ENZYME IMMOBILISATION

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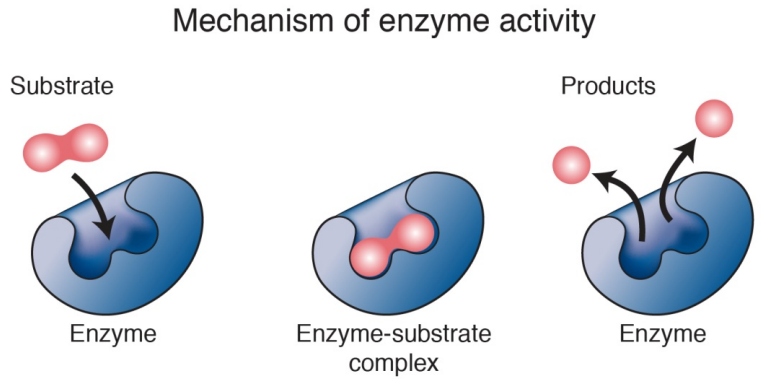
ENZYME ???

A substance produced by a living organism which acts as a catalyst to bring about a specific biochemical reaction & shows its activity even when it is independent.



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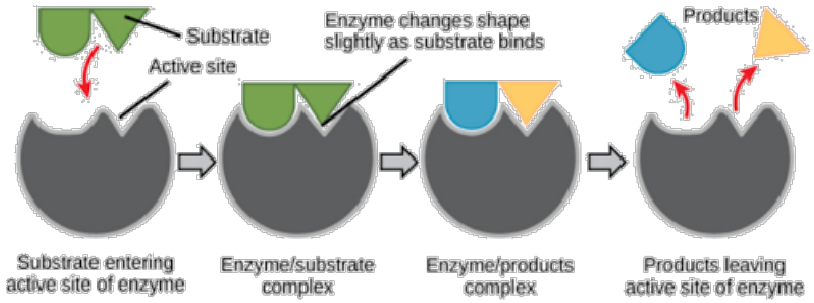
- Enzymes are biological catalysts which enhance the rate of biochemical reactions from 10^6 - 10^{12} times when compared to those of uncatalyzed reactions.
- All enzymes are proteins and contain an active site where substrates are converted to products. They possess both speed of action and precision.



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Mechanism of enzyme action

- In biochemical reaction, the molecules possessing high internal energy can go to a reactive form instantly. This reactive form is called **transition state**. To reach transition state, all the molecules must possess activation energy.
- The enzyme can accelerate the reaction by combining with substrate. The substrate-enzyme complex is then converted to products and enzyme is liberated. The transition state of this enzyme substrate complex has much lower activation energy than transition state of substrate alone.

- Due to this reduction in activation energy, more substrate molecules participate in the reaction and hence reaction rate will be increased.
- The enzyme recognizes substrate which fits into its active site perfectly.
- Once enzyme-substrate complex is formed, making and breaking of chemical bond occurs which converts the substrates into products and the enzyme is set free.
- Thus the enzyme and substrate complex can be compared with a lock and key model.

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Sources of enzymes

- Enzymes are usually obtained from three major sources-
- **Plants**
- **Animals**
- **Micro-organisms**
- The advent of fermentation technology led to the preparation of purer form of enzymes from microbes. Today, most of the industrially used enzymes are from microbial source.

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Advantaged of microbes over other sources of enzymes:

- 1. Microbes have a short generation time and hence the times required for enzyme production in large quantities is less compared to plants and animals.
- 2. Enzymes can be easily extracted from microbes. If the enzymes are extracellular, they will be secreted into the medium and can be purified easily.
- 3. Micro-organisms produce enzymes throughout the year and no seasonal variation is found which is common in case of plants and animals.

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- 4. The enzymes obtained from microbes are stable under extreme environmental conditions.
- 5. Micro-organisms can produce a large varieties of enzymes which is not possible with plants and animals. They can be manipulated easily by mutation or by genetic engineering to give high yield of enzymes.

