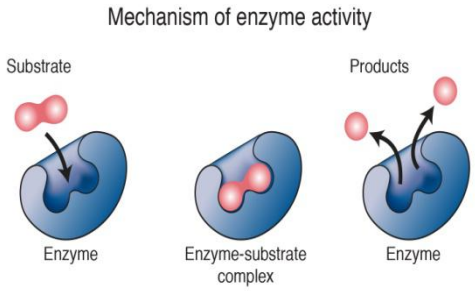
Discipline Specific Elective Course

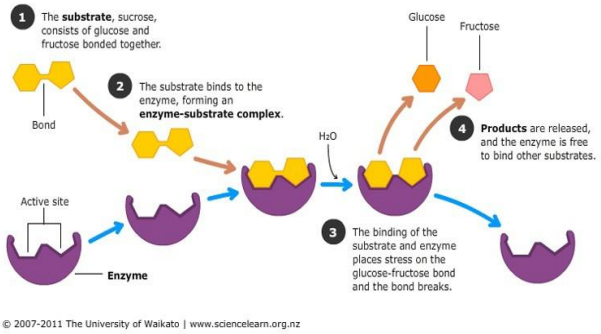
Industrial and Environmental Microbiology
Course Code: BOTDSC03T

Unit: 4 Microbial Enzymes of Industrial Interest and Enzyme Immobilization (Part-2)

Enzyme Immobilization:

- As definition, Enzymes are the type of bio catalysts which can not initiate or finish the biological reactions but can alter the rate of reaction and itself remain unchanged after the reaction - it is well known to us. But the enzyme substrate reaction always takes place in aqueous medium and after the reaction although product and enzyme get separated from each other, yet they are difficult to separate. Especially in industrial purposes, enzymes are needed to separate from the product because of application of same enzyme for next round of reactions. In industrial purposes mostly microbial enzymes are used. Production costs of these enzymes are too much because of why reuses of enzymes are needed. It is the main aim of introduce the process enzyme immobilization.

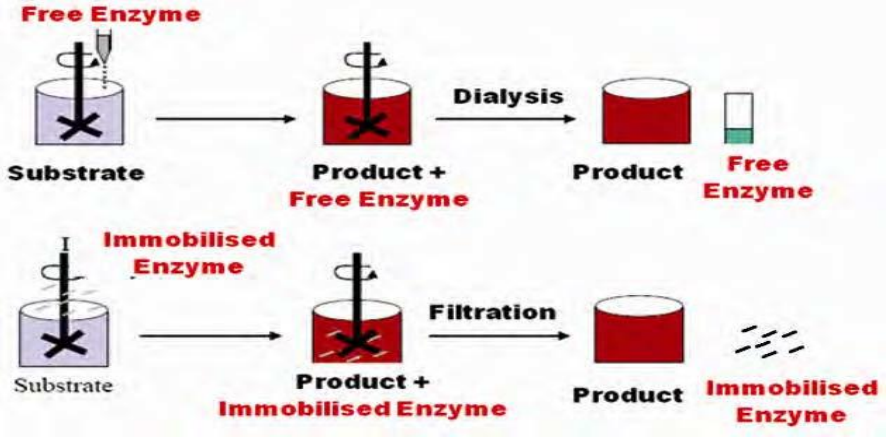




Enzyme	Source	Application	Industry
Amylase (starch-digesting)	Fungi	Bread	Baking
	Bacteria	Starch coatings	Paper
	Fungi	Syrup and glucose manufacture	Food
	Bacteria	Cold-swelling laundry starch	Starch
	Fungi	Digestive aid	Pharmaceutical
	Bacteria	Removal of coatings (desizing)	Textile
	Bacteria	Removal of stains; detergents	Laundry
Protease (protein-digesting)	Fungi	Bread	Baking
	Bacteria	Spot removal	Dry cleaning
	Bacteria	Meat tenderizing	Meat
	Bacteria	Wound cleansing	Medicine
	Bacteria	Desizing	Textile
	Bacteria	Household detergent	Laundry
Invertase (sucrose-digesting)	Yeast	Soft-center candies	Candy
Glucose oxidase	Fungi	Glucose removal, oxygen removal	Food
		Test paper for diabetes	Pharmaceutical
Glucose isomerase	*Bacteria*	High-fructose corn syrup	Soft drink
Pectinase	Fungi	Pressing, clarification	Wine, fruit juice
Rennin	Fungi	Coagulation of milk	Cheese
Cellulase	*Bacteria*	Fabric softening, brightening; detergent	Laundry
Lipase	Fungi	Breaks down fat	Dairy, laundry
Lactase	Fungi	Breaks down lactose to glucose and galactose	Dairy, health foods
DNA polymerase	*Bacteria*	DNA replication in polymerase chain	Biological research;
	Archaea	reaction (PCR) technique (Section 7.9)	forensics

