Cell & Enzyme Immobilization

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What is Immobilization?

- Immobilization of enzymes (or cells) refers to the technique of confining/anchoring the enzymes (or cells) in or on an inert support for their stability and functional reuse.
- The support or matrix on which the enzymes are immobilized allows the exchange of medium containing substrate or effector or inhibitor molecules.
- The practice of immobilization of cells is very old and the first immobilized enzyme was amino acylase of *Aspergillus oryzae* for the production of L-aminoacids in Japan.

Support/ matrix used for immobilization

- The matrix holds the enzyme/cells.
- The matrix used should be cheap and easily available.
- Their reaction with the product should be minimum.
- Various types of matrix are used and the major category includes:
  - Natural polymers
  - Synthetic polymers
  - Inorganic materials

Natural Polymers

- ALGINATE: Derived from algal cell wall (calcium or magnesium alginate)
- CHITOSAN AND CHITIN: Used for enzyme attachment; binds to the free -OH group
- COLLAGEN: Protein derived
- CARRAGEENAN: A sulphated polysaccharide obtained from algae
- GELATIN: Partially hydrolysed collagen with high water holding capacity.
- CELLULOSE: Cheapest support available
- STARCH: Good water holding capacity
- PECTIN: Good water holding capacity

Synthetic Polymers

- They are ion exchange resins or polymers
- They are insoluble supports with porous surface
- The porous surface trap and hold the enzymes/cells
- Examples:
  - DEAE CELLULOSE
  - POLYVINYL CHLORIDE
  - UV ACTIVATED POLYETHYLENE GLYCOL

Inorganic Materials

(a). Zeolites: They are microporous, aluminosilicate minerals with good adsorbing properties and extensively used for immobilizing enzymes and whole cells.

(b). Ceramics: They are nonmetallic solids consisting of metal and nonmetal atoms held in ionic and covalent bonds. The composition and bonding pattern varies with different types.

(c). Diatomaceous earth: They are silicious sedimentary rocks formed by fossilized accumulations of the cell wall of diatoms. Celite is the trade name of diatomaceous earth. It is a good adsorbent and are resistant to high pH and temperature.

(d). Silica:

(e). Glass:

(f). Activated carbon

(g). Charcoal

Methods of Immobilization

Based on support or matrix and the type of bonds involved, there are five different methods of immobilization of enzyme or whole cells.