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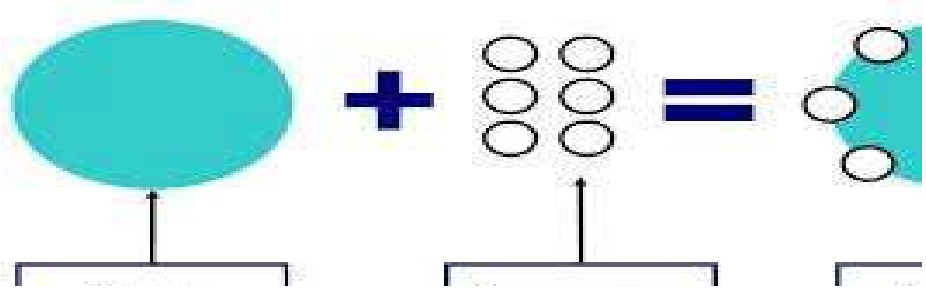
ENZYME IMMOBILISATION???

- Enzyme immobilization can be defined as a process where the movement of enzymes in space is completely or severely restricted and which can be used repeatedly and continuously .

- Immobilized enzymes are also referred as matrix linked enzymes.

- By employing this technique enzymes become more efficient. It allows enzyme to be held in place during reactions.



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Why is enzyme immobilization necessary?

- Most of the enzymes are expensive and hence they should be used as efficiently as possible. An important function of enzyme is their capacity to perform same function again and again.
- But if the enzymes are used in pure or crude form, it is not possible to separate them from the products after completion of catalysis since both of them are in dissolved state. If they are separated, the process becomes very costly and difficult.
- Some enzymes are too costly and the process of enzymatic catalysis becomes cost effective only if they are reused.
- Hence, immobilization techniques have been developed.
- The immobilization involves the conversion of water soluble enzymes into a solid form of catalyst which is insoluble.

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Advantages of immobilization of enzymes.....

- Enzymes can be reused.
- Favourable sterile characteristics.
- Accelerates the chemical reaction.
- Not difficult to separate.
- Catalytic operation can be operated continuously.

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Why immobilized enzyme

- Protection from degradation and deactivation.
- Re-use of enzymes for many reaction cycles, lowering the total production cost of enzyme mediated reactions.
- Ability to stop the reaction rapidly by removing the enzyme from the reaction solution.
- Enhanced stability.
- Easy separation of the enzyme from the product.
- Product is not contaminated with the enzyme

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CARRIERS.....

- The substances used for immobilizing of enzymes are termed as “carriers”.

- The form, shape, density, porosity, pore size distribution, operational stability and particle size distribution of the supporting matrix will influence the reactor configuration in which the immobilised biocatalyst may be used.

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CLASSIFICATION OF CARRIERS

Inorganic Carriers

- High pressure stability.
- May undergo abrasion

Examples:

1.Commercialy SiO2 available materials-
- Porous glass.
- Silica.

2. Mineral materials - (clays)
Celite ,Centonite

Organic Natural Carriers

- Favourable compatibility with proteins.

Examples:

1.cellulose derivatives-
- DEAE-cellulose
- CM-cellulose.

2. Dextran.

3.Polysacharides
Agarose, Starch
Pectine ,Chitosan

Organic Synthetic Carriers

- High chemical and mechanical stability.

Examples:

1.Polystyrene
2.Polyvinylacetate
3. Acrylic polymers

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An Ideal Carrier Matrices For Enzyme Immobilization

- Inert.
- Physically strong and stable.
- Cost effective.
- Regenerable.
- Reduction in product inhibition.

