Applied and Industrial biochemistry

PHY-403

UNIT-37 (MODULE-IV)

4TH SEM STUDY MATERIAL-II

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IMMOBILIZATION OF ENZYMES(BIOCATALYSTS)

Immobilization is one of the most effective and powerful tools used in industry, which has been studied and improved since the last century. The term immobilized enzymes refers to "***enzymes physically confined or localized in a certain defined region of space with retention of their catalytic activities, and which can be used repeatedly and continuously.***" In almost every industry, the term productivity has a vital significance for every single step of the entire process carried out to produce a specific product. The importance of the productivity increases due to the restriction of production time and final product volume since both can alter total cost of the whole process. Every industrial bioprocess is catalysed by a specific biocatalyst. Therefore, the recovery of these molecules during downstream processes and improving operational stabilities is highly crucial and of high cost. Improving recovery and operational stabilities will result in reduced cost and higher overall bioprocess efficiency.

- Immobilization systems allow us to overcome most of the process restrictions, improve recovery of biocatalysts
  - for reuse,
  - offer better stability,
  - activity, and selectivity of the molecules,
  - higher resistance against inhibition,
  - help the elimination of unnecessary separation and purification steps, and
  - consequently produce desired products with more efficiency.

- **Immobilization**, by definition, is the term that expresses of making something immobile or fixed. In the first Enzyme Engineering Conference that was held at Henniker, NH, USA, in 1971, it was defined that immobilized biocatalysts, enzymes, or cells are physically fixed in a defined region in order to catalyze a specific reaction with no loss of catalytic activity and with repeated use (Katchalski-Katzir and Kraemer 2000). Immobilization can also be defined as a key to optimization of the operational performance of biocatalysts in industrial processes (Sheldon 2007).

WHY TO IMMOBILIZE ENZYMES?

- To enhance stability
- To protect from degradation and deactivation
- To stop the reaction rapidly by removing the enzyme from the reaction solution
- To easily separate the enzyme from the new product and reactants
- To reduce the contamination
- To re-use the enzymes for many reaction cycles
- To lower the total production and cost of enzyme mediated reactions.

Major products obtained using immobilized enzymes

Enzyme	Product
Glucose isomerase	High-fructose corn syrup
Amino acid acylase	Amino acid production
Penicillin acylase	Semi-synthetic penicillins
Nitrile hydratase	Acrylamide
β-Galactosidase	Hydrolyzed lactose (whey)

