BP 605 T. Pharmaceutical Biotechnology (Theory)

Enzyme Biotechnology- Methods of Enzyme Immobilization and Applications

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Overview

- Definition and Introduction
- Enzyme uses and application
- Enzyme immobilization

ENZYME TECHNOLOGY

Enzyme technology is the study of industrial enzymes and their uses

- ✓ **Enzymes** are the **functional proteins** or **nucleic acids (Ribozymes)**, also known as **biocatalysts** that facilitate the execution of biochemical reactions at the rates which are suitable for the normal functioning, growth, and proliferation of any living system, including unicellular or multicellular plants as well as animals.

- ✓ The enzymes works in **mild temperature, pressure, pH, substrate specificity under suitable reaction conditions** and for the **production of desired products without any intermediate products** as contaminations.

- ✓ Enzyme are used in **variety of application** such as cosmetics, paper industry, textile industry, food industry, **pharmaceutical industry**, laundry and in detergents etc

THE USE OF ENZYMES IN MEDICINE

Due to the catalytic activity of enzymes they are able to be used in a variety of ways.

- ✓ The uses of important **enzymes in medicine** include

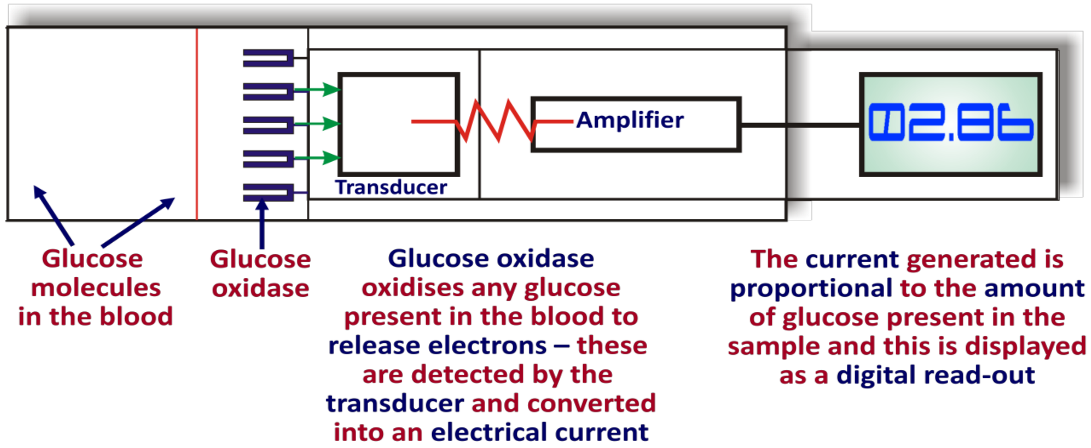
- killing disease-causing micro-organisms,
- prompting wound healing. Eg. Collagenases
- diagnosing certain diseases. Eg. Diabetes

- ✓ Pharmaceutical enzyme applications generally require small quantities of highly purified enzymes.
- ✓ Enzyme-generated products are administered to patients in very small doses; this is in order to avoid possible side effects

ANALYTICAL USES

- ✓ Enzymes can be used to detect and measure amounts of glucose in blood.
- Amount of glucose in blood and urine is an indicator for diagnosis of diabetes.
- Detected by using **enzyme glucose oxidase**.
- Catalyses reaction between glucose and oxygen to form gluconic acid.
- Biosensor measures the produced gluconic acid and then indicated by a colour change.

A biosensor has been developed for detecting glucose in the blood of diabetics



ANALYTICAL USES ..Contd.

- ✓ **Alcohol dehydrogenase** one of the most useful enzymes for bioanalytical applications. There are many
- ✓ Other examples of enzymes that can be used in diagnosis.
- **Arginase:** for L-arginine levels in plasma and urine
- **Cholesterol esterase:** for serum cholesterol levels
- **Creatine kinase:** for cardiac and skeletal malfunction

THERAPEUTIC

- ✓ Can be administered individually or along with other drugs and/or treatments.
- ✓ Enzyme supplements for enzyme deficiencies.

- **Prolactazyme** treats lactose intolerance.
- **Collagenase** treats skin ulcer.
- **Asparaginase** used to treat leukemia.
- **Streptokinase** administered to patients immediately after heart attacks.