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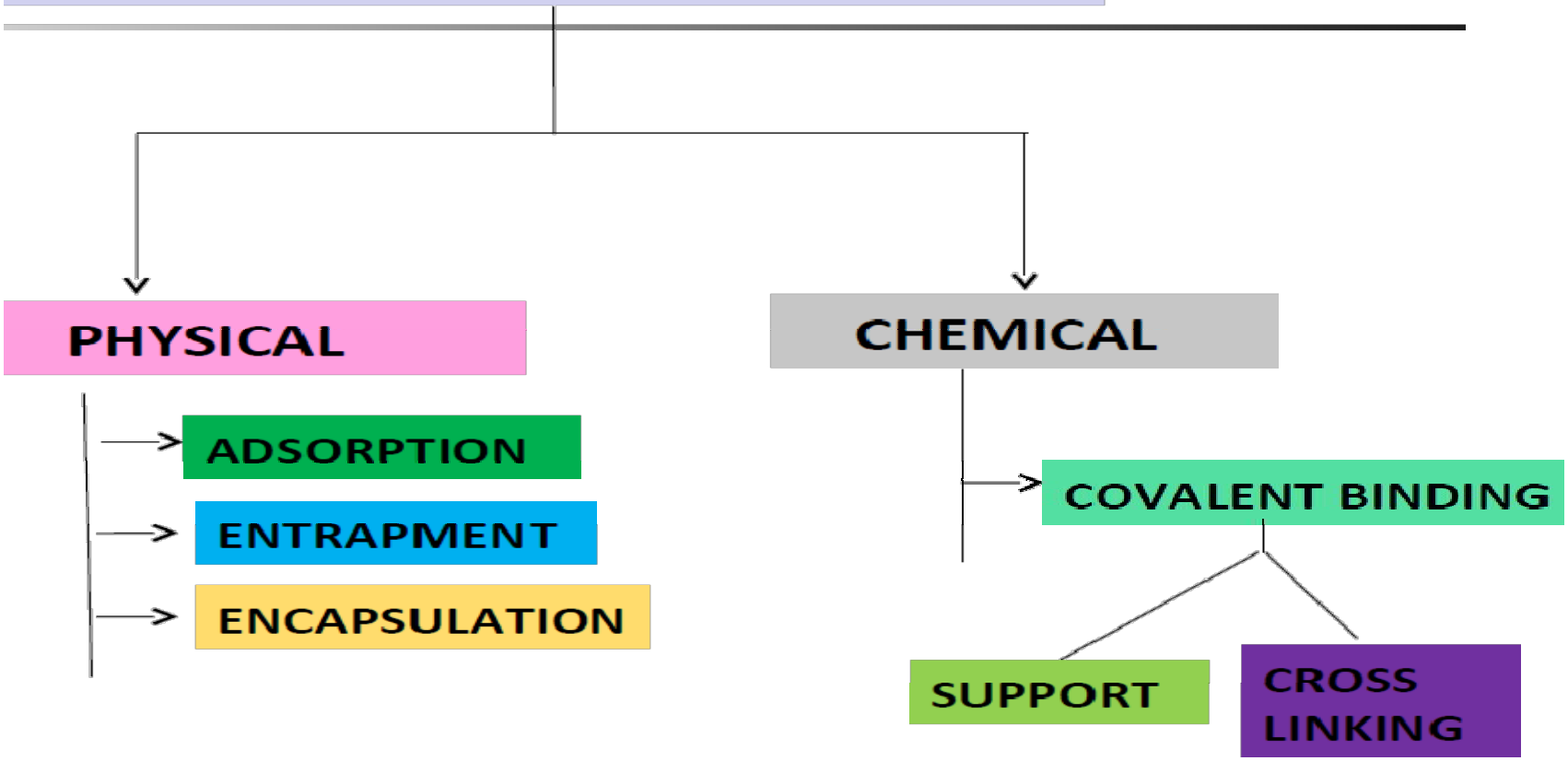
METHODS FOR ENZYME IMMOBILIZATION



