SNS COLLEGE OF TECHNOLOGY

(An Autonomous Institution)
Approved by AICTE, New Delhi, Affiliated to Anna University, Chennai
Accredited by NAAC-UGC with 'A++' Grade (Cycle III) &
Accredited by NBA (B.E - CSE, EEE, ECE, Mech & B.Tech.IT)
COIMBATORE-641 035, TAMIL NADU

DEPARTMENT OF FOOD TECHNOLOGY

23FTT204- BIOCHEMISTRY & NUTRITION

UNIT V – ENZYMES

Factors affecting enzyme action, Immobilization methods & Selected application of enzymes in food Industries

Enzyme activity is affected by a number of factors including:

- **The concentration of enzyme**: Assuming a sufficient concentration of substrate is available, increasing enzyme concentration will increase the enzyme reaction rate.
- **The concentration of substrate**: At a constant enzyme concentration and at lower concentrations of substrates, the substrate concentration is the limiting factor. As the substrate concentration increases, the enzyme reaction rate increases. However, at very high substrate concentrations, the enzymes become saturated with substrate and a higher concentration of substrate does not increase the reaction rate.
- **The temperature**: Each enzyme has an optimum temperature at which it works best. A higher temperature generally results in an increase in enzyme activity. As the temperature increases, molecular motion increases resulting in more molecular collisions. If, however, the temperature rises above a certain point, the heat will denature the enzyme, causing it to lose its three-dimensional functional shape by denaturing its hydrogen bonds. Cold temperature, on the other hand, slows down enzyme activity by decreasing molecular motion.
- **The pH**: Each enzyme has an optimal pH that helps maintain its three-dimensional shape. Changes in pH may denature enzymes by altering the enzyme's charge. This alters the ionic bonds of the enzyme that contribute to its functional shape.
- **The salt concentration**: Each enzyme has an optimal salt concentration. Changes in the salt concentration may also denature enzymes.

Some relationships between bacterial enzymes and the use of disinfectants and extremes of temperature to control bacteria.

1. Many disinfectants, such as chlorine, iodine, iodophores, mercurials, silver nitrate, formaldehyde, and ethylene oxide, inactivate bacterial enzymes and thus block metabolism.

2. High temperatures, such as autoclaving, boiling, and pasteurization, denature proteins and enzymes.
3. Cold temperatures, such as refrigeration and freezing, slow down or stop enzyme reactions.

Immobilization methods:

Definition: Immobilization can define as the enticement of an enzyme to a solid support. Enzyme immobilization is a process, which encloses the enzyme molecules to an absolute phase from a bulk phase. The bulk phase consists of substrates, effectors and inhibitors. Enzyme molecule impounds in or on some suitable matrix that is having a definite porosity.

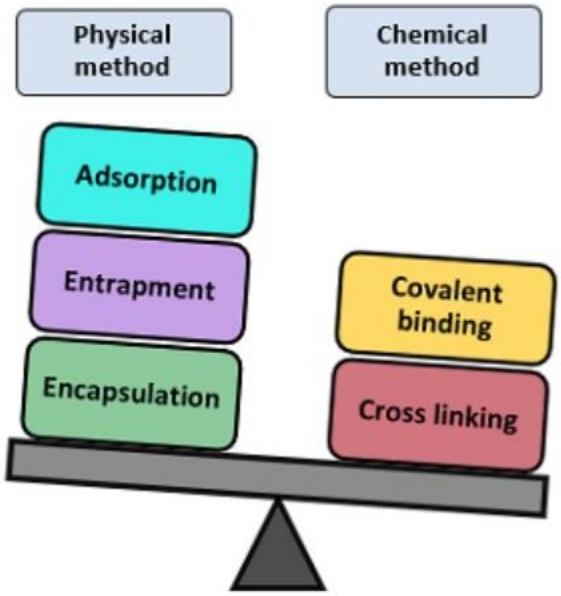
The carrier or matrix allows the exchange of medium (contains substrate and product), to which an enzyme molecule can be confined. Immobilization restricts the movement of enzymes. It is a prevalent method, which is now used in many fields like industrial, medical, bioresearch, food science etc.

Therefore, the biocatalyst that gets confined to the inert material will be termed as an immobilized enzyme. The methods which facilitate the enzyme entrapment into or on the support matrix is called immobilization of an enzyme. Amino acylase was the first immobilized enzyme.

Here, we will look into some important topics related to the context like the discoveries behind the introduction of the immobilization technique, the components, physical and some chemical methods of immobilization. Besides, we will also discuss some of the advantages, disadvantages and applications of the immobilization technique.

