it is non-specific. Further, adsorption of other proteins or other substances as the immobilized enzyme may occur. This may alter the properties of the immobilized enzyme or, if the substance adsorbed is a substrate for the enzyme, the rate will probably decrease, depending on the surface mobility of enzyme and substrate. Stabilization of enzymes temporarily adsorbed onto a matrix has been achieved by cross linking the protein in a chemical reaction subsequent to its physical adsorption (Sankaran et al., 1989). Since the enzyme is in a stable particulate form, it can easily be separated from the reaction mixture and reused.

### Entrapment within the lattice of a polymerized gel (synthetic/non-synthetic)

Immobilization by entrapment differs from adsorption and covalent binding in that enzyme molecules are free in

solution, but restricted in movement by the lattice structure of a gel (Figure 4).

Confining enzymes within the lattices of polymerized gels is another method for immobilization (Elnashar et al., 2008). This allows the free diffusion of low molecular weight substrates and reaction products. The usual method is to polymerize the hydrophilic matrix in an aqueous solution of the enzyme and break up the polymeric mass to specific particle size as there is no bond formation between the enzyme and polymer matrix. However, free radical generated on the course of the polymerization may affect the activity of entrapped enzymes. Another disadvantage is that only low molecular weight substrates can diffuse rapidly in the enzyme rendering the method unsuitable for enzymes that act on macromolecular substrates such as ribonuclease, trypsin and dextranase (Sankaran et al., 1989).

### Cross-linking of the protein

Immobilization of enzymes has been achieved by intermolecular cross-linking of the protein, either to other protein molecules/polymerized gel (synthetic and non-synthetic) or to functional groups on an insoluble support intra matrix. This type of immobilization is support-free, as shown in Figure 5 and involves joining enzyme molecules to each other to form a large, three-dimensional complex structure, and can be achieved by chemical or physical methods.

Cross-linking of enzyme to itself has also been achieved. In this case, the protein material will invariably be acting as a support. Furthermore, an artificial cross link can be formed within a polypeptide chain (an intra molecular crosslink). Internal cross-link can stabilize the protein by preventing unfolding under stress conditions. It is possible to link two polypeptides via an intermolecular cross-link (Ciaron, 2003). A particular form of this involves the cross-linking of small enzyme crystals with the bifunctional reagent. Since the enzyme is in a stable particulate form, it can easily be separated from the reaction mixture and reused (Dariush, 2003).

### Covalent bonding to a reactive insoluble support

The immobilization of enzymes on solid supports by covalent coupling usually leads to very stable preparations with extended active life when compared with immobilized enzyme preparations obtained with other coupling methods. The advantage of enzyme immobilization by covalent bonding is based on strong covalent bonding, so that the stable immobilized enzyme preparations have been obtained, which do not lose protein in the solution, even in the presence of high ionic strength solution.

The disadvantages include; selection of conditions for immobilization by covalent binding is more difficult than in other carrier binding methods. The reaction conditions required are somewhat complicated. To achieve higher activities in the resulting immobilized enzyme preparations, by preventing inactivation reactions with the essential amino acid residues of the active site, several attempts have been made: (1) covalent attachment of the enzymes in the presence of a competitive inhibitor or substrate, (2) a reversible covalently linked enzyme inhibitor complex, (3) a chemically modified soluble enzyme whose covalent linkages to the matrix is achieved by newly incorporated residues, and (4) a zymogen precursor. This method of immobilization involves formation of a covalent bond between the enzyme and support material as shown in Figure 6.

The main factors taken into account for covalent immobilization of enzymes are that;

1. The functional group of proteins is suitable for covalent binding under mild conditions (Taylor, 1991). The reactive residues of enzymes involved in covalent immobilization, are shown in Table 1.
2. The coupling reactions between the proteins and the supports. The variable characteristics of proteins supports are shown in Table 2.
3. The functionalized supports suitable for protein immobilization are presented in Figure 1.
4. The coupling of protein molecules to solid supports involved mild reactions between amino acid residues of the protein and several groups of functionalized carriers. The major classes of coupling reactions used for the immobilization of proteins are: diazotization, amido (peptide) bond formation, alkylation and arylation, Schiff's base formation, Ugi reaction, amidation, thiol disulfide interchange reactions, mercury enzyme interaction and  $\gamma$ - irradiation for immobilization of proteins by covalent bonding.