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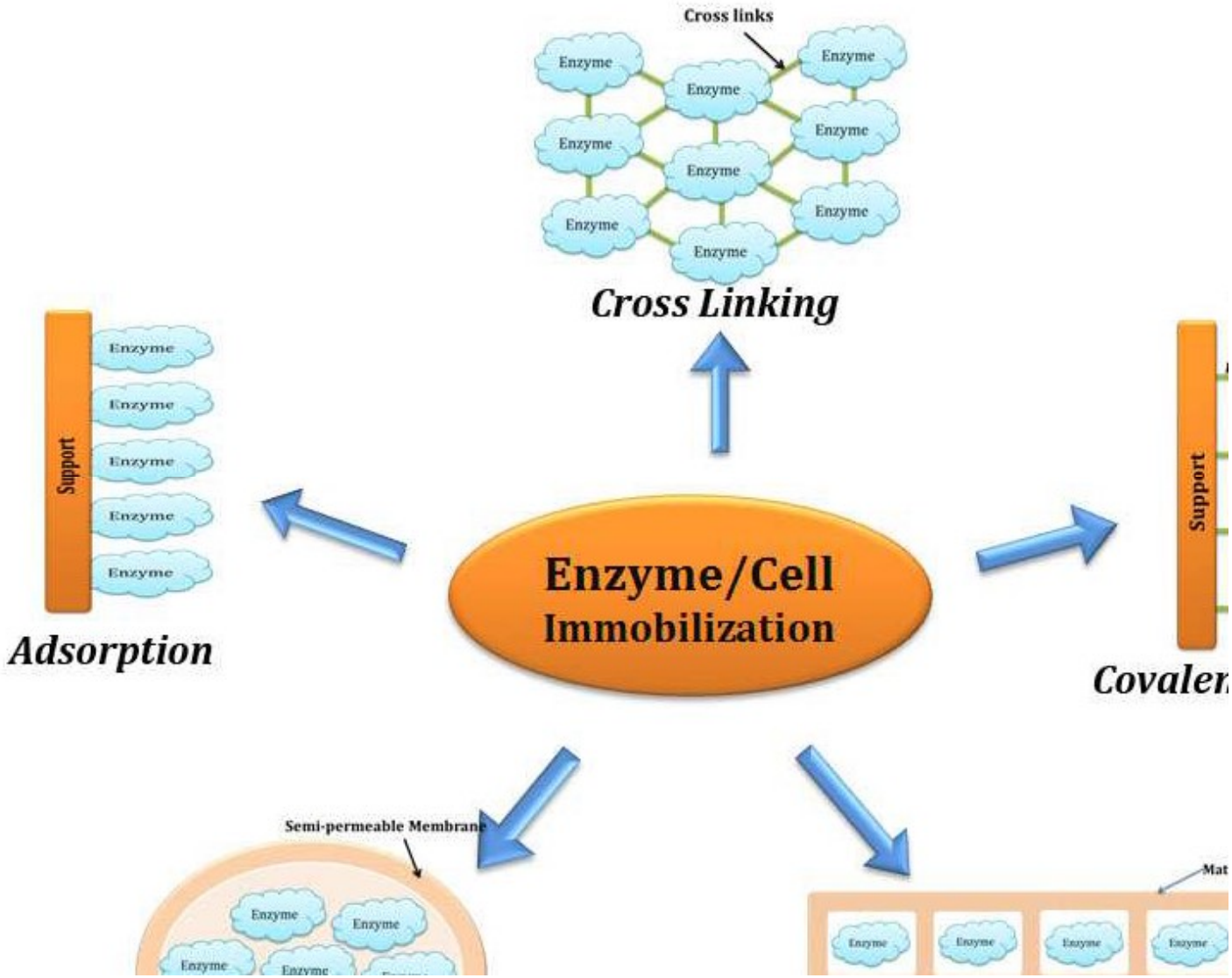