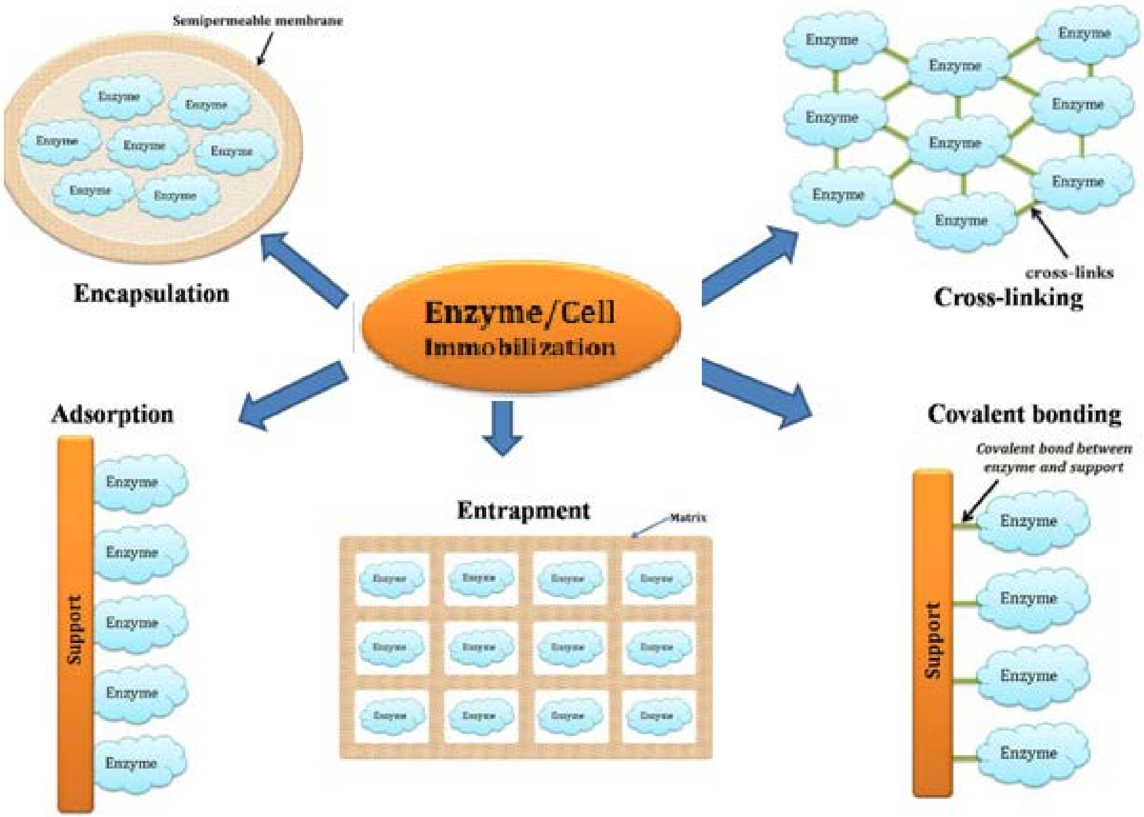
(1). Adsorption

(2). Covalent bonding

(3). Entrapment

(4). Copolymerization

(5). Encapsulation



Adsorption

Adsorption is the oldest and simplest method of enzyme immobilization. Nelson & Griffin used charcoal to adsorb invertase for the first time in 1916. In this method enzyme is adsorbed to external surface of the support. The support or carrier used may be of different types such as:

(1). *Mineral support* (Eg. aluminum oxide, clay)

(2). *Organic support* (Eg. starch)

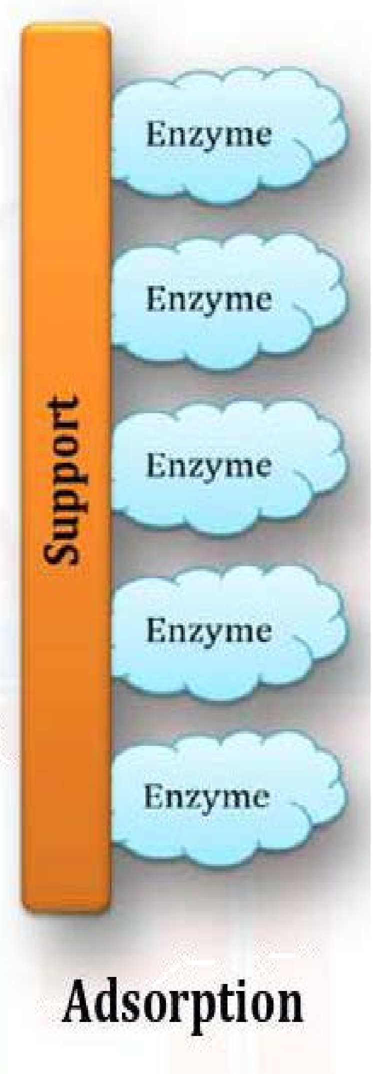
(3). *Modified sepharose and ion exchange resins*

There is no permanent bond formation between carrier and the enzyme in adsorption method. Only weak bonds stabilize the enzymes to the support or carrier. The weak bonds (low energy bonds) involved are mainly:

(a). Ionic interaction

(b). Hydrogen bonds

(c). Van der Waal forces



...Adsorption

Advantages of adsorption method:

(a). No pore diffusion limitation

(b). Easy to carry out

(c). No reagents are required

(d). Minimum activation steps involved

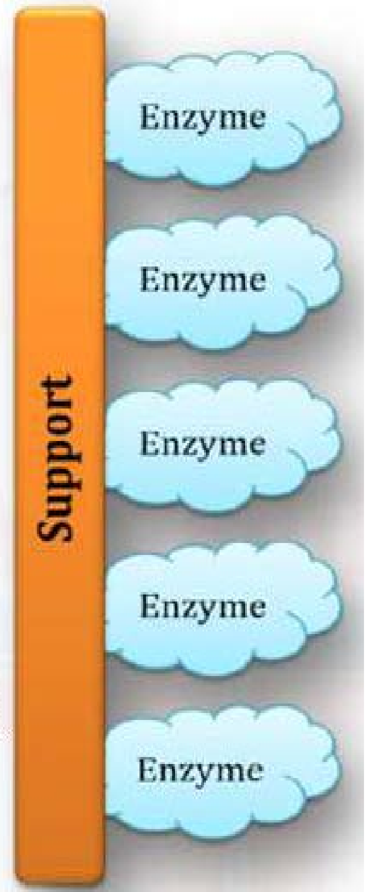
(e). Comparatively cheap method of immobilization