It is most important to choose a method of attachment aimed to reactive groups outside the active catalytic and binding site of that enzyme. Considerable knowledge of active sites of particular enzymes will enable methods to be chosen that would avoid reaction with the essential group therein. Alternatively, these active sites can be protected during attachment as long as the protective groups can be removed without loss of enzyme activity. In some cases, this protective function can be fulfilled by a substrate of the enzyme or a competitive inhibitor; this also contributes towards retention of tertiary structure of the enzyme (Norouzian et al., 2003).

The surface on which the enzyme is immobilized has several vital roles to play such as retaining of tertiary structure in the enzyme by hydrogen bonding or the formation of electron transition of tertiary structure in the enzyme or the formation of electron transition complexes. Retention of tertiary structure may also be a vital factor.

in maximizing thermal stability in the immobilized state. In this respect, it is wise to follow closely the new findings in the chemical nature of soluble thermo stable enzyme. The microenvironment of surface and the immobilized enzyme has an anionic or cationic nature of the surface that can cause a displacement at the optimum pH of the enzyme up to 2 pH units. This may be accompanied by a general broadening of the pH region in which the enzyme can work effectively (Norouzian et al., 2003).

Immobilization by cross-linking the protein enzyme in order to insolublize it or merely immobilize it in the desired location has many possibilities and is relatively cheap. Several aldehydes and other cross-linking agents are now available for this purpose (Taylor, 1991).

### **Ion-exchange**

An ion-exchanger is an insoluble material containing chemically bound charged groups and mobile counter-ions. The counter-ions can be reversibly exchanged with other ions of the same charge without any changes in the insoluble matrix.

Proteins and thus enzymes can carry a charge depending on the pH and type of protein. This property was utilized by Tosa et al. (1967) to link enzymes with ion-exchangers via electrostatic interactions. Although changes in pH and ionic strength can cause leaking of the enzymes, a judicious set of operating conditions can overcome this problem. If the enzyme is denatured after repetitive use, the process of removing the old batch of enzyme and recharging the matrix with a fresh batch can be a relative simple process.

### **Copolymerization**

Polymerization is a process of changing the molecular arrangement so as to form new compounds having the same percentage elemental composition as the original compound but of greater molecular weight and different properties. Enzymes have been copolymerized with a polymer of the matrix by Levin et al. (1964). The enzyme that is participated chemically in the formation of the copolymer is thus immobilized. The difference between entrapment and copolymerization is that in the former process, the enzyme is physically confined, whereas, in the later, the enzyme participates chemically in the formation of the polymer.

### **CARRIERS FOR IMMOBILIZATION**

A large number of polymers have been used to aid or to affect cell immobilization. Such polymers may be used alone as the cell support, in a beads or cube, membranes, fibres, or a larger scale 'native matrix' form, or in

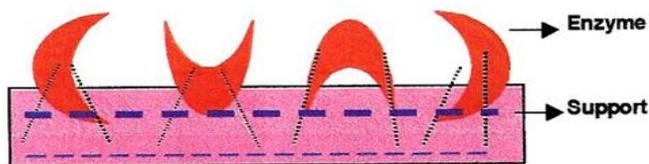
Nucleophilic group of support	Activating reagent	Active derivative
$\begin{array}{ c} \text{OH} \\ \text{OH} \end{array}$	CNBr	
$\begin{array}{ c} \text{OH} \\ \text{OH} \end{array}$	$\text{ClO}_2\text{C}_2\text{H}_5$	
$\begin{array}{ c} \text{OH} \end{array}$		
$\begin{array}{ c} \text{OH} \\ \text{OH} \end{array}$	$\text{IO}_4^-$	$\begin{array}{ c} \text{COH} \\ \text{COH} \end{array}$
$\begin{array}{ c} \text{NH}_2 \end{array}$	$\text{Cl}_2\text{CS}$	$\begin{array}{ c} \text{NCS} \end{array}$
	$\text{Cl}_2\text{CS}$	
	$\text{Cl}_2\text{CO}$	
$\begin{array}{ c} \text{CONHNH}_2 \end{array}$	$\text{NaNO}_2$	$\begin{array}{ c} \text{CON}_3 \end{array}$
$\begin{array}{ c} \text{COOH} \end{array}$	$\text{R-N}^+\text{C}^+\text{N-R}'^+$ , $\text{H}^+$	$\begin{array}{ c} \text{COOH} \end{array}$
$\begin{array}{ c} \text{COOH} \end{array}$	$\text{CH}_3\text{OH}$ , $\text{NH}_2\text{NH}_2$ , $\text{HNO}_2$	$\begin{array}{ c} \text{CON}_3 \end{array}$

Figure 1. Functionalized supports suitable for protein immobilization.