Table 30-4 Brock Biology of Microorganisms 11/e

- Enzyme Immobilization can be defined as, the process of fixing of enzyme (protein) particles on some specialized solid/ semi solid substratum (such as Calcium Alginate) or, making of larger network of enzyme (protein) components or, encapsulation of enzyme particles in some membranous structure etc. so that after the enzymatic reactions easily enzyme particles can be removed from the system for next time application.
- Here after proper immobilization of enzymes, Substrate is applied along with water over the enzyme. It results the formation of product. And after the reaction enzymes are removed from the system easily just by the application of filtration. But on other hand in case of free enzymes extraction is very difficult, mainly dialysis process is applied which are again a costly process.



- There are some differences between free and immobilized enzymes, which are listed below-

Characters	Free Enzymes	Immobilized Enzymes
Price	High	Low
Efficiency	Low	High
Activity	Unstable	Stable
Reuse ability	Not possible	Possible
Tolerance to temperature & pH	Low	High
Separation from substrate	Difficult	Easy
Separation from product	Difficult	Easy

- Hence, there are some advantages of enzyme immobilization-
  1. Easily enzyme can be reused as the separation process is easy.
  2. As immobilized enzymes have higher efficiency and high level of activity including temperature tolerance properties in long term reaction process these can be used easily as the free enzymes have the reverse characters of these parameters. Especially as the enzymes are protein they have the tendency to coagulate at high temperature which is avoided by the utilization of immobilized enzymes in industrial purposes.
  3. This type of enzymes are reused in reaction processes, hence, total productivity cost is lowered, which make the product more market friendly.
  4. Immobilized enzymes are also easy to separate from the final product of the reaction. Hence, in product there are no contaminations of enzymes which reduce the chance of allergic responses in consumers.
  5. Immobilized enzymes have the stable activity, which implies the rate of reaction remain constant throughout the duration of reaction. Ultimately this feature is helpful to predict as well as control the reaction easily.

