Enzyme Biotechnology

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Enzymes are the biocatalysts that are capable of mediating many biological reactions and conversion of element into other intermediate products and so on to the final product. Coming to their activity, total enzyme will not be involved in any conversion of substrate to a product. Only, a specific part of the enzyme named □active site□ is involved in the reaction phenomenon. Any modifications at the active site will lead to the activity change of that particular enzyme and even that might make an enzyme to lose its activity totally and permanently. In order to make an enzyme to show its maximum potency in conversion of substrate to desired product, many parameters need to be optimized which include pH, temperature, concentration of enzyme, concentration of substrate, reaction time and even many modifications need to be done at the genetic level of that particular enzyme in order to manipulate the enzyme in all the possible ways to give maximum yield of the desired product yield and desired product quality. Until all the parameters required for the better production of desired product are not optimized, that process will not be approved to scale-up studies at higher quantities. Optimizing all the parameters favouring the better production of the product will let the scale up studies to pilot scale and then to industrial level production can be done successively.

What Is Enzyme Immobilization ?

Enzyme immobilization may be defined as a process of confining the enzyme molecules to a solid support over which a substrate is passed and converted to products. The process whereby the movement of enzymes, cells, organelles, etc. in space is completely or severely restricted usually resulting in a water-insoluble form of the enzyme

What Is An Immobilized Enzyme?

An immobilized enzyme is one whose movement in space has been restricted either completely or to a small limited region.

Need for Immobilization

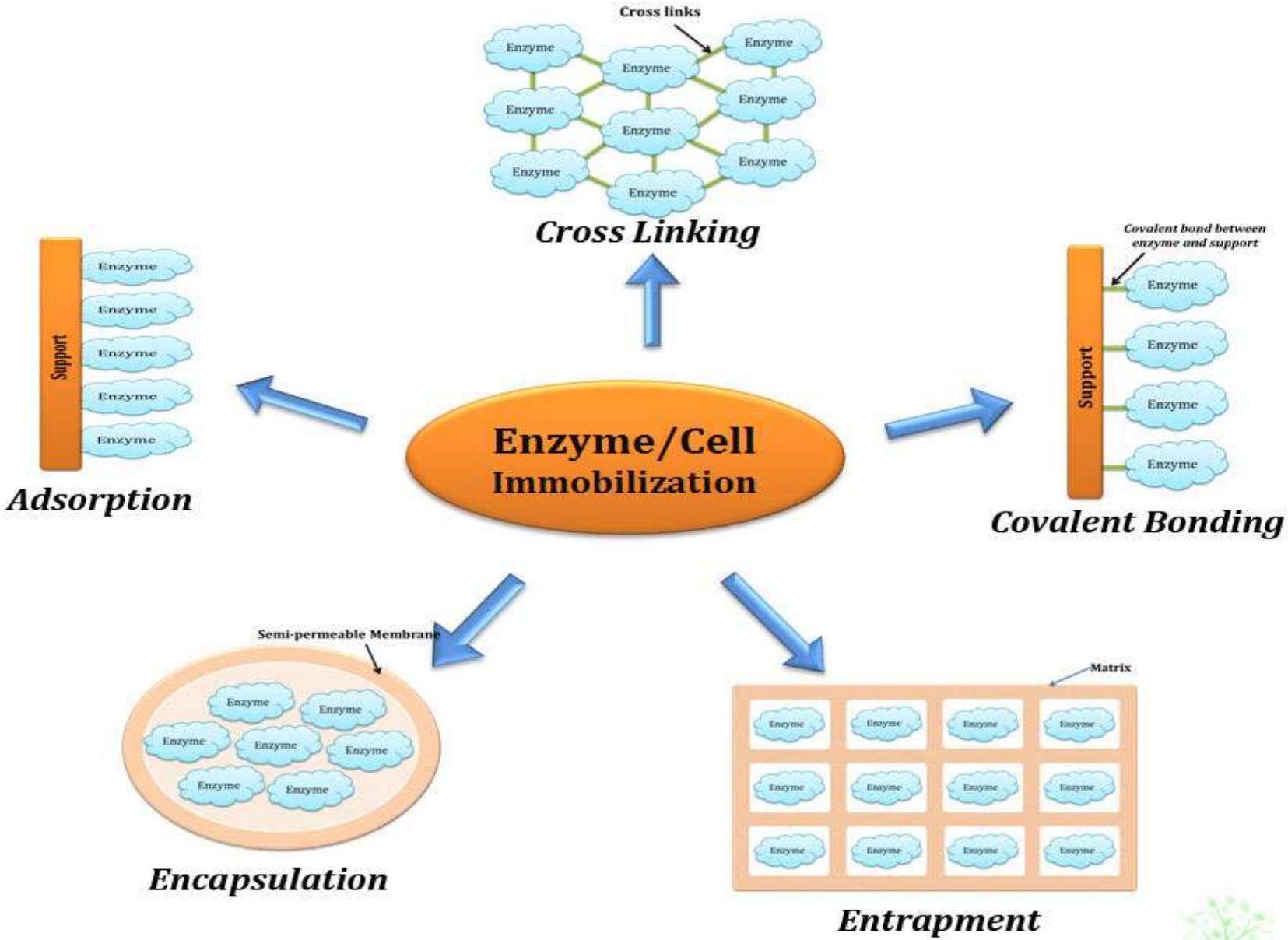
- Protection from degradation and deactivation.
- Retention of enzyme, enzyme-free products.
- Recycling, repetitive use.
- Cost efficiency.
- Enhanced stability.
- Use as controlled release agents.
- The ability to stop the reaction rapidly by removing the enzyme from the reaction Solution (or vice-versa)
- Allows development of multi-enzyme reaction system.