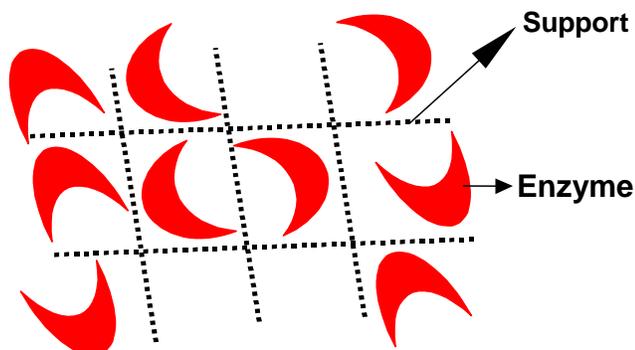
combination with another support material, to increase the overall strength or possible biomass loading of a support (Figure 6).

Inorganic supports generally have one major advantage over other materials, namely, their toughness. Most

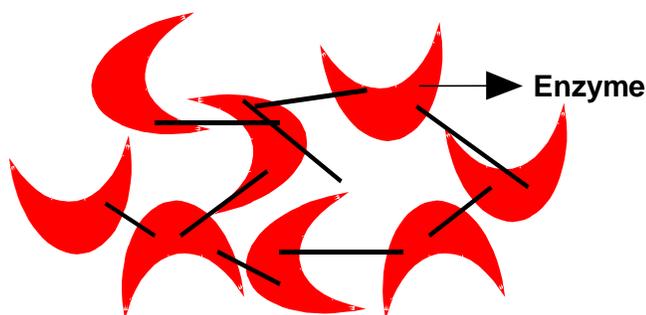
inorganic supports are totally inert, immune to temperature, pH, chemicals, microbial degradation, and highly resistant to crushing or abrasion. Given that they do not normally have to be produced by the end-user and may well be naturally occurring (for example, sand used



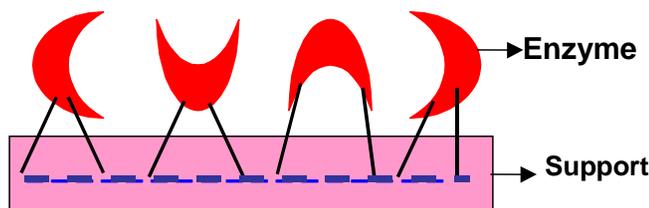
**Figure 2.** Immobilization of enzymes using the adsorption technique.



**Figure 3.** Immobilization of enzyme using the entrapment technique.



**Figure 4.** Cross linking technique.



**Figure 5.** Immobilization of enzymes using the covalent technique.

in methanogenic fluidized beds) (Latme et al., 1998), the inorganic supports also lend themselves more

conveniently to scale-up. Sintered and porous glass and ceramics have been used in immobilized cell cutler (Lin et al., 1990; Looby and Grtrnhs, 1990).

### Properties of carrier for immobilization

The choice of a carrier is dependent upon certain factors which play a vital role in industrial processing applications. According to Messing (1975), these factors include mechanical strength, microbial resistance, thermal stability, chemical durability, chemical functionality, low cost, hydrophilicity, regeneration and high capacity for enzyme. While all of these factors do not occur in any known single matrix, the selection of a carrier should be based on an optimization of these considerations. Two commonly used supports are agarose and glass beads, and neither meets all of the previous requirements. Agarose, for example, is compressible, expensive, and susceptible to microbial attack.

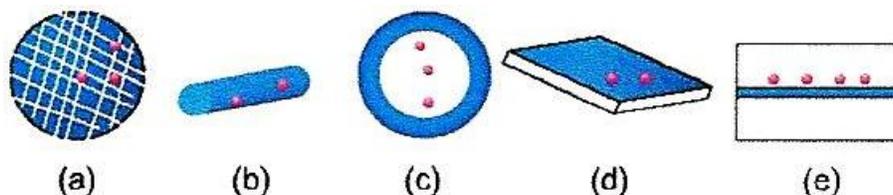
Fluidized bed reactors may overcome the problem of compressibility, and the intermittent flushing of reactors with antibacterial agents may limit the hazards of microbial growth. Porous glass, on the other hand, may have many good qualities listed, but its cost for high-volume use can be prohibitive. Derivatives of phenol formaldehyde resins and conjugates of various polymers with silica beads have been discussed as alternates to "ideal" supports (Elnashar, 2005; Denial et al., 2010).