Free Enzymes

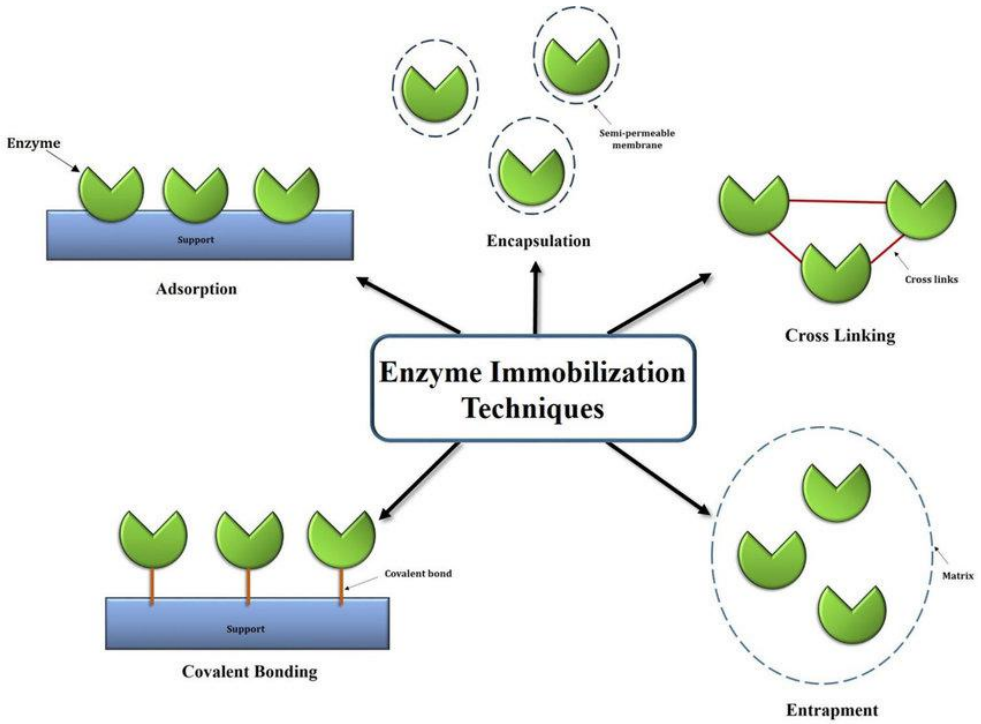
Immobilized Enzyme

- There are many advantages of using Immobilized enzymes, still there are some disadvantages also-
  1. In Immobilization process enzymes are fixed in / on some inert materials. Here during fixing inside of any compound, enzyme structure may alter i.e. active site of enzyme may entrapped inside of the material in that case enzyme will function less.
  2. Inert materials having high price, because of why production cost of this enzyme is not so less.
  3. Immobilized enzyme particle may detach from the surface of the inert materials, in that case no reaction will occur. Etc.
- The inert materials which are generally used in immobilization process are known as Carriers. Carriers must have some features –
  1. It should be an inert material.
  2. It should have strong and stable structure.
  3. The material must be cost effective. Etc.
- Different types of carriers are used in this process-

Types of Carrier	Examples
Inorganic Carrier	Silicon dioxide Containing materials (Porous glass, Silica etc), Mineral materials/ clay (Celite, Centonite etc.)
Organic (Natural) Carrier	Cellulose Derivatives (DADE Cellulose, CM Cellulose etc.), Dextran, Polysaccharides (Agarose, Starch, Pectin, Chitosan etc.).
Organic (Synthetic) Carrier	Polysystyrine, Polyvinylacetate, Acrylic Polymers etc.

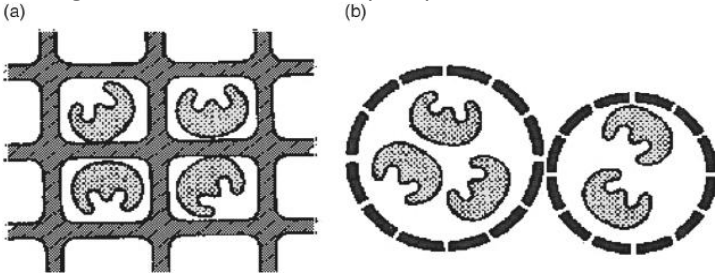
- Different strategies are used in immobilization process of enzymes-

Type of Immobilization	Name of the technique
Physical	Adsorption
	Entrapment
	Encapsulation
Chemical	Covalent bonding
	Cross Linking



- **Physical Immobilization:** This type of immobilization does not involve with formation of any kinds of fixed chemical bonds.
  1. **Adsorption:** In this case, because of Vander wall attraction force enzymes are fixed over the surface of carriers. Sometimes another type of inert material is also used to increase the surface area of adsorption, known as surface modifier.

  2. **Entrapment:** Here enzymes are not actually allowed to attach over the surface of carrier, rather they are entrapped within the polymeric matrix (like gel). In external appearance these are looks like larger beads inside of it enzyme particles are located.

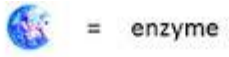


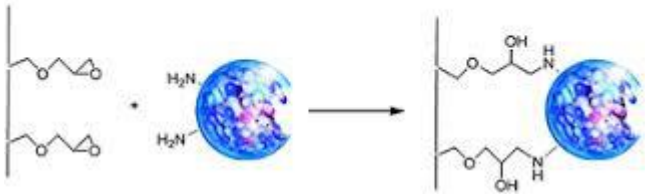
(a) Entrapment (b) Encapsulation

3. **Encapsulation:** In this type of immobilization, enzymes are allowed to enclose within a semi permeable membrane, generally these membranes form minute beads like structures inside of which enzyme particles are located. It looks like smaller version of Entrapment method.

