Immobilization of Enzymes

Enzyme immobilization is a process by which an enzyme is chemically or physically attached to a carrier to impart better physical and chemical properties than free enzymes would exhibit outside of its natural environment and give a longer life span. In addition, enzyme immobilization leads to increased stability, & ease of separation from product when applied to organic synthesis or industrial processes.

Enzyme immobilization is confinement of enzyme to a phase (matrix/support) different from the one for the substrates and products. The materials used for immobilization of enzymes, called carrier matrices which are grouped into three major categories**:**

1- Natural polymers: cellulose, gelatin, chitosan, collagen, pectin & starch.
2- Synthetic polymers: DEAE cellulose, PVC, PEG.
3- İnorganic polymers: Ceramic, Silica, Glass, Charcoal.

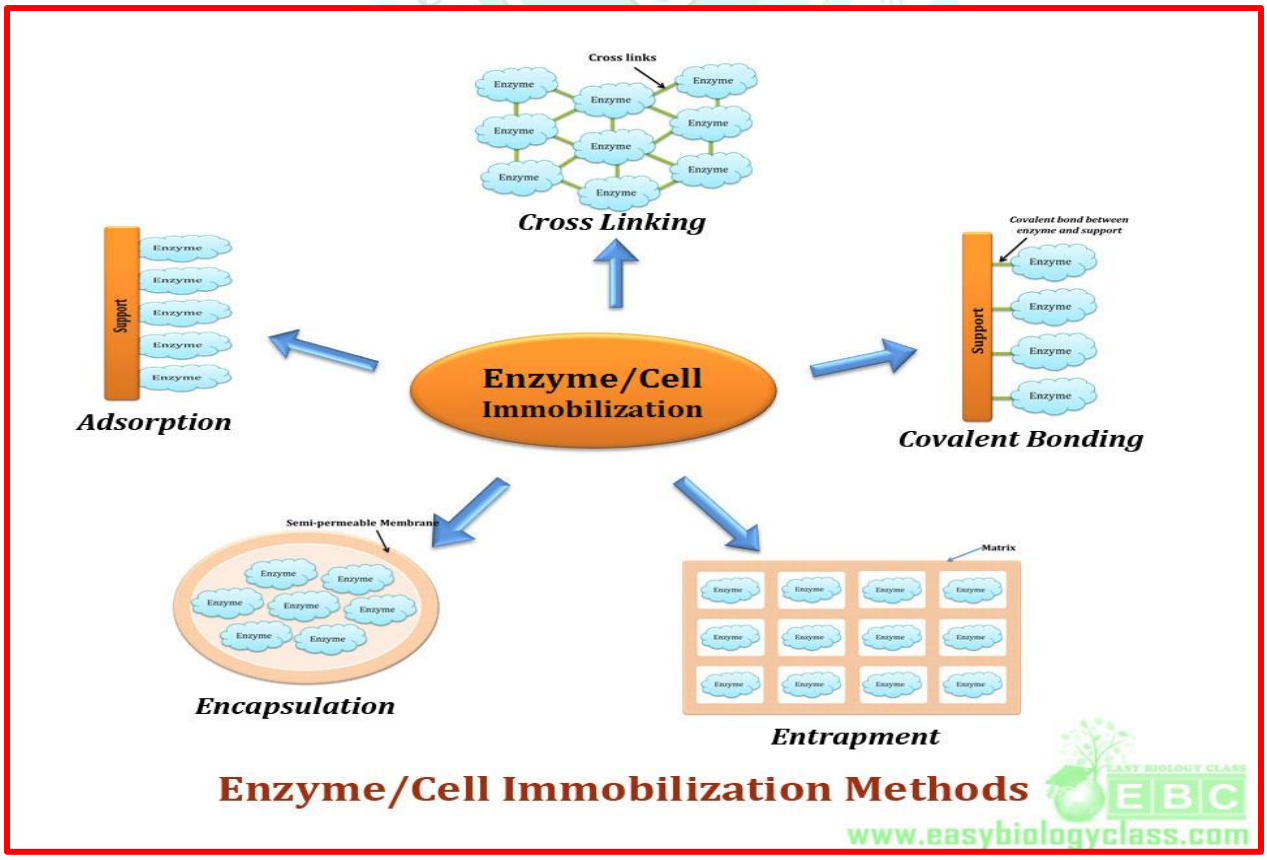
Benefits of immobilizing enzyme

1- Repetitive use of Enzymes.
2- Product is not contaminated with the enzyme.
3- Easy separation of enzyme from the product (food & pharmaceutical industries).
4- Continuous production systems can be used.
5- Thermal stability of Enzymes are usually increased by binding.
6- The ability to stop the reaction rapidly by removing the enzyme from the reaction solution, this led to improved process control.
7- Allows development of a multi-enzyme reaction system.

Classification of immobilization methods for enzymes

The various methods used for immobilization of enzymes may be grouped into two main types:

1- **Entrapment types like gel or fiber entrapment.**
2- **Microencapsulation.**
3- **Binding types like Crosslinking.**
4- **Covalent & metal binding.**
5- **Physical adsorption.**



Enzyme immobilization by gel entrapment

The major components of an immobilized enzyme system are the enzyme, the matrix, and the mode of attachment of the enzyme to the matrix. The entrapment method is based on the occlusion of an enzyme within a polymeric network that allows the substrate and products to pass through but retains the enzyme.

An excellent matrix that has been extensively used in this method is agarose. In addition to its high porosity, which leads to a high capacity for proteins, some other advantages of using agarose as a matrix are hydrophilic character, absence of charged groups (which prevents nonspecific adsorption of substrate and products), and commercial availability. However, an important limitation in the use of agarose is the high cost.

Procedure of peroxidase entrapment by agarose

1- Extraction of peroxidase enzyme from radish (1:10) by 0.1M phosphate buffer pH 7).
2- Prepare 100ml of 1% agarose.
3- Mix 2 ml of enzyme with 10 ml of 1% agarose (chilled the agar to 45 C°).
4- Pour the mixture of enzyme with immobilization material (agarose) on the petri dish then cut the hardened gel into small cubes limits of 3 mm.
5- Wash the small cubes of gel with phosphate buffer.
6- Put the small cubes of immobilized enzyme in container with substrate of peroxidase, changing the color of substrate to brown indicates that the enzyme entrapped with agarose.