### TRADITIONAL APPLICATION FOR ENHANCING IMMOBILIZATION

A vast number of methods of immobilization are currently available. Thus, the major problem in enzyme immobilization is not how to immobilize enzymes, but how to design the performance of the immobilized enzyme as well. Unfortunately, the approaches currently used to design robust industrial immobilized enzymes are, without exception, labeled as "irrational", because they often result from screening of several immobilized enzymes and are not designed. As a result, many industrial processes might be operating under suboptimum conditions because of a lack of robust immobilized enzymes.

Another difficulty in rational design is that the comparability of different methods of immobilization is often very poor, mainly owing to inconsistency in the enzymes used (source, purity, contamination), the immobilization conditions (time, pH, additives, ionic strength), the assay (substrate, concentration, temperatures), the preconditioning of the carrier and the post-treatment of catalysts.

There is, however, no doubt that many are not ideal catalysts for industrial applications, for example in the manufacture of fine chemicals (Bommarius et al., 1998) and pharmaceuticals and their intermediates (Liese and



**Figure 6.** Different shapes and types of enzyme carriers (a) bead, (b) fiber, (c) capsule, (d) film, and (e) membrane.

**Table 1.** Reactive residues of proteins.

Reaction	Support – enzyme linkage
Diazotization	Support--N=N---enzyme
Alkylation and arylation	Support--CH <sub>2</sub> -NH---enzyme
	Support--CH <sub>2</sub> -S---enzyme
Schiff's base formation	Support---CH=N---enzyme
Amide bond formation	Support---CO-NH---enzyme
Amidation reaction	Support---CNH-NH---enzyme
Thiol-disulfide interchange	Support---S-S---enzyme
Carrier binding with bifunctional reagents	Support---O(CH <sub>2</sub> ) <sub>2</sub> N=CH(CH <sub>2</sub> ) <sub>3</sub> CH=N---enzyme

**Table 2.** Characteristics of the proteins supports.

S/N	Characteristics of the proteins support
1	Large surface area
2	Permeability
3	Hydrophilic/hydrophobic characters
4	Insolubility
5	Chemical, mechanical and thermal stability
6	High rigidity
7	Suitable shape and particle size
8	Resistance to microbial attack
9	Re-generability

Filho, 1999). The enzymes are usually exposed to non-natural conditions such as high substrate concentrations, high pH, high temperature and the use of deleterious organic solvents. Accordingly, for most industrial applications, they must be modified either by genetic engineering or by chemical modification, with the objective of improving their selectivity, activity and durability under the process conditions. They must, furthermore, be used in the immobilized forms, to reduce production cost by facilitating downstream processing such as recycling and separation (Schulze and Wubbolts, 1999).

Although it has been increasingly appreciated that genetic engineering is a powerful tool for improvement of enzyme performance, enzyme immobilization is the only

technique, which can combine immobilization of an enzyme with improvement of enzyme performance, for example stability, selectivity and activity (Clark, 1994). Thus, immobilization-improvement strategies might be very attractive for enzymes designed to be used in the immobilized form anyway. In this sense, it is also increasingly recognized that rational immobilization of enzymes by combining immobilization and genetic engineering might be an alternative and complimentary technique for protein engineering. Many examples have excitingly demonstrated that even for genetically engineered enzymes performance can be further improved by immobilization techniques and many examples have revealed that enzyme-immobilization techniques are indeed an indispensable complimentary tool in enzyme engineering, due to its potential for:

1. Combination of immobilization and improvement,
2. Modulation of enzyme performance by selecting appropriate method of immobilization, and
3. Combination of different immobilization methods.

More so, information becomes available about the relationship between the performance of the immobilized enzyme and the method selected. Design of more robust immobilized enzymes at will, via the use of different immobilization techniques, might be a reality in the near future. It is currently possible to draw the conclusion that immobilized enzymes might perform better than the native enzymes (improved stability, activity and selectivity) if the method is correctly selected (Rocchietti

et al., 2002).

As already noted, the problem of enzyme immobilization is not how to immobilize the enzyme but how to achieve the desired performance for a given application by selecting an appropriate means of immobilization. Thus, it is also important to distinguish the two concepts modification and immobilization. Although enzyme immobilization and improvement of enzyme performance by immobilization share the same principles, the emphasis is different. The former is mainly associated with efforts to find suitable immobilization methods for enzymes that must be immobilized for certain applications.

