Methods of Immobilization

The commonly employed techniques for immobilization of enzymes are—adsorption, entrapment, covalent binding, crosslinking etc.

Adsorption

Adsorption of enzyme molecules (on the inert support) involves weak forces such as van der Waals forces and hydrogen bonds. Therefore, the adsorbed enzymes can be easily removed by minor changes in pH, ionic strength or temperature. This is a disadvantage for industrial use of enzymes.

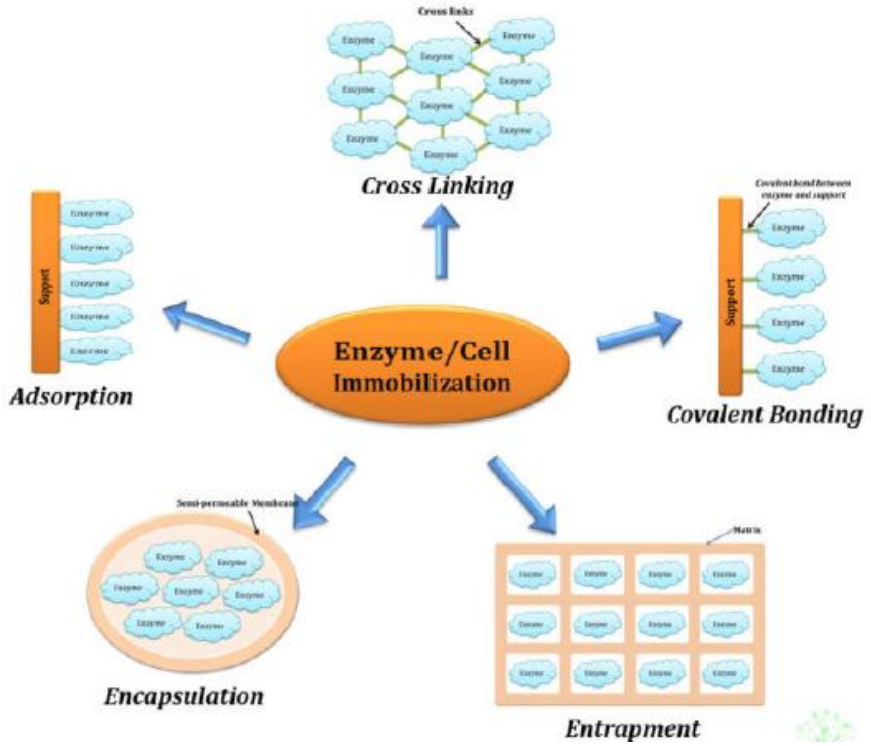
Entrapment

Enzymes can be immobilized by physical entrapment inside a polymer or a gel matrix. The size of the matrix pores is such that the enzyme is retained while the substrate and product molecules pass through. In this technique, commonly referred to as lattice entrapment, the enzyme (or cell) is not subjected to strong binding forces and structural distortions.

Some deactivation may however, occur during immobilization process due to changes in pH or temperature or addition of solvents. The matrices used for entrapping of enzymes include polyacrylamide gel, collagen, gelatin, starch, cellulose, silicone and rubber. Enzymes can be entrapped by several ways.

Encapsulation

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



Covalent binding

Covalent binding is the most widely used method for immobilizing enzymes. The covalent bond between enzyme and a support matrix forms a stable complex. The functional group present on enzyme, through which a covalent bond with support could be established, should be non essential for enzymatic activity.

The most common technique is to activate a cellulose-based support with cyanogen bromide, which is then mixed with the enzyme.

The protein functional groups which could be utilized in covalent coupling include:

Amino group, Carboxylic group, Phenol ring, Indole group, Imidazole group

Cross linking

This method is based on the formation of covalent bonds between the enzyme molecules, by means of multifunctional reagents, leading to three dimensional cross linked aggregates. The most common reagent used for cross-linking is glutaraldehyde.

Immobilization technique	Advantages	Disadvantages
Physical Adsorption	Simple and cheap	Low stability
	High catalytic activity	Possible loss of biomolecules
	No conformational change of the biocatalyst	Weak bonds might cause desorption of biocatalyst
	No need to use reagents	
	Reuse of expensive material	
Encapsulation and Entrapment	Protection of biocatalyst	Limitations on mass transfer
	Allows the transport of low molecular weight compounds	Low enzyme loading
	Enables continuous operation due to maintained cell density	
	Facilitates cell separation and simplified downstream process	
	Allows controlled release of product	
Cross-linking	Strong biocatalyst binding	Might cause alteration in active site
	Prevents leakage	Diffusion limitations
	Decreases desorption	Loss of enzyme activity
	Increases the stability of biocatalyst	
Covalent Binding	Strong binding	Limited enzyme mobility causes decreased enzyme activity
	High heat stability	Less effective for immobilization of cells
	Facilitates the enzyme contacts with its substrate	Support materials are not renewable
	Prevents elution of biocatalysts	
	Flexibility in design of support material and method	

Please refer to these links : https://www.ncbi.nlm.nih.gov/pubmed/25025272

https://www.ncbi.nlm.nih.gov/pubmed/27770861