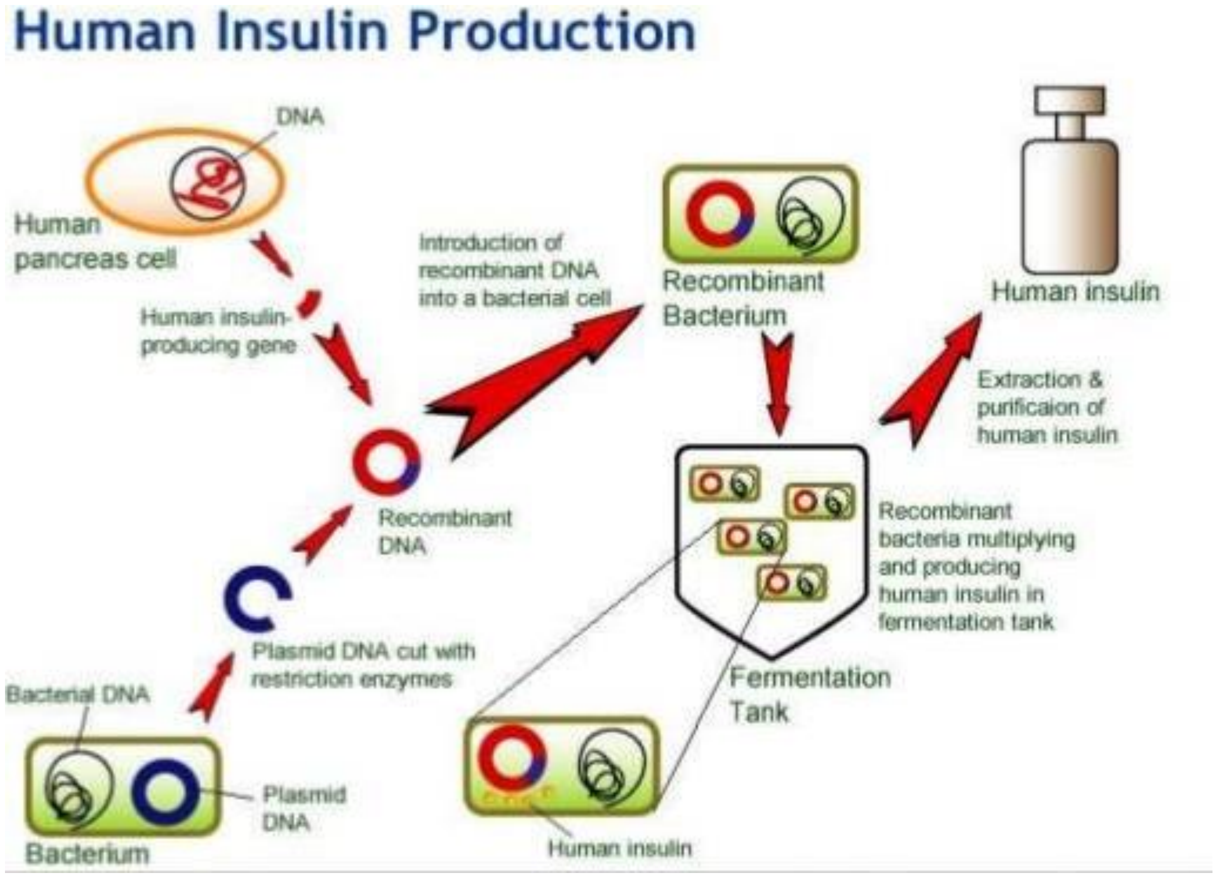
Enzyme	Reaction	Treatment
Collagenase	Collagen hydrolysis	Skin ulcers
Glutaminase	L-Glutamine H_2O → L-glutamate + NH_3	Leukaemia
Lysozyme	Bacterial cell wall hydrolysis	Antibiotic
α-Lactamase	Penicillin → penicilloate	Penicillin allergy
Streptokinase	Plasminogen → plasmin	Blood clots
Trypsin papain	Protein hydrolysis	Inflammation Breaking down venom
Urokinase	Plasminogen → plasmin	Blood clots

PHARMACEUTICALS INDUSTRIES

- ✓ The enzymes are playing important role in pharmaceutical industries. They can be used as drugs

❖ **Insulin**

- This hormone is essential for controlling blood sugar. Diabetes mellitus is a disorder which results due to deficiency of hormone insulin. Since from 1922 this hormone is being used in treatment for diabetes mellitus.



PHARMACEUTICALS INDUSTRIES…. Contd.

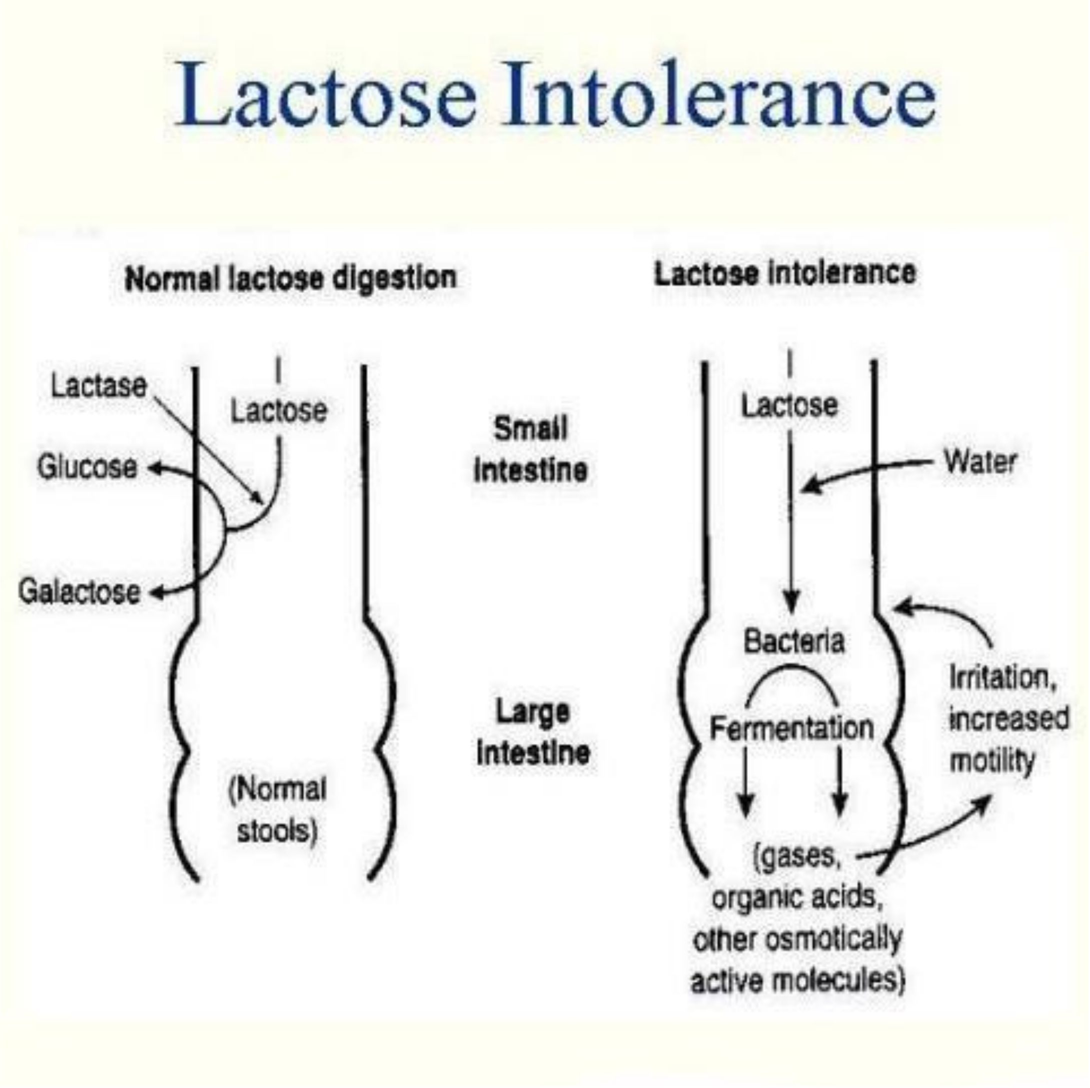
- **Protease Capsules:** Another important enzyme based pharmaceutical product is protease capsule. It provides therapeutic benefits by