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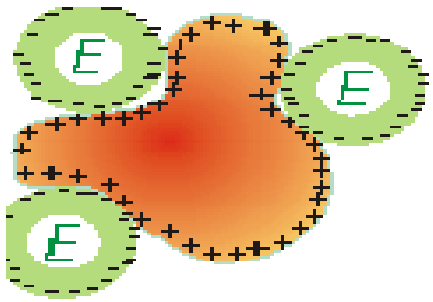
•1. Physical Methods For Immobilization

ADSORPTION

- ❖ Involves the physical binding of the enzyme on the surface of carrier matrix.
- ❖ Carrier may be organic or inorganic.
- ❖ The process of adsorption involves the weak interactions like Vander Waal or hydrogen bonds.
- ❖ Carriers: - Silica, Bentonite, Cellulose, etc.

example of enzyme: catalase & invertase



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ADVANTAGES

1. Simple and economical

2. Limited loss of activity

3. Can be Recycled, Regenerated & Reused.

DISADVANTAGES

1. Relatively low surface area for binding.

2. Exposure of enzyme to microbial attack.

3. Yield are often low due to inactivation and desorption.

Entrapment

In entrapment, the enzymes or cells are not directly attached to the support surface, but simply trapped inside the polymer matrix.

Enzymes are held or entrapped within the suitable gels or fibres.

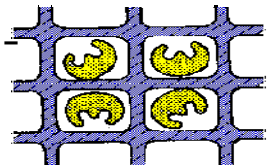
It is done in such a way as to retain protein while allowing penetration of substrate.

It can be classified into **lattice and micro capsule types**.

Inclusion in gels: Poly acrylamide gel, Poly vinyl alcohol gels.

Inclusion in fibers: Cellulose and Poly -acryl amide gels.

Inclusion in micro capsules: Polyamine, Polybasic - acid chloride monomers.



entrapped in a matrix

entrapped in droplets

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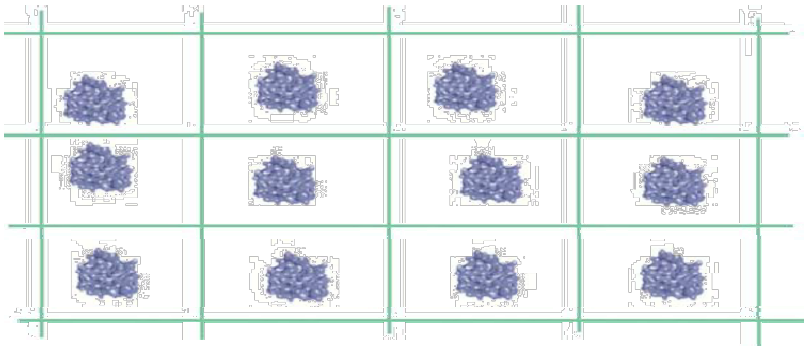
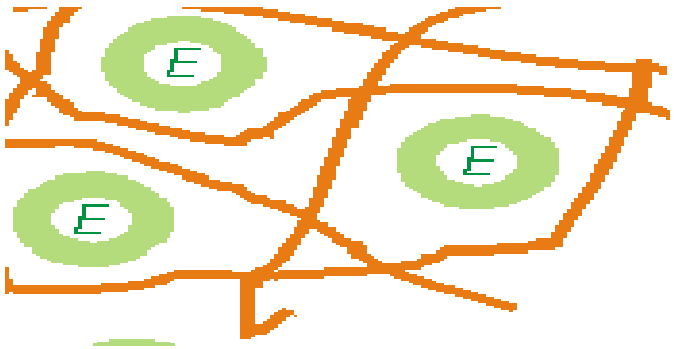
A. Lattice-Type Entrapment

- Entrapment involves entrapping enzymes within the interstitial spaces of a cross-linked water-insoluble polymer.
- Some synthetic polymers such as *polyarylamide, polyvinylalcohol, etc...* and natural polymer (starch) have been used to immobilize enzymes using this technique.