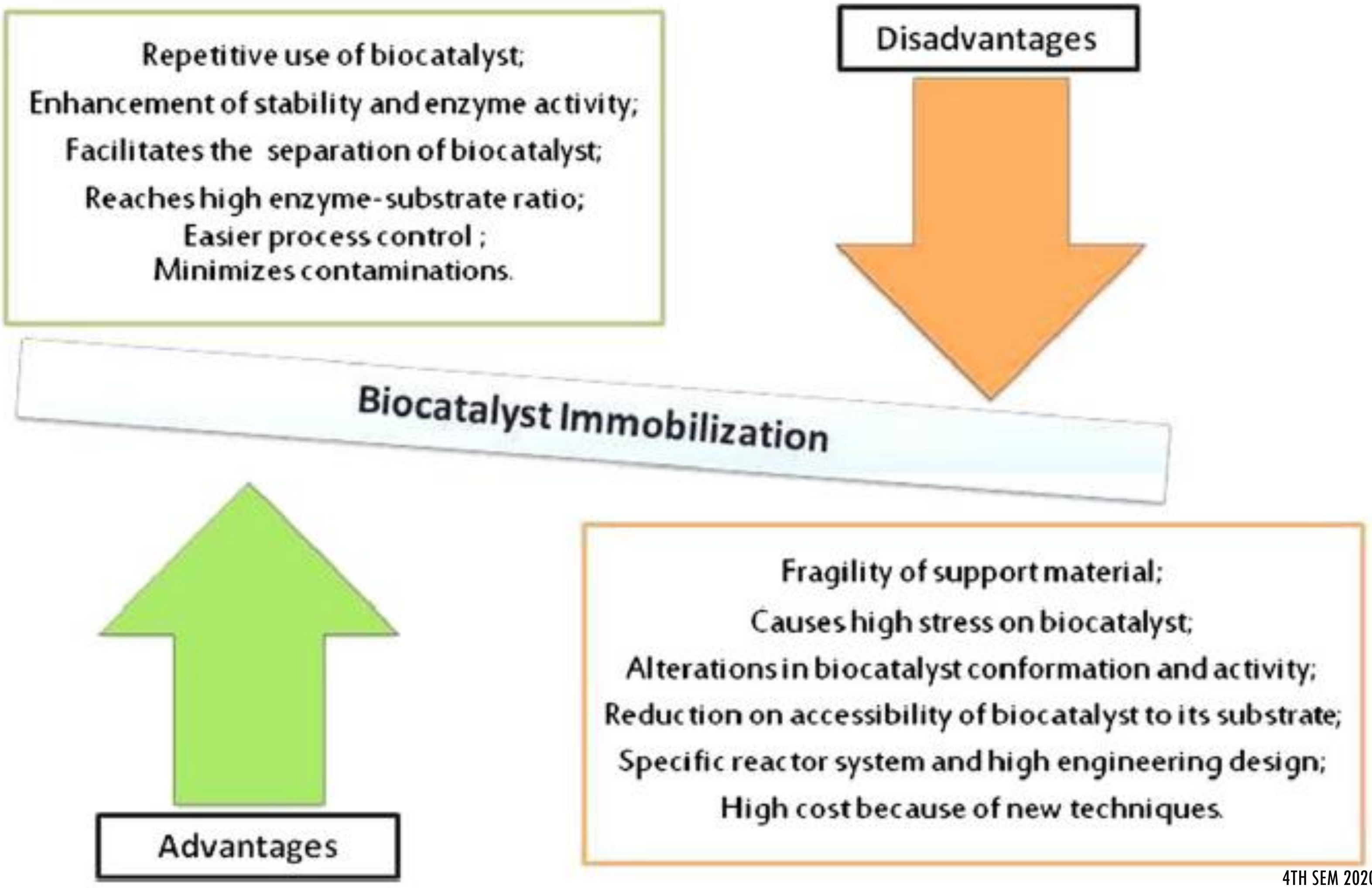
Technological properties of immobilized enzyme systems

Advantages	Disadvantages
Catalyst reuse	Loss or reduction in activity
Easier reactor operation	Diffusional limitation
Easier product separation	Additional cost
Wider choice of reactor	