- enhancing circulatory and immune systems,
- quicker healing
- better stamina.

PHARMACEUTICALS INDUSTRIES…. Contd.

❖ **Lactose Intolerance:**

- Lactose intolerance is the inability to digest lactose. So there are Lactase enzyme supplements for this disorder which are better alternative.

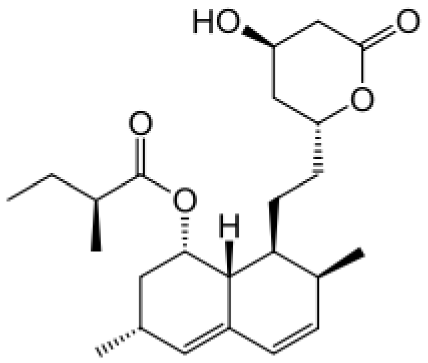


PHARMACEUTICALS INDUSTRIES.... Contd.

- ✓ **Penicillin G/V acylase and glucose isomerase:** are just two of the many enzymes used in the pharmaceutical industry.
- ✓ Penicillin acylase and glucose isomerase aid in the production of semi synthetic penicillin and fructose syrup.

- ✓ **However, Pharmaceutical enzymes may contain minor to severe side effects.**

Examples; Statin (inhibiting enzyme HMG-CoA reductase)



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ENZYME IMMOBILISATION

- ✓ Immobilization of enzyme defined as **imprisonment of cell or enzyme** in a **distinct support or matrix**
- ✓ It is a process where an enzyme makes **use of carrier phase for safe homing**.
- ✓ The support or matrix on which the enzymes are immobilized allows the exchange of medium containing substrate or effector or inhibitor molecules.
- ✓ The enzymes have various other limitations such as low stability, highly sensitive
- ✓ The process conditions and these problems can be overcome by the immobilization techniques.
- ✓ Immobilized enzymes are being used since 1916, when Nelson and Griffin discovered that invertase when absorbed to charcoal has the ability to hydrolyse the sucrose
- ✓ First immobilized enzyme was **amino acylase** of ***Aspergillus oryzae*** for the production of L-amino acids (Japan)

ENZYME IMMOBILISATION..contd.

The use of enzyme in industrial application is limited because;

- Enzyme are relatively unstable,
- Need of high cost isolation
- Purification and recovery of active enzyme from reaction mixture after the completion of catalytic process.

✓ For soluble enzyme in batch operation is uneconomical as active enzyme is lost after each reaction.

✓ Some of enzyme are rapidly inactivated by heat and become heat stable by attachment to inert polymeric supports.

- Immobilized enzyme can be reused. process can be repeated continuously and be radically controlled.
- easily separated and enzyme properties can be preserved.

ENZYME IMMOBILISATION..contd.

Advantages of the immobilized enzyme;

- Increased functional efficiency of enzyme,
- Enhanced reproducibility of the process the are undertaking
- Reuse of enzyme.
- Continuous of enzyme
- Less labour input in the processes
- Saving in capital cost and investment of the process
- Minimum reaction time
- Less chance of contamination in the final products
- More stability of products
- Improved process control with a high quality enzyme (enzyme:substrate)

ENZYME IMMOBILISATION..contd.

Disadvantages of the enzyme immobilization;

- **High cost for the isolation, purification and recovery of active enzyme**
- Limited industrial application
- Catalytic properties of some enzymes are reduced or completely lost after their immobilization on support carrier.
- Some enzymes become unstable after immobilization
- Enzymes become inactive due to heat generation

ENZYME IMMOBILISATION..contd.

✓ **Applications of enzyme immobilization:**

- **Industrial Production -** antibiotics beverages amino acids etc.
- **Biomedical applications** – For diagnosis and therapy of many diseases; e.g. for treatment of inborn metabolic disorder, - immobilization is effective in drug delivery system.
- **Food Industry** – pectinases and cellulases; immobilized on suitable carrier, e.g. Jams jellies, syrups
- **Research** – enhance the efficacy
- **Production of biodiesel** from vegetable oil
- **Waste water management**
- **Textile Industry** – scouring, biopolishing and desizing of fabrics.
- **Detergent industry** – immobilization of lipase enzyme for effective dirt removal.

METHODS OF ENZYME IMMOBILIZATION

Supports or Matrix used in immobilization technology:

The matrix or support immobilizes the enzyme by holding it permanently or temporarily for a brief period of time. There are a wide variety of matrixes or carriers or supports available for immobilization. The matrix used should be cheap and easily available. Their reaction with the components of the medium or with the enzyme should be minimum as possible. The matrixes or supports for immobilization of enzymes or whole cells are grouped into three major categories

1. ***Natural polymers***
2. ***Synthetic polymers***
3. ***Inorganic materials***

METHODS OF ENZYME IMMOBILIZATION……Contd..

✓ **Natural Polymers:**

- **Alginate** – A natural polymer **derived from the cell wall of some algae**.

  - **Calcium or magnesium alginate** is the most commonly used matrix

  – Inert and have good water holding capacity.

- **Chitosan and chitin – structural polysaccharides**

  - occurs in cell wall of fungi and exoskeleton of Arthropods.