Advantages of enzyme immobilization:-

- Multiple or repetitive use of a single batch of enzymes.
- Immobilized enzymes are usually more stable.
- Ability to stop the reaction rapidly by removing the enzyme from the reaction solution.
- Product is not contaminated with the enzyme.
- Easy separation of the enzyme from the product.
- Allows development of a multi-enzyme reaction system.
- Reduces effluent disposal problems.

Disadvantages of enzyme immobilization:-

- It gives rise to an additional bearing on cost.
- It invariably affects the stability and activity of enzymes.
- The technique may not prove to be of any advantage when one of the substrate is found to be insoluble.
- Certain immobilization protocols offer serious problems with respect to the diffusion of the substrate to have an access to the enzyme.

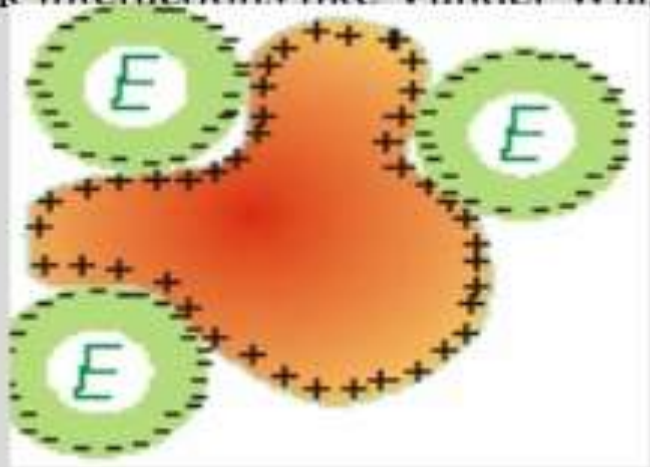


Enzyme/Cell Immobilization Methods

Physical Methods For Immobilization

ADSORPTION

- Involves the physical binding of the enzyme on the surface of carrier matrix.
- Carrier may be organic or inorganic.
- The process of adsorption involves the weak interactions like Vander Waal or hydrogen bonds.