Thus, the immobilization technique developed is mainly intended to retain the major catalytic functions of the native enzymes. In contrast, improvement by immobilization is focused mainly on utilization of available immobilization techniques to alter (or improve) enzyme performance, to suit the desired application. Thus, the native enzyme might not be suitable for a desired process, because of its poor performance such as lower activity, or stability or selectivity. Consequently, the technique to be developed should improve the performance of the enzyme besides immobilizing it. Based on the fact that the success of the later largely depends on knowledge acquired from experimental information from the former application, it is recognized to be essential to provide detailed analysis of the results so far obtained from improvement, by immobilization, of three catalytic characteristics, that is activity, stability and selectivity, of the enzymes.

### Application for enhancing bio-product industry

Current advancements in biotechnology have promoted the usage of immobilized enzymes for a wide range of applications. This increase in the number of applications of immobilized enzymes has allowed for an even wider range of research relating to the field of enzyme immobilization. Innovative research has recently been completed relating to the invention of improved immobilization techniques, new methods for the production or break down of desired compounds using biocatalytic reactions, methods of drug delivery and tumor identification utilizing immobilized enzymes. The use of immobilized enzymes in biosensors improved the reactors used to efficiently and cost-effectively carry out the reactions catalyzed by these enzymes.

### Application in medicine

Medicine has benefited from enzyme immobilization. Immobilized enzymes have proven valuable for many applications in the field including drug delivery systems, diagnosis and treatment of diseases, as well as in sensors for the management of weight and diabetes (Allen, 1998).

1. Drug delivery systems: In drug delivery system, it is possible to release drug according the nature of disease in response to a chemical signal. Insulin released in response to rising glucose concentration can be achieved by using this system (Soetan et al., 2010).

2. Diagnosis and treatment of diseases: Many immobilized enzyme are used for diagnosis and treatment of diseases. Examples for diagnosis of diseases are listed in Table 3. Lactose intolerance is an example of diseases that can be treated using an immobilized enzyme (Richmond et al., 1981). The consumption of foods with a high content of lactose is causing a medical problem for lactose intolerance people. Since the naturally present enzyme ( $\beta$ -galactosidase) in the human intestine, loses its activity during lifetime, thus hydrolysis of lactose to glucose and galactose is largely affected. Elnashar and Yassin (2009) obtained the immobilized enzyme  $\beta$ -galactosidase on thermo stable biopolymers of grafted carrageenan. Whereas, Kettering et al. (2009) used magnetic iron nanoparticles with cisplatin adsorbed on them for drug release in magnetic heating treatments for cancer.

3. Sensors: Analytic sensors are an extremely popular application of enzyme immobilization at the current time. Immobilized enzymes are used as biocatalysts to convert previously electrochemically unreactive compounds into compounds producing an electrical signal which is in turn calibrated to certain scale (Kettering et al., 2009). The most popular biosensors include those to help monitor glucose levels for diabetics (Whiteley et al., 2003).

### Application in organic compound production

The application of biocatalysts can suffer from several general drawbacks. In addition to the often relatively high cost compared to current chemical catalysts, enzymes may display instability towards temperature, pH, solvents, oxidation and shear resulting in limited suitability or shelf life. Moreover, soluble enzymes cannot be easily recovered from aqueous media and hence often cannot be reused. Thus, although enzymes such as lipases in particular have found extensive application in organic synthesis (Reetz and Jaeger, 1998), effective stabilization remains a technical hurdle limiting use of enzymes such as *Pseudomonas* lipases in industrial-scale bio transformations (Cao, 2005). However, conventional support-based immobilization techniques involve the use of costly resins, and are usually associated with a large loss in enzyme activity compared to the native enzyme, as well as a reduction in the specific and volumetric activity of the biocatalyst to approximately 10% due to the limited loading capacity of these supports (Domínguez et al., 2006).