  - Functional groups of enzyme binds to - OH group of chitin.

- **Collagen –** proteinaceous and porous- good water holding capacity – side chain of amino acid forms covalent bonding.

METHODS OF ENZYME IMMOBILIZATION……Contd..

- **Carrageenan –** Sulfated polysaccharide from red Algae

  – gelling property makes it good support

- **Gelatin –** partially hydrolyzed collagen and good support

- **Cellulose** – Most abundant polymer from nature

  - cheapest support

  – OH group on Glucose monomer forms covalent bonds with AA of enzymes.

- **Starch –** A natural polymer of amylose and amylo-pectin

  - Good water holding capacity

- **Pectin –** structural polysaccharides of plants found in their primary cell wall

  - Also act as inter-cellular cementing material in plant tissues

  - Gelling agent with good water holding capacity

METHODS OF ENZYME IMMOBILIZATION……Contd..

- ✓ **Synthetic Polymers:**
  - – Ion exchange resins or polymers
  - - Insoluble supports with porous surface
  - - Porous sulfate can trap and hold the enzyme and/or cells
  - – Example: Diethylaminoethyl (DEAE) cellulose,

    Polyvinyl chloride (PVC),

    UV activated Polyethylene glycol (PEG).

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METHODS OF ENZYME IMMOBILIZATION……Contd..

✓ **Inorganic Materials:**

- **Zeolites** – Microporous aluminosilicate minerals with good adsorbing properties.

  - extensively used for enzyme immobilization.

- **Ceramics –** non metallic solids consists of metal and nonmetal atoms held in ionic and covalent bond

  – composition and bonding pattern varies differently.

- **Diatomaceous earth –** Silicious sedimentary rocks formed by fossilized accumulations of the cell wall of diatoms

  – Celite is the trade name

  - good adsorbent and are resistant to high pH and temperature.

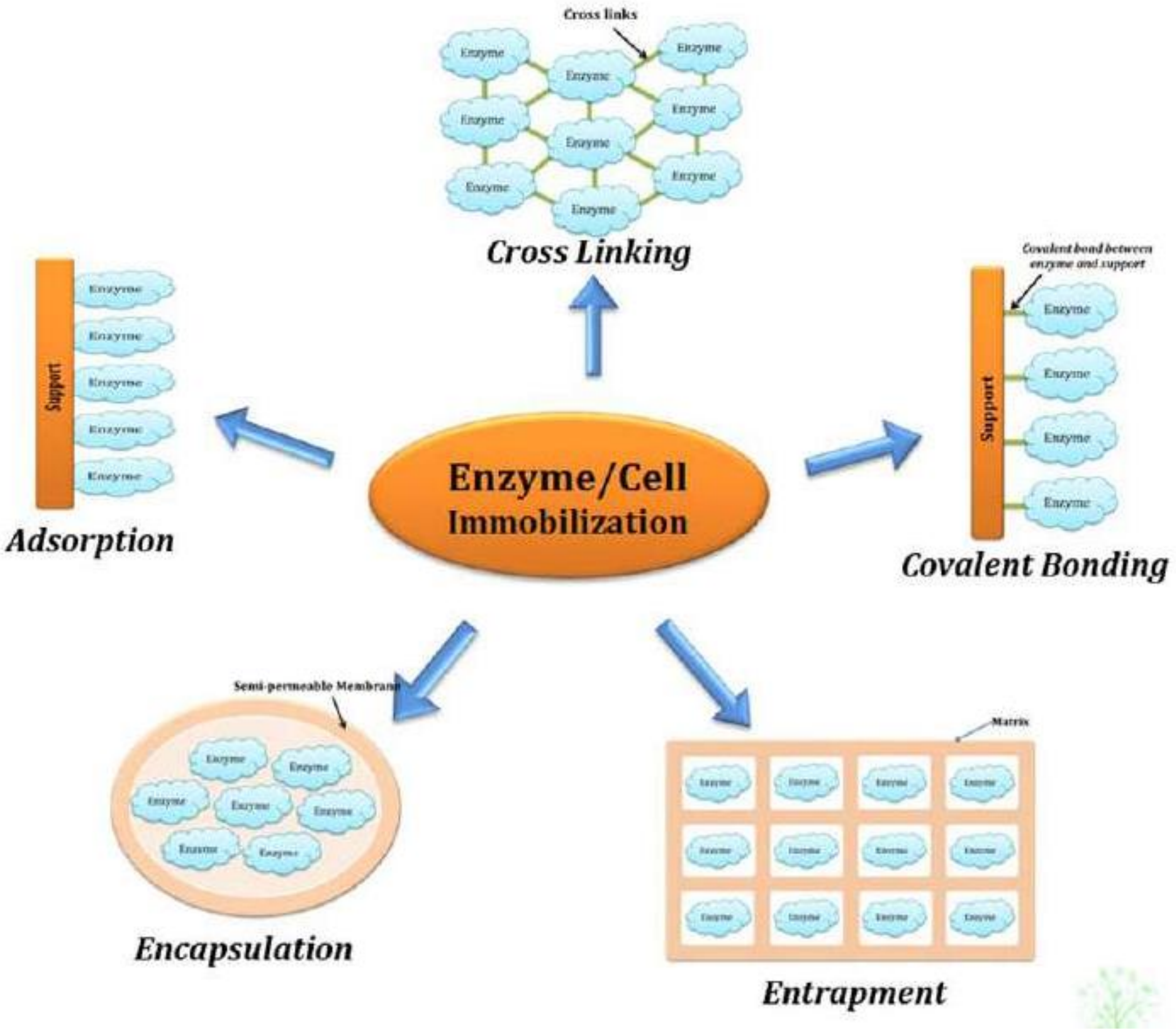
- **Silica –**
- **Glass –**
- **Activated Carbon –**
- **Charcoal -**

METHODS OF ENZYME IMMOBILIZATION……Contd..

