IMMOBILIZED ENZYMES

PRESENTED TO:

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INTRODUCTION

- As enzymes are biological catalyst that promote the rate of reactions but are not themselves consumes in the reactions; they may be used repeatedly for as long as they remain active.
- However in most of the processes, enzymes are mixed in a solution with substrates and cannot be economically recovered after the reaction and are generally wasted.
- Thus, there is an incentive to an immobilized or insolubilized form so that they may be retained in a biochemical reactor for further catalysis.

What is enzyme immobilization?

- Immobilization is defined as the imprisonment of cell or enzyme in a distinct support or matrix. The support or matrix on which the enzyme are immobilized allows the exchange of medium containing substrate or effectors or inhibitor molecules.
- e.g: AMINO ACYLASE of Aspergillus oryzae for the production of L-amino acids in Japan.

Advantages of immobilized enzymes-

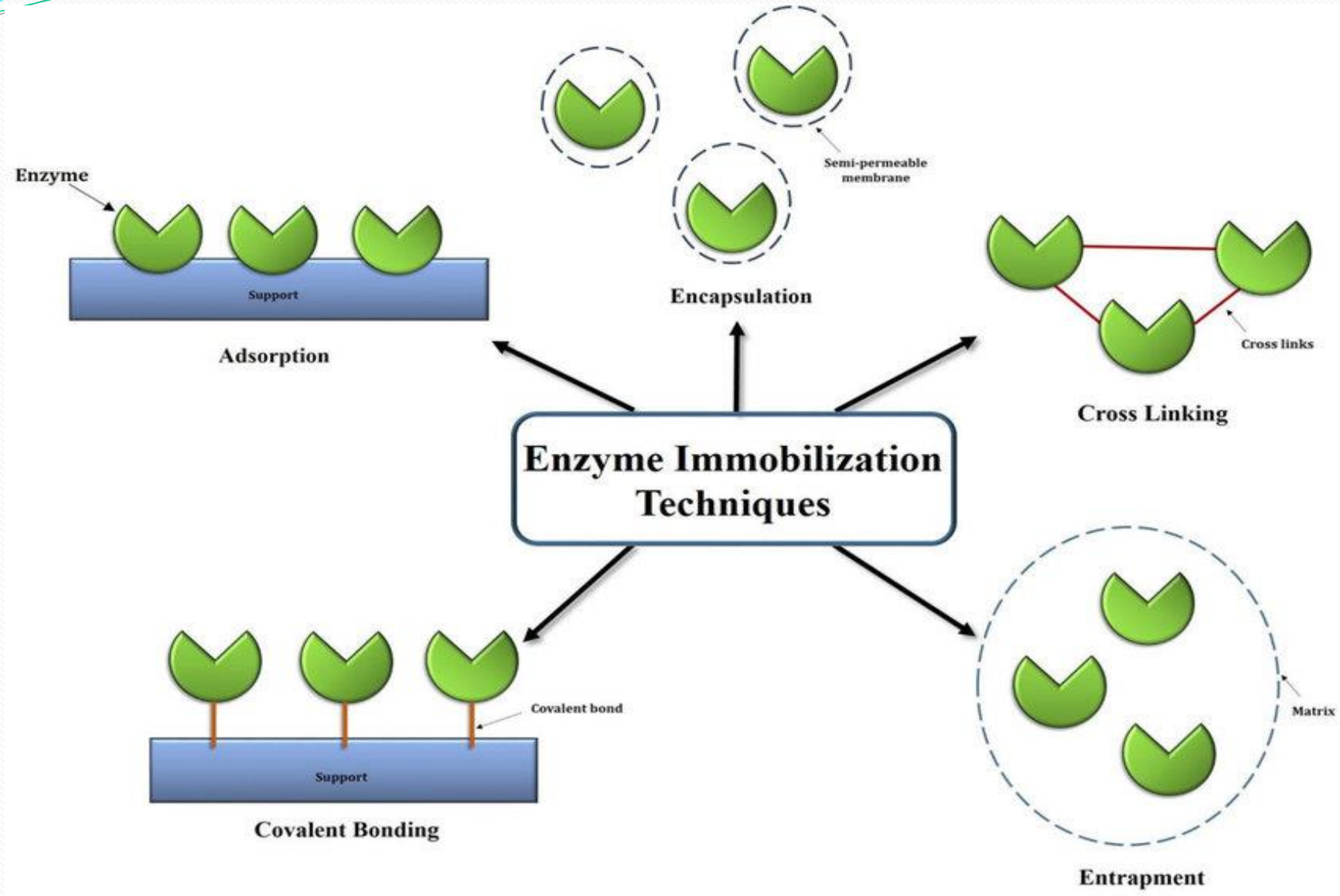
- Increased functional efficiency of enzyme.
- Enhanced reproducibility of the process they are undertaking.
- Reuse of enzyme.
- Saving in capital cost and investment of the process.
- Minimum reaction time.
- Less chance of contamination in product.
- More stability of product.
- High enzyme substrate ratio.
- Improved process control.