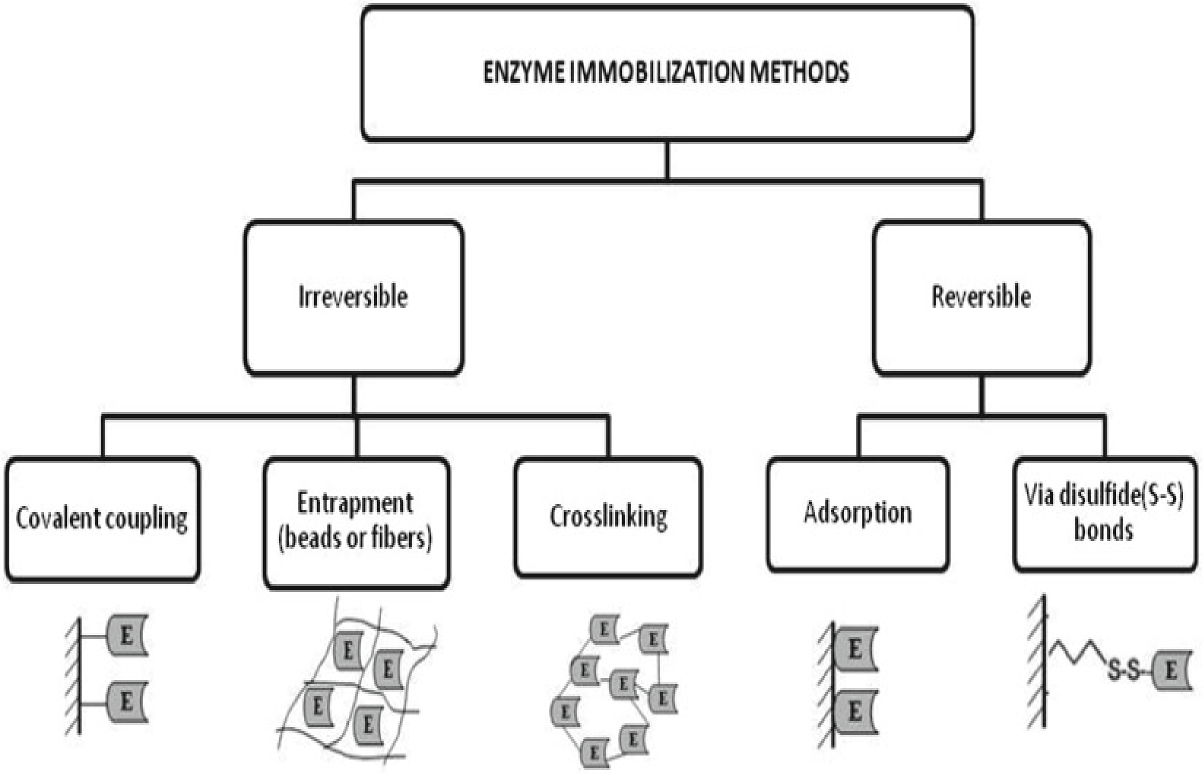
Classification of supports

Organic
Natural polymers
• Polysaccharides: cellulose, dextrans, agar, agarose, chitin, alginate
• Proteins: collagen, albumin
• Carbon
Synthetic polymers
• Polystyrene
• Other polymers: polyacrylate, polymethacrylates, polyacrylamide, polyamides, vinyl and allyl-polymers
Inorganic
Natural minerals
Bentonite, silica
Processed materials
Glass (non-porous and controlled pore), metals, controlled pore metal oxides

COMPARISON OF ADVANTAGES AND DISADVANTAGES OF BIOCATALYST IMMOBILIZATION:



METHODS



Advantages and disadvantages of the main enzyme immobilization methods

Methods and binding nature	Advantages	Disadvantages
Physical adsorption		
Weak bonds: hydrophobic, Van der Waals or ionic interactions.	Simple and cheap Little conformational change of the enzyme	Desorption Nonspecific adsorption
Affinity		
Affinity bonds between two affinity partners	Simple and oriented immobilization Remarkable selectivity	High cost
Covalent binding		
Chemical binding between functional groups of the enzyme and support	No enzyme leakage Potential for enzyme stabilization	Matrix and enzyme are not regenerable Major loss of activity
Entrapment		
Occlusion of an enzyme within a polymeric network	Wide applicability	Mass transfer limitations Enzyme leakage
Cross-linking		
Enzymes molecules are cross-linked by a functional reactant	Biocatalyst stabilization	Cross-linked biocatalysts are less useful for packed beds. Mass transfer limitations Loss of activity

✓ Each production requires a specific operational condition since process pathway varies for each product. Hence, selecting proper immobilization technique becomes more important for overall process yield. Additionally, when industrial scale is in the question, choosing the right immobilization technique saves time and money to industry. Immobilization techniques improve enzyme properties by increasing stabilization and rigidity of 3D structure, causing chemical modification, generating hyper-hydrophilic microenvironments and reducing enzyme inhibitions.

❑ **Physical adsorption ---**

- It occurs by dipole-dipole and hydrophobic interactions, van der Waals forces, or hydrogen bonding, and one of these interactions is used for immobilization depending on the natural properties of the substrate surface and the adsorbate
- Physical adsorption is the one of the easiest techniques used for immobilization in industry. Physical adsorption is a simple and less expensive technique, which retains high catalytic activity; therefore, it is used more commonly than other methods.
- This technique also allows the reuse of expensive support materials; as a result, this reusability of the support material brings economic advantages for industrial productions.
- On the other hand, this technique does not offer high stability and might cause loss of biomolecules that is immobilized during washing and operation.