(f). Less disruptive to enzyme than chemical methods

Disadvantages of adsorption method:

(a). Desorption of enzymes from the carrier

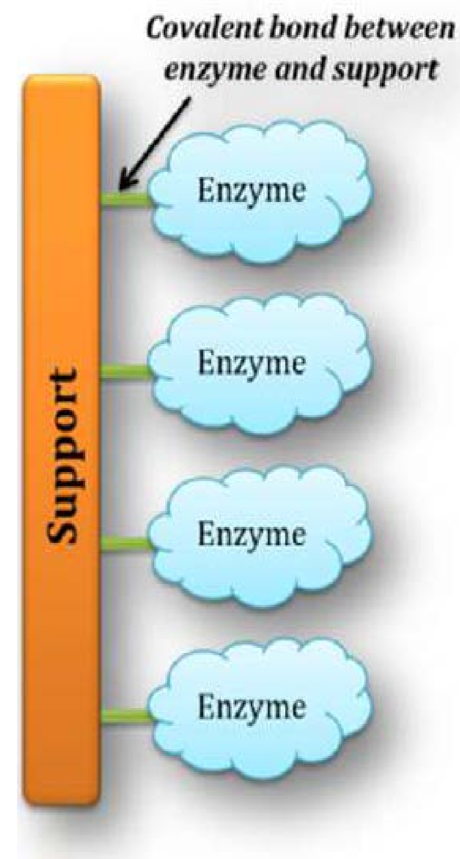
(b). Efficiency is less



Adsorption

Covalent Bonding

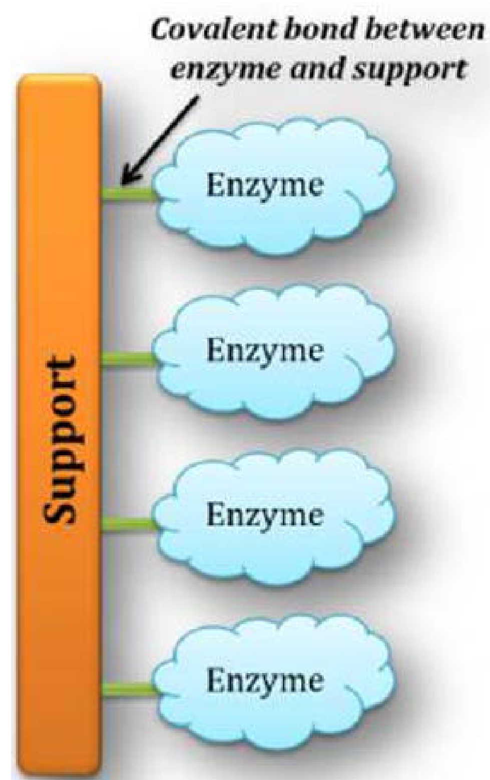
- Involves the formation of covalent bonds between enzyme/cell and support
- Widely used method for enzyme immobilization
- Chemical bonds in enzymes that forms covalent bonds with support are:
  - Amino groups and imino groups
  - Hydroxyl groups
  - Carboxyl groups
  - Thiol groups and methylthiol groups
  - Guanidyl groups and imidazole groups
  - Phenol rings



Covalent Bonding

Support Materials for Covalent Bonding

- **Carbohydrates:**
  - Cellulose,
  - DEAE cellulose,
  - Agarose
- **Synthetic agents:**
  - Polyacrylamide
- **Amino group bearing carriers:**
  - Amino benzyl cellulose
- **Inorganic carriers:**
  - Porous glass,
  - Silica
- **Cyanogen bromide:**
  - CNBr-agarose
  - CNBr-sepharose
- **Protein carriers:**
  - Collagen
  - Gelatin