Disadvantages of immobilized enzyme-

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

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**


Enzyme
Support
Adsorption
Semi-permeable membrane
Encapsulation
Cross links
Cross Linking
Enzyme Immobilization Techniques
Covalent bond
Support
Covalent Bonding
Matrix
Entrapment

1.ADSORBTION-

- 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)

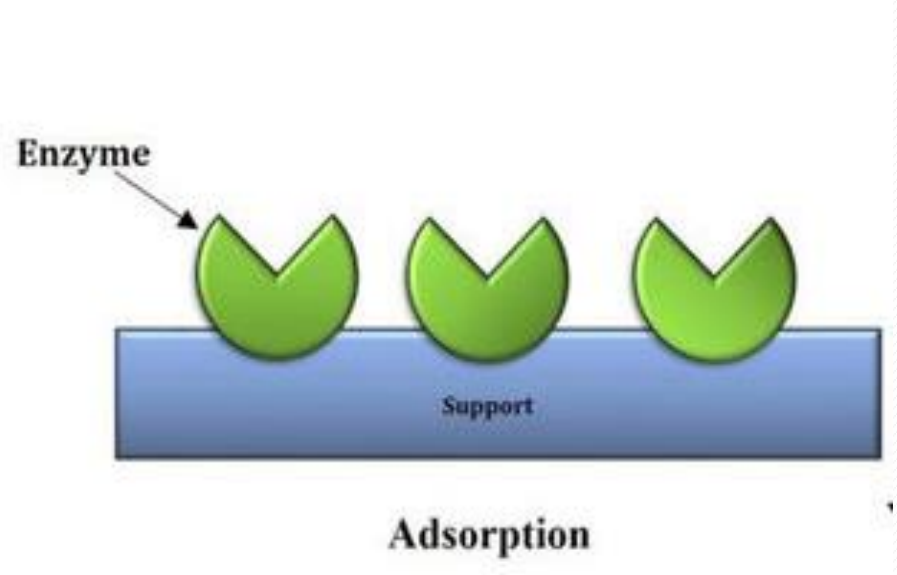
(3). Modified sepharose and ion exchange resins

- 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 :

(a). Ionic interaction

(b). Hydrogen bonds

(c). Vander Waal forces



2. COVALENT BONDING-

- This method involves the formation of covalent bonds between the chemical groups in enzyme and to the chemical groups on the support or carrier.
- It is one of the widely used methods of enzyme immobilization.
- Hydroxyl groups and amino groups of support or enzyme form covalent bonds more easily.
- Support or carrier that can form covalent bonds are amino groups, imino groups, hydroxyl groups, carboxyl groups, thiol groups, methylthiol groups, guanidyl groups, imidazole groups and phenol ring.