Methods of Enzyme Immobilization:

Based on the binding property, it classifies into physical and chemical processes.

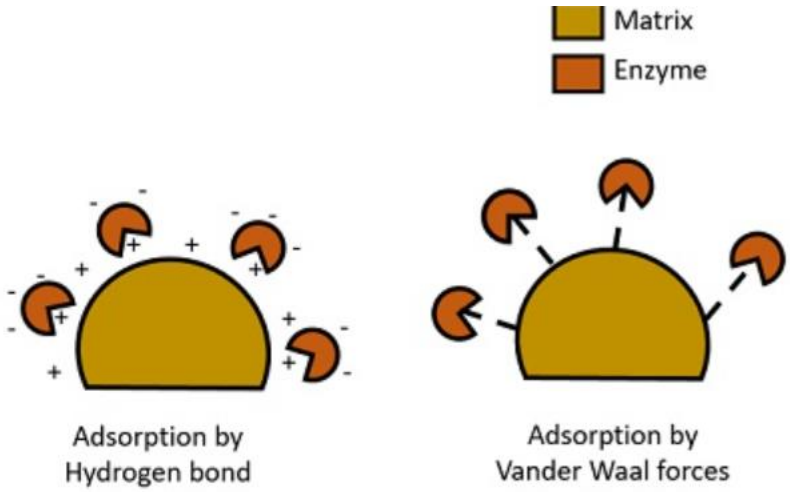


Adsorption:

It is the oldest and simplest method. In this type, enzyme adheres to the surface of the water-insoluble carrier matrix. The binding is nonspecific like electrostatic or hydrophobic affinity binding to a particular ligand. The binding between enzymes and the carrier matrix is usually firm, but it gets weakened by many factors like:

- Addition of substrate
- pH or ionic strength

In enzyme adsorption, the bonding is non-permanent and accomplished by the weak bonds, mainly like hydrogen bond and Vander Waal forces.



The matrix used: The matrix's particle size must be small (500Å-1mm D).

Examples: Different carrier materials are used in this type like:

- Mineral support (E.g. Aluminium oxide, clay)
- Organic support (E.g. Starch)
- Modified sepharose and ion exchange resins

Methods of Immobilization by Adsorption:

1. Static method: It is an efficient but time-consuming method. It involves immobilization of enzyme and carrier molecule without agitation.

2. Dynamic process: It involves the mixing of an enzyme with the carrier, under constant agitation.

3. Reactor loading: It involves transferring of both enzyme and carrier in the reactor with the agitation of the whole content. It is widely used for the commercial production of the immobilized enzyme.

4. Electro-deposition: Here, a carrier is kept proximal to the electrode in an enzyme bath, after which an electric current is passed through it. It results in the movement of an enzyme towards the carrier. At last, the enzyme gets deposited on the surface.

Advantages of Adsorption:

- It has no pore diffusion limitation.
- It is a simple and economical method to conduct.
- No reagents are required in this method.
- There is a limited loss of enzyme activity.
- It causes less disruption to an enzyme.
- It requires minimum activation steps.
- The adsorped enzyme can be recycled, regenerated and reused.
- It has a high loading efficiency of an enzyme.

Disadvantages of Adsorption:

- It provides low surface area for the enzyme binding.
- Desorption of an enzyme from the carrier usually occurs.
- Also, the yield is low.

Entrapment

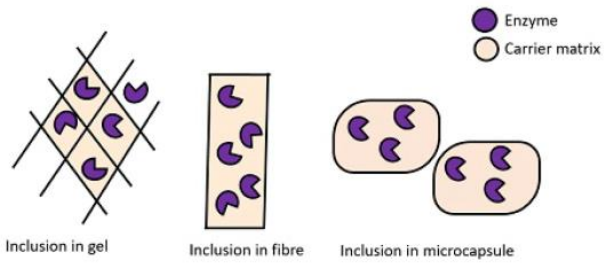
An enzyme traps inside a porous polymer or gel matrix during this method. It is also called lattice entrapment. The bonding between an enzyme and matrix can be covalent or non-covalent. The matrix used: It is water-soluble, and nature varies with different enzymes.

Examples: It makes the use of the following carrier materials like polyacrylamide gels, cellulose triacetate, agar, gelatine, alginate etc. Methods of enzyme entrapment: It involves the inclusion of an enzyme into the following matrices:

1. Gels: Involves entrapment of an enzyme inside the gel matrix.

2. Fibres: Entrapment of an enzyme inside the fibre matrix.

3. Microcapsules: Involves entrapment inside a microcapsule.



Advantages of Entrapment Method:

- Its enzyme loading capacity is high.
- It's a rapid method.
- Here, the enzyme distortion is low.
- It is easy to practice.