❑ **Encapsulation and entrapment----**

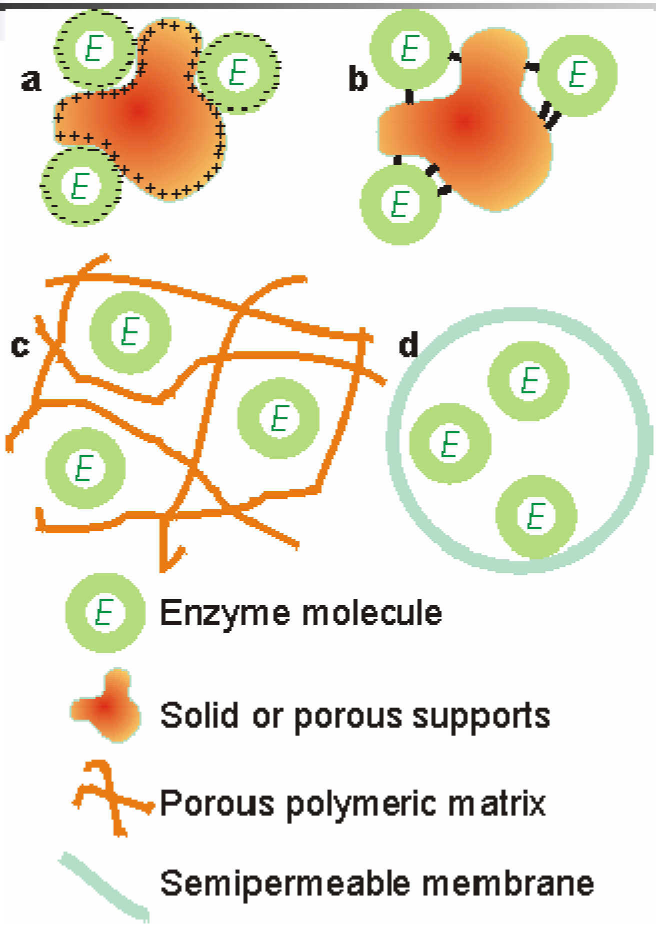
- The encapsulation process is based on the entrapment of the biomolecule in a polymeric matrix. The main advantage of this technique is that it allows the transport of low molecular weight (LMW) compounds through the permeable matrix.
- Entrapment matrix is generally formed during the immobilization process. Therefore, the properties of the gel matrix and the conditions used for the encapsulation should be compatible with the enzyme to be immobilized.
- Among the immobilization methods applicable to plant cells, the most common and effective methods are entrapment or encapsulation of cells within a gel or in a solid support.

❑Cross-linking method----

- Cross-linking is an immobilization technique that combines both covalent bonding and entrapment.
- Immobilization by this method requires cross-linking agents such as glutaraldehyde and bisisodiacetamide.
- The enzyme activity of immobilized preparations cross-linked with these reagents is dependent upon the degree of cross-linking.

❑Covalent binding------

- Covalent binding is based on the formation of a covalent bond between the biomolecule and the support material. It causes a tight binding so the biomolecule to be immobilized does not separate from supports during utilization. Because of this strong interaction between enzyme molecules and supports, there is high heat stability.
- However, this interaction, unfortunately, does not allow the enzyme molecules to have free movement, resulting in decreased enzyme activity.
- Enzymes immobilized with this technique also can easily contact with substrates since the enzymes are localized on the surface of the support material.
- This technique is less effective for immobilization of cells, and support materials are not renewable.