Covalent Bonding

3.ENTRAPMENT-

- In this method enzymes are physically entrapped inside a porous matrix. Bonds involved in stabilizing the enzyme to the matrix may be covalent or non-covalent.
- The matrix used will be a water soluble polymer. The form and nature of matrix varies with different enzymes.
- Pore size of matrix is adjusted to prevent the loss of enzyme. Pore size of the matrix can be adjusted with the concentration of the polymer used
- Examples of commonly used matrixes fc

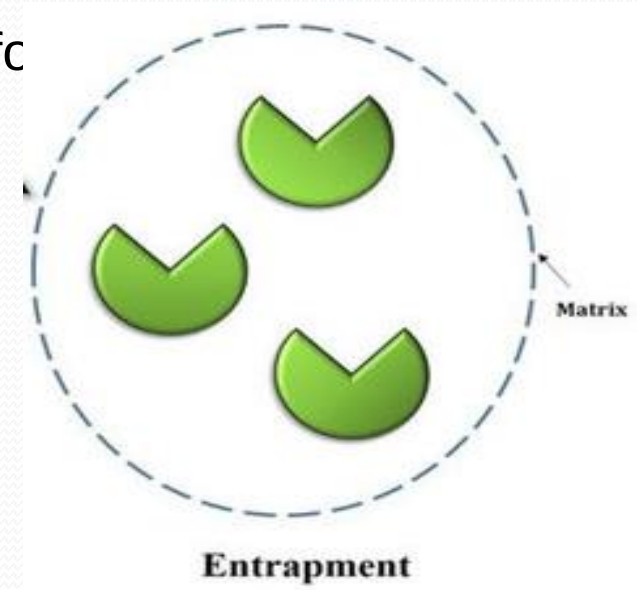
(1). Polyacrylamide gels

(2). Cellulose triacetate

(3). Agar

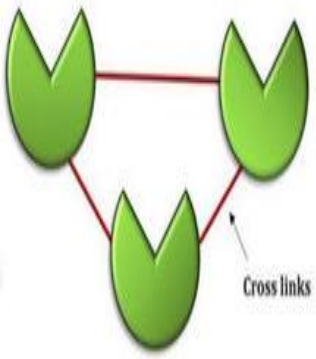
(4). Gelatin

(5). Carrageenan



4.CROSS LINKAGE (COPOLYMERIZATION)-

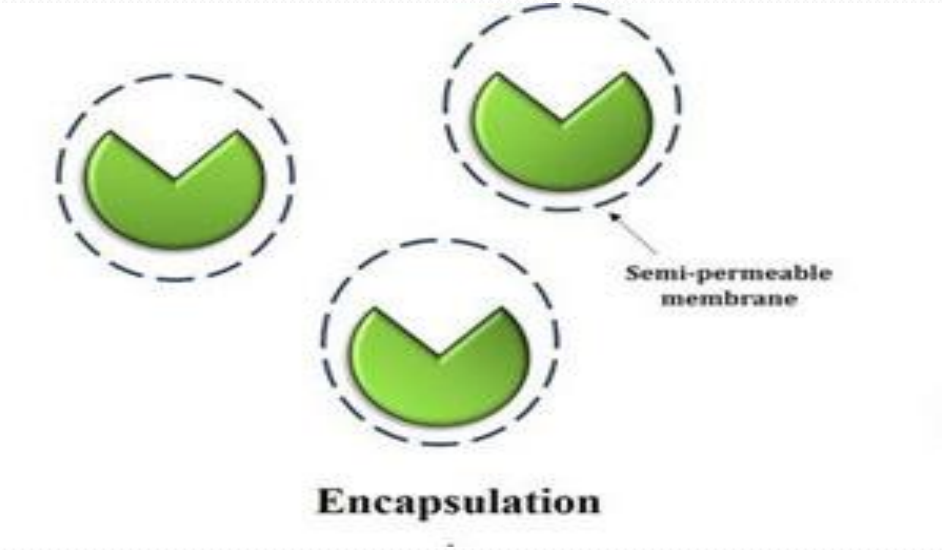
- This method is also called as copolymerization. In this method of immobilization enzymes are directly linked by covalent bonds between various groups of enzymes via polyfunctional reagents.
- Commonly used polyfunctional reagents are glutaraldehyde and diazonium salt.
- This technique is cheap and simple but not ofter used with pure enzymes.
- This method is widely used in commercial preparations and industrial applications.



Cross Linking

5.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.



Applications of Enzyme Immobilization-

1. Industrial production of antibiotics, beverages, amino acids etc. uses immobilized enzymes or whole cells.
2. In food and drink industry.
3. 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.
4. Treatment of sewage and industrial effluents.
5. In washing powder industry.

THANK YOU