✓ **Methods of immobilization:**

Based on the support or matrix types of chemistry (Bonds), there are five different methods of immobilization of enzyme or whole cells

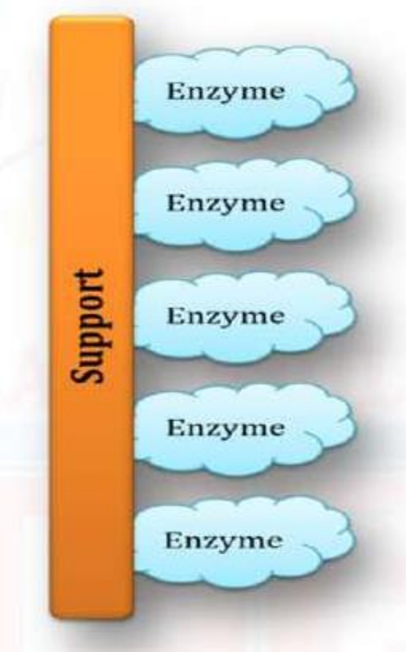
1. **Adsorption**
2. **Covalent Bonding**
3. **Entrapment**
4. **Copolymerization**
5. **Encapsulation**



METHODS OF ENZYME IMMOBILIZATION……Contd..

✓ **Adsorption:** Easiest and oldest immobilization techniques.

- Simple method of preparing an immobilized enzymes
- Materials used for adsorption are **activated charcoal, Alumina, Ion exchange resins**,
- cheap and easy for use.
- Interaction between the enzyme and the surface of the matrix through weak **forces by salt linkage, hydrogen bonds, hydrophobic bonds, ionic bonds and van der waals forces**.
- Based on the charges of the matrix and the protein arrangements the strongly bound, but not distorted enzyme will be formed.
- The advantage of enzyme adsorption is minimum activation step and as a result of minimum activation, no reagents required. It is cheap and easy way of immobilization.
- Disadvantage is a weak binding force between the carrier and the enzyme.

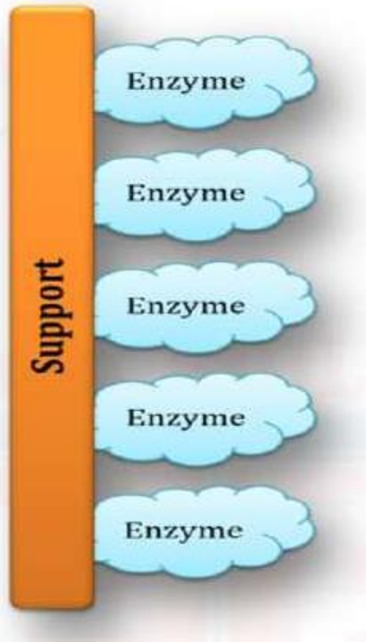


Adsorption

METHODS OF ENZYME IMMOBILIZATION……Contd..

✓ **Methods of Adsorption:**

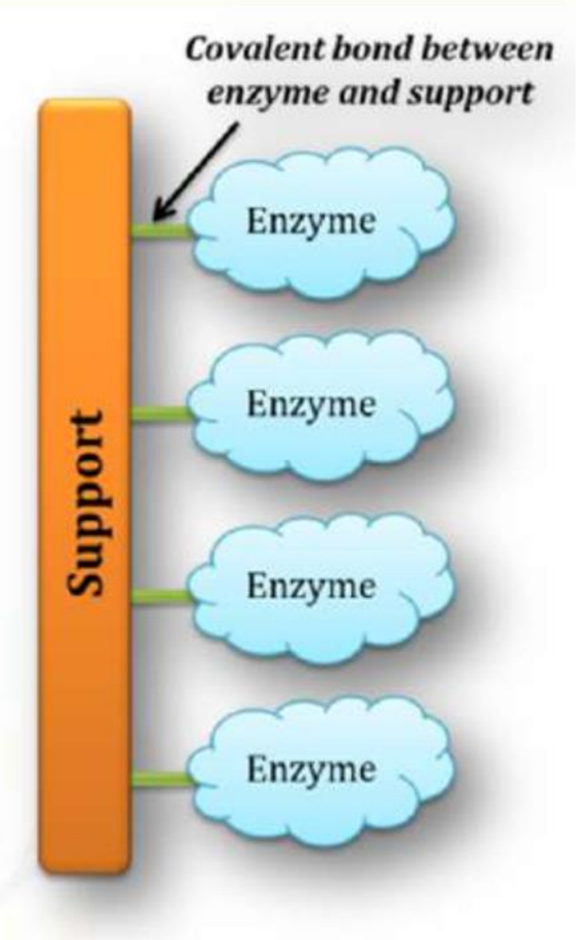
- **Static process –** immobilization to carrier by **allowing the solution containing enzyme to contact the carrier without steering**
- **Dynamic batch process –** Carrier is placed in the enzyme solution and mixed by agitation or stirring
- **Reactor loading process –** Carrier is placed in the reactor, and then the enzyme solution is transferred to the reactor with continuous agitation.
- **Electrode position process –** Carrier is placed near to an electrode in an enzyme bath and then the current is put on, under the electric field the enzyme migrates to the carrier and deposits on surface.



Adsorption

METHODS OF ENZYME IMMOBILIZATION……Contd..

- ✓ **Covalent Bonding:** Involves the formation of covalent bonds between the chemical groups of enzyme with carrier or support
- Widely used method of enzyme immobilization
- **Hydroxyl group (-OH) and amino groups (-NH_2)** of enzyme with carrier or support forms covalent bonds more easily.
- **Other chemical groups** of enzyme and support or carrier for covalent bonding are imino groups, hydroxyl groups, carboxyl groups, thiol groups, guanidyl groups, imidazole groups and phenol ring .