Disadvantages of Entrapment Method:

- The diffusion of substrate and product create difficulties.
- It causes leakage of low molecular weight enzymes.
- There might some chances of microbial contamination.
- It also causes enzyme inactivation and sometimes loss of enzyme activity.
- It has limited industrial use.

Encapsulation

It is the membrane confinement method. An enzyme confines within the semipermeable membrane of a capsule in an aqueous solution. This process allows the exchange of medium (substrate & product), but not an enzyme. The effectiveness of encapsulation relies upon the enzyme stability.

The matrix used: The capsule is made of a semi-permeable membrane, and it can be polymeric, lipoid, non-ionic etc. in nature. Examples: It includes nitrocellulose, nylon semi-permeable matrix etc.

Methods of encapsulation: It can be achieved by the following ways:

1. Encapsulation in a reaction vessel: It involves the partitioning of a chamber by a semipermeable membrane. One chamber contains enzymes, whereas the other contains substrate and product.

2. Encapsulation by hollow fibre membrane: It involves entrapment of an enzyme inside a semipermeable matrix (cellulose, triacetate etc.). Here, an enzyme traps inside the space of the matrix.

3. Microencapsulation: By chemical polymerization, the enzyme molecules enclose inside a microcapsule by the use of 1-6- diaminohexane.

4. Encapsulation by liposomes: Here, an enzyme binds to the concentric lipoidal membrane of the liposome by the use of phospholipid.

Advantages of Encapsulation:

- There is no enzyme leakage.
- It does not affect enzyme activity.
- It’s a simple method to conduct.
- It possesses a high loading efficiency of an enzyme.

Disadvantages of Encapsulation:

- It makes the use of a carrier that has a pore size limitation.
- It is not so cost-effective.

Covalent Binding

It is a widely used method. An enzyme molecule binds to the carrier by a covalent bond during this process. Here, the binding strength is powerful or a complex form through this bonding is stable. Also, there is no enzyme loss during the process. Covalent binding occurs between the active part, i.e. the functional group of an enzyme and the carrier molecule. The functional group that are participating in the binding process are -NH , -NH -COO, -OH, -SH, -O, -S etc. The order of reactivity of these functional groups to the carrier depends upon their charged status:

-S > -SH > -O > -NH > -COO > -OH >> -NH

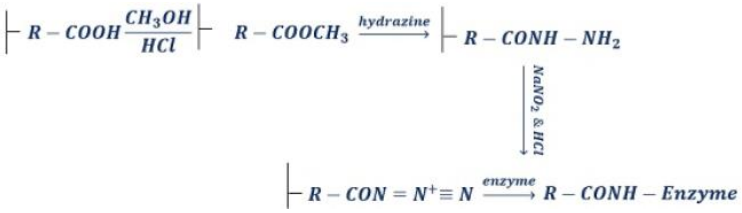
Examples of a polymeric carrier used in covalent binding are as follows: Carboxylic acid and related groups of polyglutamic acid, Amide group of a polypeptide, Amino and related groups of polysaccharides

Some most commonly used polymers are the polysaccharide (celluloses, agarose, sepharose, etc.), polyvinyl alcohol, silica and porous glasses.

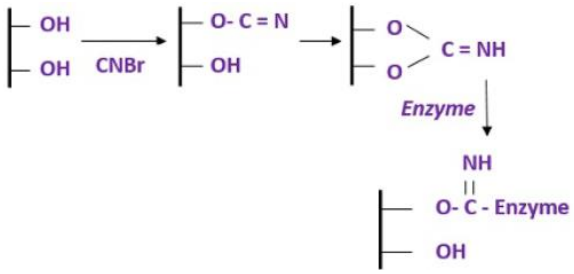
Methods used for Covalent Binding: It involves the following methods that are given below.

1.Diazotation: It involves bonding between the amino group of the matrix and tyrosyl or histidyl group of an enzyme on the reaction with NaNO and HCl.

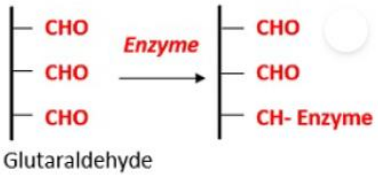
2. By peptide bond: It involves bonding between amino or carboxyl group of the matrix to the carboxyl or amino group of an enzyme. During this, a matrix is chemically treated to bind with the active functional group.



3.Cyanogen bromide activation: It involves the binding of glycol groups of a matrix with an enzyme by the activation of CNBr.



4. By polyfunctional reagents: This process involves bonding between the amino group of the matrix and amino group of an enzyme.