An alternative to immobilization onto supports is self-immobilization through cross-linking of the enzyme. This negates the need for an immobilization matrix, thereby reducing the cost, and increasing specific and volumetric

**Table 3.** The relation between enzymes and diseases.

<b>Enzyme</b>	<b>Disease</b>
Heparinase	Extracorporeal therapy
Urate oxidase	Hyperuricemia
Xanthine oxidase	LESCH–Nyhan disease
Glucose-6-phosphate dehydrogenase	Glucose-6-phosphate dehydrogenase deficiency
Glucoamylase	Glycogen storage disease
Carbonate dehydratase and catalase	Artificial lungs
Glucose oxidase	Artificial pancreas
Urease	Artificial kidney, uraemic disorders
Arginase	Cancer
Asparaginase	Leukemia

activity. To overcome some of these problems, enzymes are frequently immobilized, primarily by attachment onto supports. Immobilization has several advantages including effective recovery and recycles through centrifugation or filtration and improved enzyme stability (Mateo et al., 2007).

### **Application in laboratory techniques**

#### ***Tissue immobilization staining***

Staining or detection protocols frequently include numerous steps which can involve the use of acids, bases, detergents, hydrolytic agents or combinations of such agents. Before now, a significant percentage of tissue sections or other samples have been lost from glass slides at some step in the rather lengthy procedures used in diagnostic/analytical staining, resulting in frustration and delay. Therefore, the immobilization of tissue sections, cells and tissue components for histological and immunohistochemical staining or detection systems is applicable nowadays. This process takes place by preparations. It is useful to use suitable adhesive factors to promote attachment efficiency, rate and/or strength of adhesion (Mansour et al., 2007).

#### ***Enzyme-linked immunosorbent system assays (ELISA)***

ELISA is a test used as a general screening tool for the detection of antibodies or antigens in a sample (Farre et al., 2007). ELISA technology links a measurable enzyme to either an antigen or antibody. However, ELISA technique in some cases is regarded as time consuming and it needs special equipment to run the assay (not portable). Thus many techniques have been developed to fasten the process such as that of Xin et al. (2009), where they developed a chemiluminescence enzyme immune assay using magnetic particles to monitor 17  $\beta$ -

estradiol (E2) in environmental water samples. Another technique is using simple/rapid (S/R) test. The development of S/R tests has been extended from pregnancy detection of HIV antibodies in whole blood in addition to serum and plasma (WHO, 2002).

#### ***Cell culturing system***

The harvest of cells from tissue for maintenance and propagation *in vitro* by tissue culture is a major tool in medical and biochemical research. Tissue culture is the technique or process of propagating and/or supporting the metabolism of tissues or cells derived from organisms (plant or animal) in a formulated nutritive environment. Once isolated by gentle tissue dissociation, cells are incubated in nutritive media capable of supporting life functions. With few exceptions, cells require attachment to a substrate in order to perform normal metabolic functions, grow and divide. In tissue, the substrate which provides the matrix for cell growth consists of collagen, laminin, and fibronectin. *In vitro*, the substrate is most often plastic, although glass and microporous cellulosic filters are sometimes used as substitutes (Doaa and Wafaa, 2009).

### **RECOMMENDATION FOR FUTURE IMMOBILIZATION TECHNOLOGY**

At present, a vast number of methods of immobilization are currently available. Unfortunately, there is no universal enzyme support, that is, the best method of immobilization might differ from enzyme to enzyme, from application to application and from carrier to carrier. Accordingly, the approaches currently used to design robust industrial immobilized enzymes are, without exception, labeled as “irrational”, because they often result from screening of several immobilized enzymes and are not designed. As a consequence, some of the industrial enzymes are working below their optimum

conditions. Cao (2005) in his book "Carrier bound immobilized enzymes" tackled this problem as he suggested that the major problem in enzyme immobilization is not only the selection of the right carrier for the enzyme immobilization but it is how to design the performance of the immobilized enzyme.

Inventions have recently been patented in disciplines as different as cancer treatment and materials engineering, but still relating to immobilized enzymes for use in biotechnology. This demonstration provides evidence that increasing research efforts will continue in the field of enzyme immobilization for biotechnology. As methods for the immobilization of enzymes continue to improve and become commercially widespread, the availability of immobilized enzymes to industry has the opportunity to increase significantly. This increase in the availability of immobilized enzymes would allow for a growth in the application of immobilized enzymes throughout the chemical and medical fields. Continuing research on drug delivery and tumor location analysis, along with the use of immobilized enzymes in biosensors, makes an escalating use of immobilized enzymes in medicine inevitable.

Finally, the ongoing development of bioreactors employing immobilized enzymes continues to improve the efficiency of reactions utilizing immobilized biocatalysts. These improvements will likely result in a growth in the application of immobilized enzymes to new fields increasingly realistic.

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