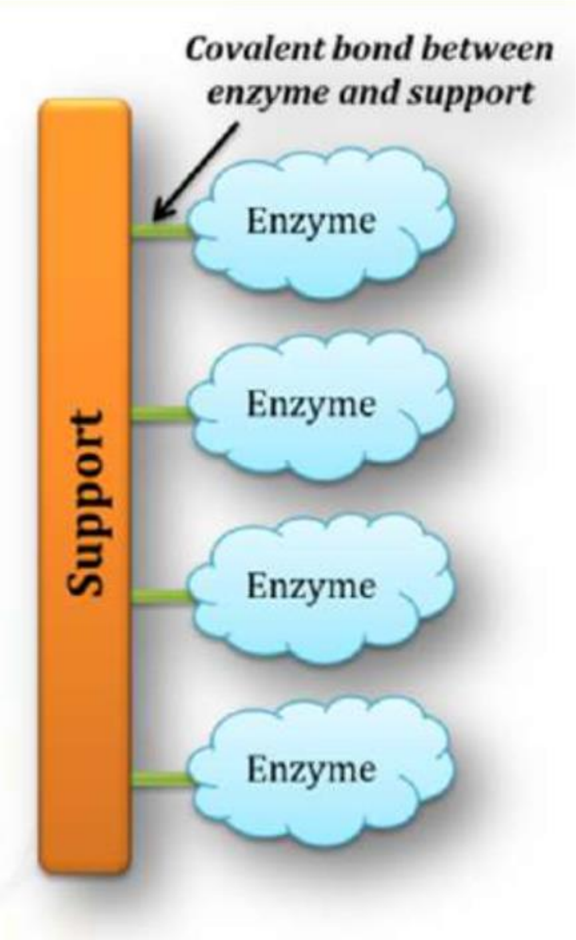


Covalent Bonding

METHODS OF ENZYME IMMOBILIZATION……Contd..

✓ **Important Functional groups of Enzymes:**

- Alpha carboxyl group (C-terminal)
- Alpha amino group (N-terminal)
- Epsilon amino groups of Lysine and Arginine in the enzyme
- B and γ carboxyl groups of Aspartate and Glutamate
- Phenol ring of Tyrosine
- Thiol group of Cysteine
- Hydroxyl groups of Serine and Threonine
- Imidazole group of Histidine
- Indole ring of Tryptophan

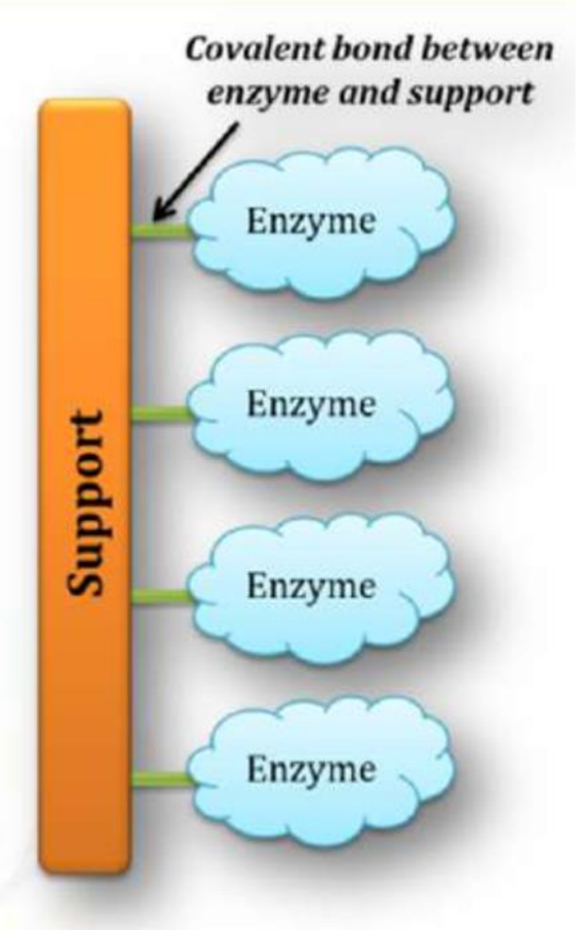


Covalent Bonding

METHODS OF ENZYME IMMOBILIZATION……Contd..

✓ **Carrier support commonly used for covalent bonding:**

- Carbohydrates: Eg. Cellulose DEAE cellulose , Agarose (C-terminal)
- Synthetic agents: Eg. Polyacrylamide
- Protein Carriers: Eg. Collagen, Gelatin
- Amino group bearing carriers: Eg. Amino benzyl cellulose
- Inorganic carriers: Porous glass, silica
- Cyanogen bromide (CNBr)-agarose and CNBr-Sepharose

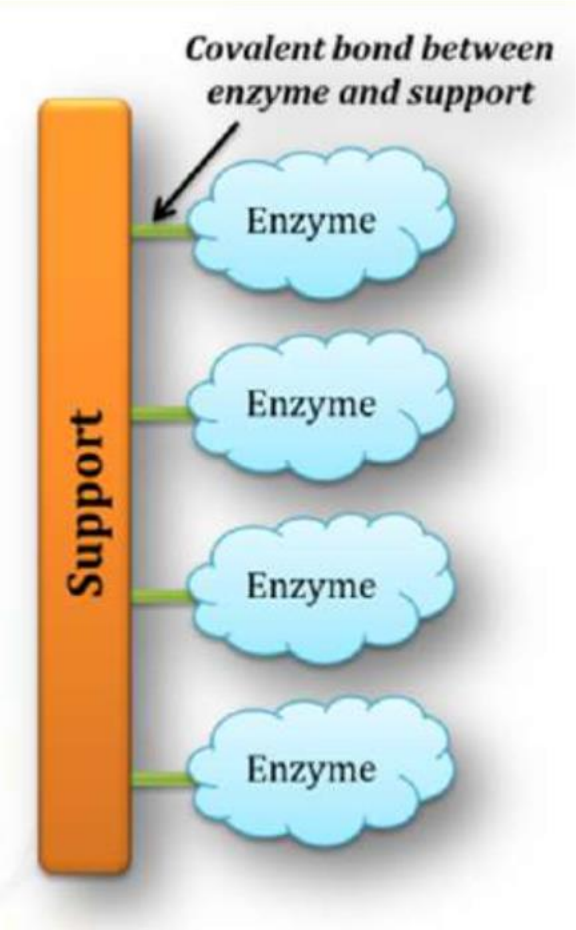


Covalent Bonding

METHODS OF ENZYME IMMOBILIZATION……Contd..