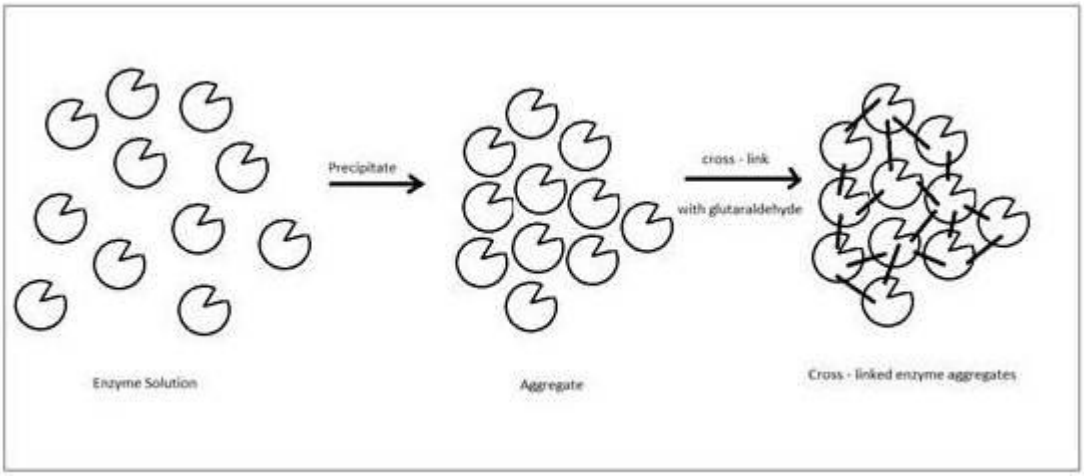
❖ **Chemical Immobilization:** This type of immobilization involves with the formation of fixed chemical bonds between carrier particle and enzyme ones.

1. **Covalent Bonding**: Here, water insoluble chemical bonds are developed in between carrier and enzymes. Formation of chemical bonds are depends upon some specific functional group of protein (enzyme) like- Amino group, Carboxyl group, Hydroxyl group etc. These groups react with the surface material of carriers. So, for this technique specialized carrier components which bears reacting groups on their surface.





2. **Cross Linking**: It is the most unique type of immobilization method where no carrier components are needed. It takes place through the allowing the enzymes particles to react with each other to form a macro molecular form which is easily separated from the system. But here aggregation of enzyme particles should takes place such a way so that their active sites remain free.



- All methods of immobilization have some advantages as well as disadvantages –

Name of the Process	Advantages	Disadvantages
Adsorption	Simple most process.	Less surface area for adsorption.
	Enzymatic activity almost consistent.	De-adsorption results less productivity.
Entrapment + Encapsulation	No chemical modification of enzyme	Enzymes may leak the process.
	Relatively stable form of attachment	Presence of active site inside reduces productivity.
Covalent Bonding	No leakage of enzyme	Sometimes protein structure

		of enzyme is modified due to reaction.
Cross Linking	Stable structure.	It may change the active site of the enzyme.

- **Applications of Immobilized enzymes In Industrial purpose:**

A. Application of Glucose Isomerase:

1. It is used in preparation of syrup in pharmaceuticals. This type of syrup is generally known as High Fructose Corn Syrup (HFCS).
2. Also it is applicable for industrial production of Ethanol.
3. Xylitol is the principal sugar component of the chewing gums which is obtained from the Glucose isomerase.

B. Application of Penicillin Acylase:

1. It is used in production of 6 - Amino Penicillanic Acid (6-APA) which is functionally acts as semi synthetic antibiotic.
2. This enzyme is responsible for the maintenance of peptide bonds between amino acids through reacting with amino groups.
3. It resolves the raceme mixtures of chiral compounds including amino acids, amine, β - amino esters and secondary alcohols in aqueous and anhydrous organic media.

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