Covalent Bonding

Methods of Covalent Bonding

- **DIAZOATION:** Bonding between amino group of support and tyrosil or histidyl group of enzyme
- **PEPTIDE BOND:** Between amino and caboxyl groups of support and enzyme
- **POLY FUNCTIONAL REAGENTS:** Use of a bi-functional or multifunctional reagent like glutaraldehyde which forms a bonding between the amino group of the support and the amino group of the enzyme

...Covalent Bonding

Advantages of covalent bonding method

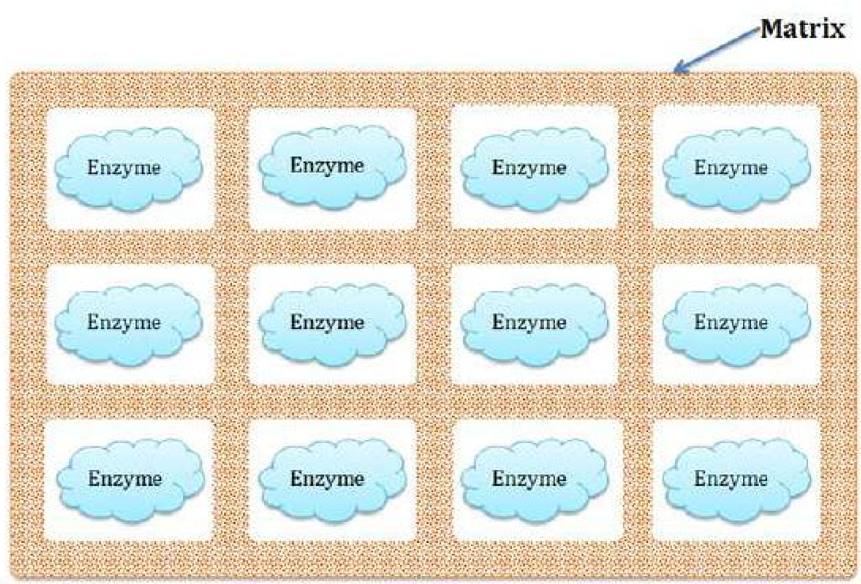
- Strong linkage of enzyme to the support
- No leakage or desorption problem
- Comparatively simple method
- A variety of support with different functional groups.
- Wide applicability

Disadvantages of covalent bonding method

- Chemical modification of enzyme leading to functional conformation loss.
- Enzyme inactivation can occur due to conformation changes when undergoes reactions at active site.

Entrapment

- Cells/ enzymes are physically entrapped in a matrix.
- Bonds involved can be covalent or non covalent.
- Matrix used will be a water soluble one.
- Pore size of matrix is adjusted to prevent the loss of enzymes.
- Pore size can be adjusted with the concentration of the polymer used.
- Agar-agar and carrageenan have comparatively large pore size.
- It has not much application in industrial level
- Easy to practice at small scale level
- Examples of matrix:
  - Polyacrylamide gels
  - Cellulose triacetate
  - Agar
  - Gelatin
  - Carageenan
  - Alginate



Entrapment

Methods of Entrapment

- **Inclusions in gel:** Cells/enzymes are trapped in GEL
- **Inclusions in fibres:** Cells/enzymes are supported on fibre matrix
- **Inclusions in microcapsules:** Cells/enzymes are trapped in microcapsules formed by monomer mixtures such as polyamine, calcium alginate etc.

...Entrapment

Advantages of entrapment:

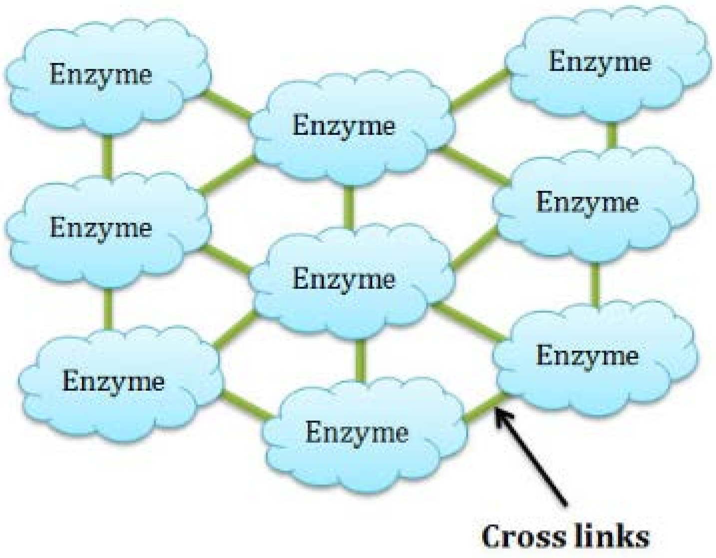
- Fast method of immobilization
- Cheap (low cost matrixes available)
- Easy to practice at small scale
- Mild conditions are required
- Less chance of conformational changes in enzyme
- Can be used for sensing application

Disadvantages of entrapment:

- Leakage of enzyme
- Pore diffusion limitation
- Chance of microbial contamination
- Not much success in industrial process

Cross linking (Copolymerization)

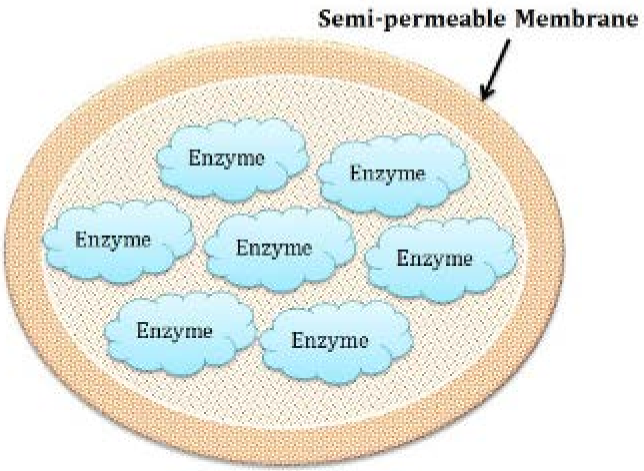
- In this method enzymes are directly linked by covalent bonds between various groups of enzymes via polyfunctional reagents.
- Commonly used polyfunctional groups includes:
  - Glutaraldehyde
  - Diazonium salt
- This technique is cheap and simple but not used with pure enzymes.
- It is widely used in commercial preparations and industrial applications.
- A disadvantage of polyfunctional group is that, they may denature the enzyme leading to the loss of catalytic activity.



Cross Linking (Copolymerization)

Encapsulation

- This type of immobilization is done by enclosing the enzymes in a membrane capsule.
- The casule will be made up of semi-permeable membrane like nitro-cellulose or nyon.
- The effectiveness of method depends upon the stability of enzymes inside the capsule.



Encapsulation

Advantages of encapsulation:

(a). Cheap and simple method

(b). Large quantity of enzymes can be immobilized by encapsulation

Disadvantages of encapsulation:

(a). Pore size limitation

(c). Only small substrate molecule is able to cross the membrane

Advantages of Immobilized Enzymes

(1). *Increased functional efficiency of enzyme*

(2). *Enhanced reproducibility of the process they are undertaking*

(3). *Reuse of enzyme*

(4). *Continuous use of enzyme*

(5). *Less labour input in the processes*

(6). *Saving in capital cost and investment of the process*

(7). *Minimum reaction time*

(8). *Less chance of contamination in products*

(9). *More stability of products*

(10). *Stable supply of products in the market*

(11). *Improved process control*

(12). *High enzyme substrate ratio*

Disadvantages of Immobilized Enzymes

- High cost for the isolation, purification and recovery of active enzyme (most important disadvantage)
- Industrial applications are limited and only very few industries are using immobilized enzymes or immobilized whole cells.
- Catalytic properties of some enzymes are reduced or completely lost after their immobilization on support or carrier.
- Some enzymes become unstable after immobilization.
- Enzymes are inactivated by the heat generated in the system