✓ **Methods of covalent bonding:**

- **Diazoation:** bonding between amino groups of support and tyrosine or histidine of enzyme
- **Peptide Bonds:** bonding between amino or carboxyl group of the enzyme and of the support
- **Poly functional reagents**: Use of bi-functional or multifunctional reagent (glutaraldehyde) which forms covalent bonds between amino group of the support and amino groups of enzyme.



Covalent Bonding

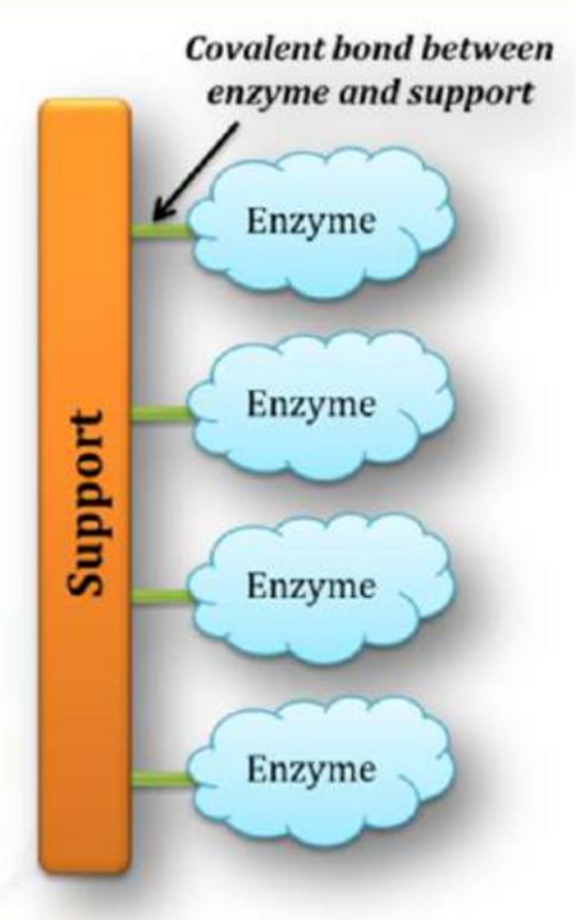
METHODS OF ENZYME IMMOBILIZATION……Contd..

✓ **Advantages of covalent bonding:**

- Strong linkage of enzyme to the support
- No leakage and desorption problem
- Comparatively simple method.
- Availability of variety of support with different functional groups
- Wide applicability

✓ **Disadvantages of covalent bonding:**

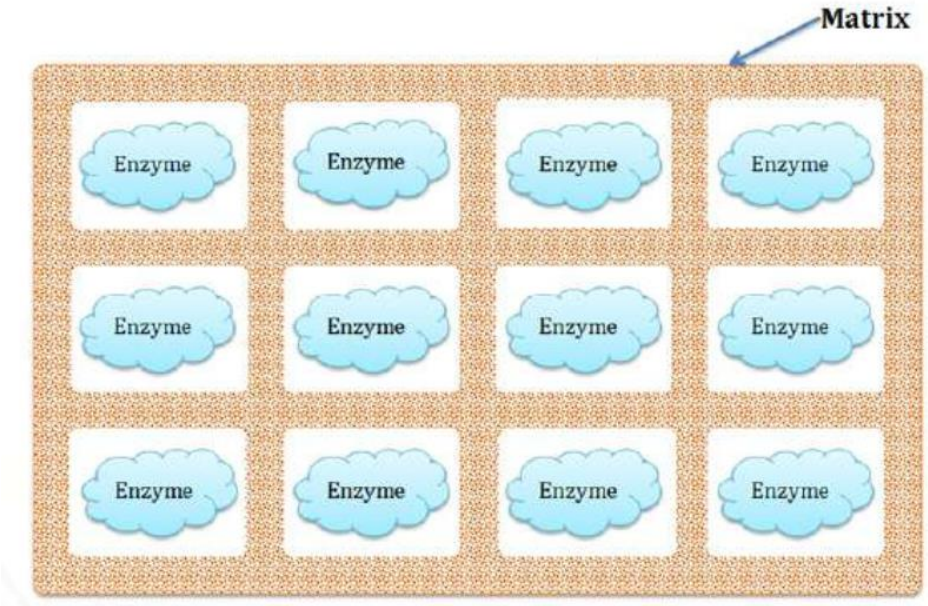
- **Chemical modification leads to conformation changes of enzyme**



Covalent Bonding

METHODS OF ENZYME IMMOBILIZATION……Contd..

- ✓ **Entrapment:** Enzymes entrapped inside the porous matrix
- Bonds for stabilization are covalent or non covalent
- Matrix used are water soluble polymers
- Form and nature of matrix varies with different enzymes.
- Pore size of matrix is adjusted with concentration of polymer used
- ✓ **Commonly used matrix for entrapment:**
- Polyacrylamide gel
- Cellulose triacetate
- Agar
- Gelatin
- Carrageenan
- Alginate