- Carriers: - silica, bentonite, cellulose, etc.
- e.g. catalase & invertase

ADVANTAGES

- 1. Simple and economical
- 2. Limited loss of activity
- 3. Can be Recycled, Regenerated & Reused.

DISADVANTAGES

- 1. Relatively low surface area for binding.
- 2. Exposure of enzyme to microbial attack.
- 3. Yield are often low due to inactivation and desorption.

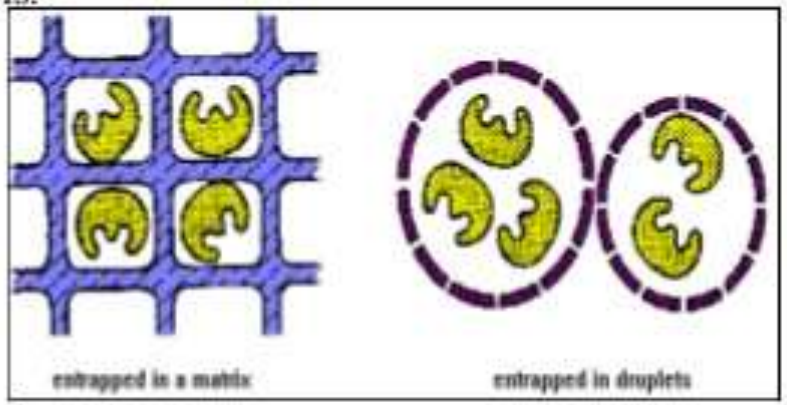
Entrapment

- In entrapment, the enzymes or cells are not directly attached to the support surface, but simply trapped inside the polymer matrix.
- Enzymes are held or entrapped within the suitable gels or fibres.
- It is done in such a way as to retain protein while allowing penetration of substrate. It can be classified into lattice and micro capsule types.

Inclusion in gels: Poly acrylamide gel, Poly vinyl alcohol gels.

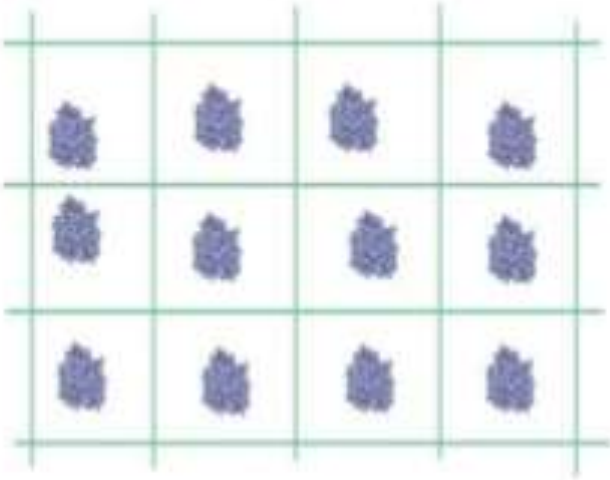
Inclusion in fibers: Cellulose and Poly -acryl amide gels.

Inclusion in micro capsules: Polyamine, Polybasic - acid chloride monomers.



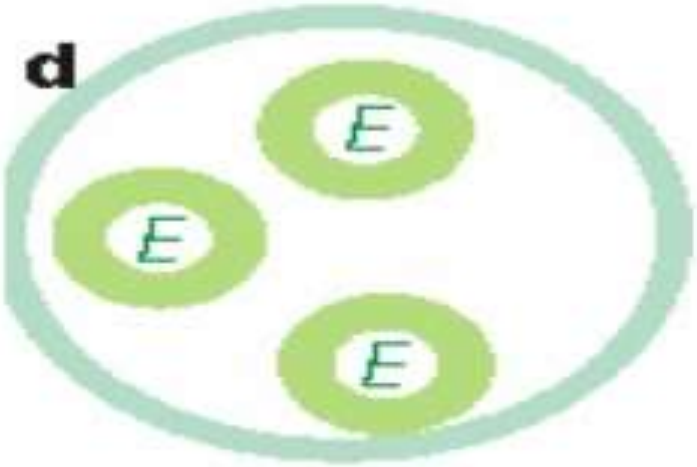
Lattice-Type Entrapment

- Entrapment involves entrapping enzymes within the interstitial spaces of a cross-linked water-insoluble polymer. Some synthetic polymers such as *polyarylamide, polyvinylalcohol, etc...* and natural polymer (starch) have been used to immobilize enzymes using this technique.