1. Absorption
2. Covalent linkage
3. Enzyme entrapment
4. Encapsulation of enzyme

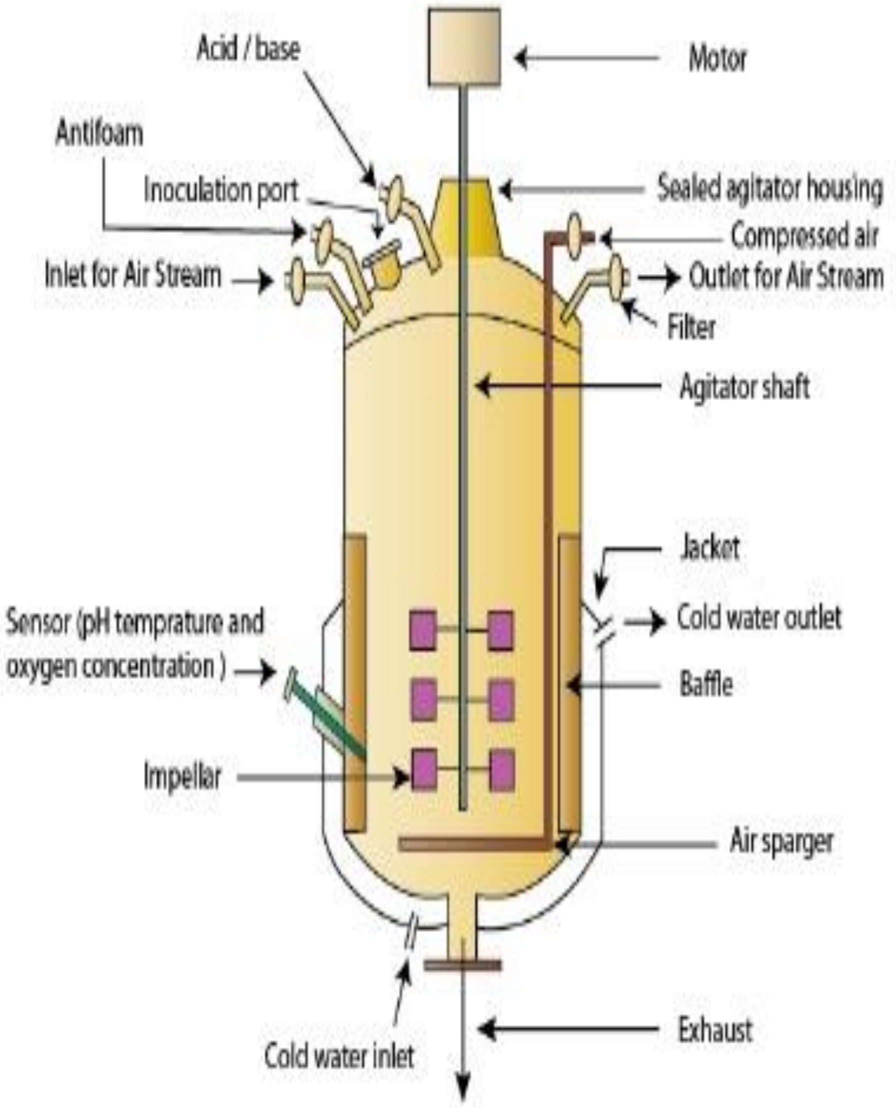
❖ Comparison between different methods:

Characteristics	Adsorption	Covalent binding	Entrapment	Membrane confinement
Preparation	Simple	Difficult	Difficult	Simple
Cost	Low	High	Moderate	High
Binding force	Variable	Strong	Weak	Strong
Enzyme leakage	Yes	No	Yes	No
Applicability	Wide	Selective	Wide	Very wide
Running Problems	High	Low	High	High
Matrix effects	Yes	Yes	Yes	No
Large diffusional barriers	No	No	Yes	Yes
Microbial protection	No	No	Yes	Yes

BIOREACTOR

- Most of the bioprocess has no great significance if it is not applicable on industrial scale. Therefore, it is very crucial to scale-up a laboratory production into industrial production. Bioreactor is the main equipment that allows us to increase the production volume.
- Bioreactor – can be described as a vessel which has provision of cell cultivation under sterile condition & control of environmental conditions e.g., pH, Temperature, Dissolved oxygen etc.
- It can be used for the cultivation of microbial plant or animal cells.
- This process can either be aerobic or anaerobic.
- The bioreactors are commonly cylindrical, ranging in size from litres to cubic metres, and are often made of stainless steel.

DIAGRAM OF A TYPICAL BIOREACTOR



SPECIFICATIONS OF A BIOREACTOR

A typical bioreactor consists of following parts:

- **Agitator** – used for the mixing of the contents of the reactor which keeps the "cells" in the perfect homogenous condition for better transport of nutrients and oxygen to the desired product(s).