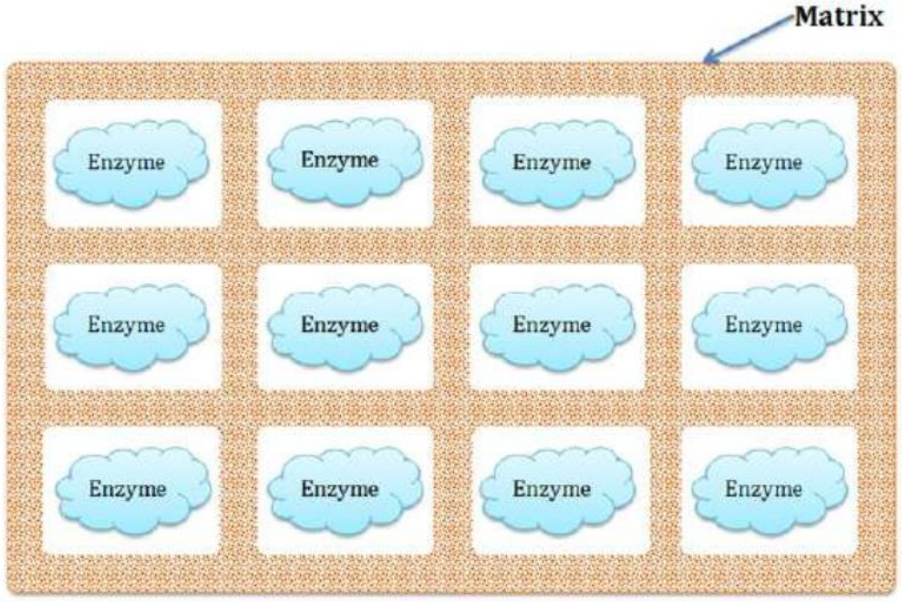


Entrapment

METHODS OF ENZYME IMMOBILIZATION……Contd..

✓ **Methods of entrapment:**

- **Inclusion in the gels -** enzyme trapped inside the gels
- **Inclusion in fibers –** enzymes supported on fibers made up of support materials
- **Inclusion in microcapsules –** Enzyme trapped in microcapsules formed by monomer mixtures such as polyamine and calcium alginate



Entrapment

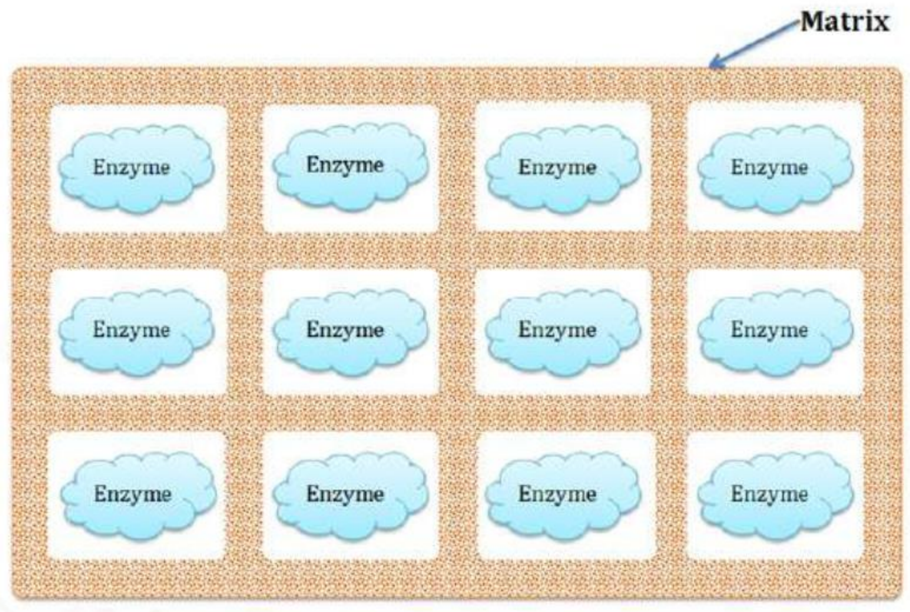
METHODS OF ENZYME IMMOBILIZATION……Contd..

✓ **Advantages of entrapment:**

- Fast method of immobilization
- Cost-effective
- Easy to practice at small scale
- Mild conditions are required
- Less chance of conformational changes in enzyme
- Can be used for sensing application

✓ **Disadvantages of entrapment**

- Leakage of enzyme
- Pore diffusion limitation
- Chance of microbial contamination
- Not much success in industrial process



Entrapment

METHODS OF ENZYME IMMOBILIZATION……Contd..

- ✓ ***Affinity Binding:*** The immobilization of enzyme linked to the matrix through the specific interactions.
- The Two methods are being followed in affinity immobilization.
- The first method is the activation of the support material which contains the coupled affinity ligand, so that the enzyme will be added.
- The advantage of this method is the enzyme is not exposed to any harsh chemicals conditions.
- The second method, the enzyme modified to another molecule which has the ability to bind towards a matrix.

METHODS OF ENZYME IMMOBILIZATION……Contd..

- ***Metal Linked immobilization:*** In metal linked enzyme immobilization, the metal salts are precipitated over the surface of the carriers and it has the potential to bind with the nucleophilic groups on the matrix.

- The precipitation of the ion on the carrier can be achieved by heating.
- This method is simple and the activity of the immobilized enzymes is relatively high (30-80%).
- The carrier and the enzyme can be separated by decreasing the pH, hence it is a reversible process.
- The matrix and the enzyme can be regenerated, by the process.

METHODS OF ENZYME IMMOBILIZATION……Contd..

- ✓ ***Biomedical Application:*** Immobilized enzymes are used in medicine from 1990 immobilized enzymes are used for diagnosis and treatment of diseases in the medical field.
- ✓ The inborn metabolic deficiency can be overcome by replacing the encapsulated enzymes (i.e, enzymes encapsulated by erythrocytes) instead of waste metabolites, the RBC acts as a carrier for the exogenous enzyme drugs and the enzymes are biocompatible in nature, hence there is no immune response (Johnson et al.,1998).
- ✓ The enzyme encapsulation through the electroporation is a easiest way of immobilization in the biomedical field and it is a reversible process for which enzyme can be regenerated (Lizano et al., 1998).
- ✓ The enzymes when combined with the biomaterials it provides biological and functional systems.

Pharmaceutical Biotechnology

Concepts and Applications

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