MicrocapsuleType Entrapmet/ Encapsulation/Membrane Confinement

- It involves enclosing the enzymes within semi -permeable polymer membranes e.g. semi permeable collodion or nylon membranes in the shape of spheres.



ADVANTAGES

- 1. No chemical modification.
- 2. Relatively stable forms.
- 3. Easy handling and re-usage.

DISADVANTAGES

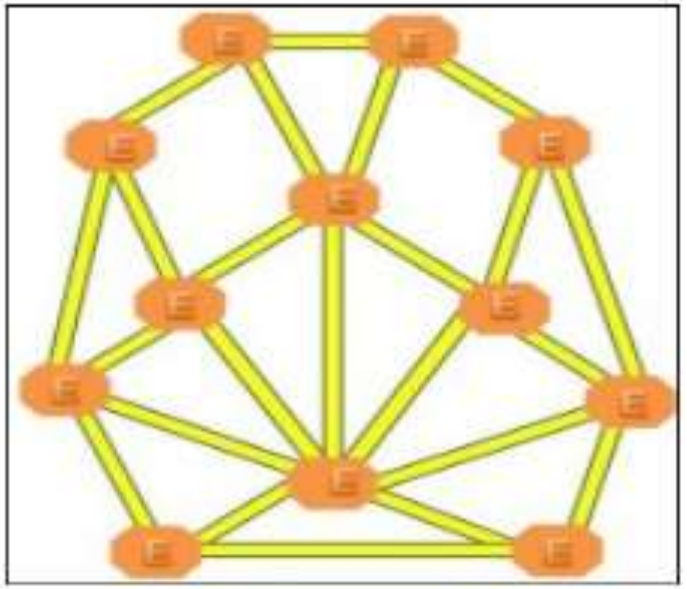
- 1. The enzyme may leak from the pores.

Covalent Binding

- Based on the binding of enzymes and water-insoluble carriers by covalent bonds
- The functional groups that may take part in this binding are Amino group, Carboxyl group, Sulfhydryl group, Hydroxyl group, Imidazole group, Phenolic group, Thiol group, etc
- Disadvantages : covalent binding may alter the conformational structure and active center of the enzyme, resulting in major loss of activity and/or changes of the substrate
- Advantages : the binding force between enzyme and carrier is so strong that no leakage of the enzymes occurs, even in the presence of substrate or solution of high ionic strength.

Cross Linking

- Cross linking involves intermolecular cross linking of enzyme molecules in the presence/absence of solid support.
- The method produces a 3 dimensional cross linked enzyme aggregate (insoluble in water) by means of a multifunctional reagent that links covalently to the enzyme molecules.



Advantages of cross linking:-

1. Very little desorption(enzyme strongly bound)
2. Higher stability (i.e. ph, ionic & substrate concentration)

Disadvantages of cross linking:-

1. Cross linking may cause significant changes in the active site.
2. Not cost effective.

Limitations Of Enzyme Immobilization

- Cost of carriers and immobilization.
- Changes in properties (selectivity).
- Mass transfer limitations.
- Problems with cofactor and regeneration.
- Problems with multienzymes systems.
- Activity loss during immobilization.

Applications

1: Production of antibiotics.

e.g; Penicillin

2: Biomedical application (to overcome metabolic deficiency with the use of encapsulated enzymes).

3: Pharmacy (this technique is used in the production of syrups).

4: Production of amino acids.