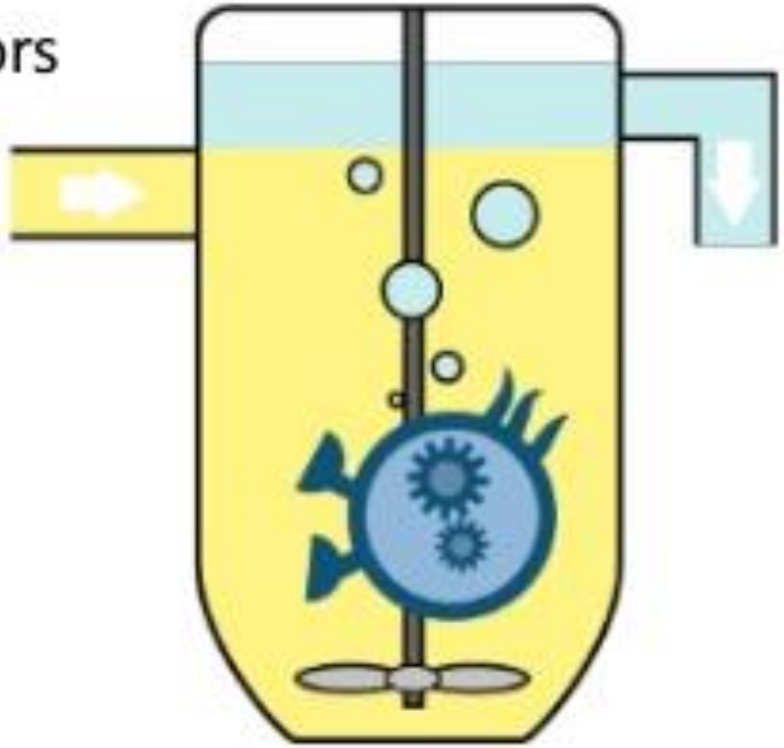
- **Baffle** – used to break the vortex formation in the vessel, which is usually highly undesirable as it changes the center of gravity of the system and consumes additional power.

- **Sparger** – In aerobic cultivation process, the purpose of the sparger is to supply adequate oxygen to the growing cells.

- **Jacket** – The jacket provides the annular area for circulation of constant temperature of water which keeps the temperature of the bioreactor at a constant value

BIOREACTORS TYPES

1. Continuous Stirred Tank Bioreactors
2. Bubble Column Bioreactors
3. Airlift Bioreactors
4. Fluidized Bed Bioreactors
5. Packed Bed Bioreactors
6. Photo-Bioreactors



❖ PROPERTIES OF IMMOBILIZED ENZYMES

- ✓ The properties of immobilized enzymes are determined by the characteristics of carrier material as well as by the nature and number of interactions between the enzyme and the support. As a consequence of enzyme immobilization, the stability and kinetic properties of enzymes are usually changed, mostly due to the microenvironment and modifycations imposed by the supporting matrix.

- ✓ This modification in the properties may be caused either by changes in the intrinsic activity of the immobilized enzyme or by the fact that the interaction between the immobilized enzyme and the substrate takes place in a micro-environment that is different from the bulk solution.

- ✓ So, one of the main problems associated with the use of immobilized enzymes is the loss of catalytic activity, especially when the enzymes are acting on macromolecular substrates. Because of the limited access of the substrate to the active site of the enzyme, the activity may be reduced to accessible surface groups of the substrate only. This steric restriction may in turn, change the characteristic pattern of products derived from the macromolecular substrate.

- ✓ There are several strategies to avoid these steric problems such as: the selection of supports composed by networks of isolated macromolecular chains, the careful choice of the enzyme residues involved in the immobilization, and the use of hydrophilic and inert spacer arms.

BIOLOGICAL ROLE OF ENZYME IMMOBILIZATION

- There is experimental evidence that the immobilized state might be the most common one for enzymes in their natural environment.
- In an attempt to mimic biology, co-immobilization of a number of sequential or cooperating biocatalysts on the same support has been used as a strategy to improve stability and enhance reaction kinetics. The attachment of enzymes to the appropriate surface ensures that they stay at the site where their activity is required.
- This immobilization enhances the concentration at the proper location, and it may also protect the enzyme from being destroyed.
- Numerous bi-enzyme systems have been reported; a remarkable example is the co-immobilization of peroxidase and glucose oxidase onto carbon nanotubes to be used as a glucose biosensor.

HOME ASSIGNMENT:

- Prepare a note on the different types of bioreactors mentioned in this study material mentioning their advantages and limitations.