Applications of Enzyme immobilization

1. ***Industrial production***: Industrial production of antibiotics, beverages, amino acids etc. uses immobilized enzymes or whole cells.
2. ***Biomedical applications***: Immobilized enzymes are widely used in the diagnosis and treatment of many diseases. Immobilized enzymes can be used to overcome inborn metabolic disorders by the supply of immobilized enzymes. Immobilization techniques are effectively used in drug delivery systems especially to oncogenic sites.
3. ***Food industry***: Enzymes like pectinases and cellulases immobilized on suitable carriers are successfully used in the production of jams, jellies and syrups from fruits and vegetables.
4. ***Research:*** A Research activity extensively uses many enzymes. The use of immobilized enzyme allow researcher to increase the efficiency of different enzymes such as Horse Radish Peroxidase (HRP) in blotting experiments and different Proteases for cell or organelle lysis.
5. ***Production of biodiesel*** from vegetable oils.
6. ***Waste water management***: treatment of sewage and industrial effluents.
7. ***Textile industry***: scouring, bio-polishing and desizing of fabrics.
8. ***Detergent industry***: immobilization of lipase enzyme for effective dirt removal from cloths.

Advantages & Disadvantages of Immobilized Whole Cells

Advantages of whole cell immobilization:

(a). Multiple enzymes can be introduced to a single step

(b). Extraction and purification of enzymes are not required

(c). Enzymes are stable for long time

(d). Native conformation of enzyme is best maintained

(e). Cell organelles like mitochondria and chloroplasts can be immobilized

(f). Cost effective method

Disadvantages of whole cell immobilization:

(a). Concentration of enzymes will be less

(b). Production of unwanted enzymes and unwanted products

(c). Modification of end products by other enzymes produced by immobilized cells

Applications of Immobilized Whole Cells

Method	Support Material	Cells	Reaction
Adsorption	Gelatin	*Lactobacilli*	Lactose ⟹ lactic acid
	Porous glass	*Saccharomyces*	Glucose ⟹ ethanol
	Cotton fibers	*Zymomonas*	Glucose ⟹ ethanol
	DEAE Cellulose	*Nocardia*	Steroid conversion
Covalent bonding	Cellulose + cyanuric chloride	*S. cerevisiae*	Glucose ⟹ ethanol
	Titanium oxide	*Acetobacter*	Vinegar
Cross linking	Glutaraldehyde	*E. coli*	Fumaric acid
Entrapment	Aluminium alginate	*Candida tropicalis*	Phenol degradation
	Calcium alginate	*S. cervisiae*	Glucose ⟹ ethanol
Encapsulation	Polyester	*Streptomyces sps.*	Glucose ⟹ fructose
	Alginate polylysine	Hybridoma cells	Monoclonal antibodies

...Applications of Cell immobilization

- **Antibiotics production:** The most widely studied system is the production of penicillin G using immobilized cells of *Penicillium chrysogenum*.
- The adsorbed cells exhibited maximum specific reaction rates over free-cells.
- It has been reported that the adsorption on celite was five times more productive than entrapment in carrageenan.
- **Organic acids Production:** *Aspergillus niger* is the widely used microorganism for the synthesis of citric acid.
- The fungal fermentations have serious disadvantage of rising viscosity during growth, leading to poor oxygen supply to the cells.
- In case of immobilized cells, since growth is restricted, it is possible to operate the fermentor without affecting the viscosity.
- The methods most widely used for immobilization of *A. niger* cells are the entrapment in alginate gels, agarose and polyacrylamide.
- In addition, adsorption on various supports, such as polyurethane foam and entrapment in hollow fibres.
- **Enzymes Production:** *Aspergillus strains* and *Bacillus cereus* have been immobilized for the production of glucoamylase and a-amylase enzyme.

Questions

- What is immobilization technique?
- What are the methods used for the immobilization of cells and enzymes of industrial importance.
- What is the significance of immobilization technology?
- What are the merits and demerits associated with immobilization of cells and enzymes?
- Write short note on application of:
  - Immobilized enzymes
  - Immobilized whole cells
  - Support materials for cell/enzyme immobilization
  - Adsorption method of cell/enzyme immobilization
  - Covalent bonding method of cell/enzyme immobilization
  - Copolymerization method of cell/enzyme immobilization
  - Cross linking method of cell/enzyme immobilization
  - Entrapment method of cell/enzyme immobilization
  - Encapsulation method of cell/enzyme immobilization