Example: Glutaraldehyde (Bi-functional reagent).

Advantages of Covalent Binding:

- It provides a high binding strength between an enzyme and a carrier.
- There is no enzyme leakage.
- It's a simple and widely used method.
- The process is not affected by the pH or ionic strength.

Disadvantages of Covalent Binding:

- Denaturation of the enzyme occurs during the immobilization.
- Only a small amount of enzyme can be immobilized.
- It is not so cost-effective.

Cross Linking

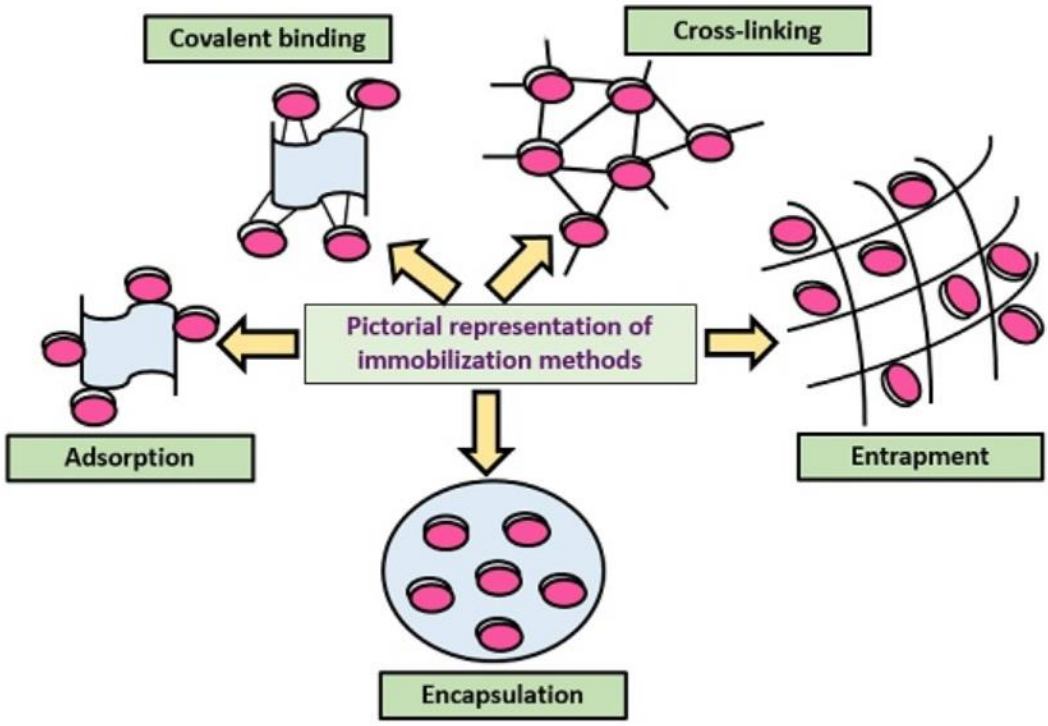
It is also called "Copolymerization". Here, the immobilized enzymes covalently link to the various groups of an enzyme via polyfunctional reagents. It does not require a support matrix. Cross-linking leads to the formation of 3D crosslinked aggregates. The most commonly used polyfunctional agents are glutaraldehyde and diazonium salts etc.

Advantages of Cross Linking Method:

- There is little or no enzyme leakage.
- It yields a highly stabilized enzyme.
- It's a simple and cheap method to carry out.
- It has wide applicability in the commercial production of the enzyme.

Disadvantages of Cross Linking Method:

- It causes enzyme inactivation.
- The polyfunctional reagents used in this process generally cause enzyme denaturation.
- It is not so cost-effective.



Selected application of enzymes in food Industries

Enzymes are vital for all biological processes, aiding in digestion, and metabolism. Besides, these are also involved in several other processes;

1. Enzymes like kinases and phosphatases are important for cell regulation and signal transmission.

2. Different enzymes are produced throughout the body for the regulation of reactions involved in various metabolic pathways.

3. The activation and inhibition of enzymes resulting in negative feedback mechanism adjust the rate of synthesis of intermediate metabolites according to the demands of the cells.

4. They also catalyze post-translational modifications involving phosphorylation, glycosylation, and cleavage of the polypeptide chain.

5. Some enzymes are also involved in the regulation of enzyme levels by changing the rate of enzyme degradation.

6. Since a tight regulation of enzymes is essential for homeostasis, any changes in the enzyme structure and production might result in diseases.

7. Enzymes synthesized in various organisms are also utilized in various industries for wine production, cheese production, bread whitening, and designing fabrics.