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- **ADVANTAGES:**
- Cost is low.
- Industrially useful.
- Can be reused many times.

- **DISADVANTAGES:**
- Preparation is difficult.
- The enzyme may leak from the pores.
- Lack of control.

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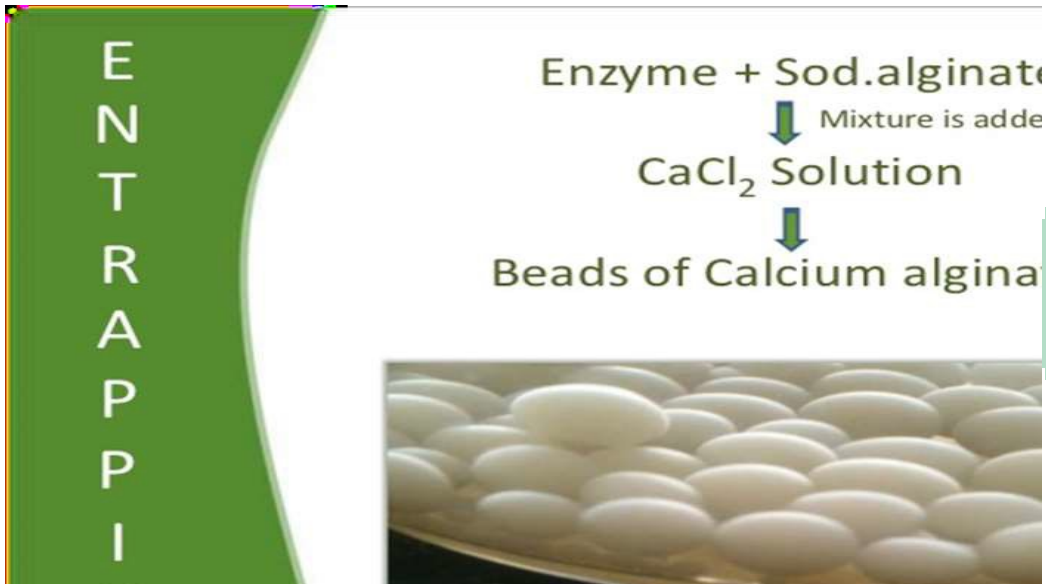
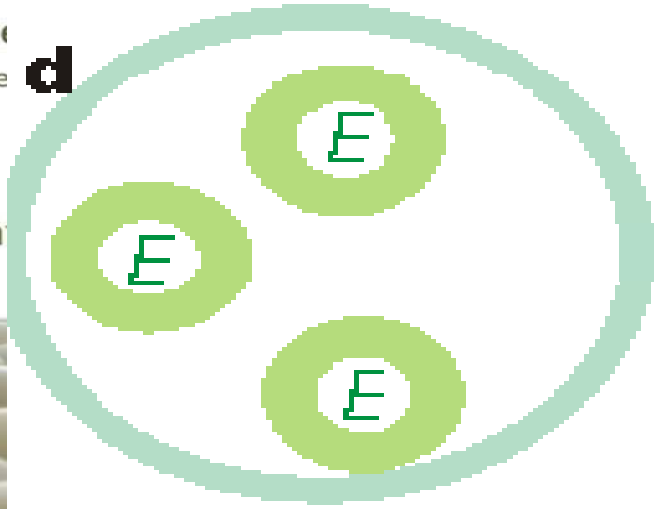
B. Microcapsule Type Entrapment/ Encapsulation/Membrane Confinement

- It involves enclosing the enzymes within semi permeable polymer membranes
- e.g. semi permeable collodion or nylon membranes in the shape of spheres.





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- **ADVANTAGES**
- 1. No chemical modification.
- 2. Relatively stable forms.
- 3. Easy handling and reusage.

- **DISADVANTAGES**
- 1. The enzyme may leak from the pores

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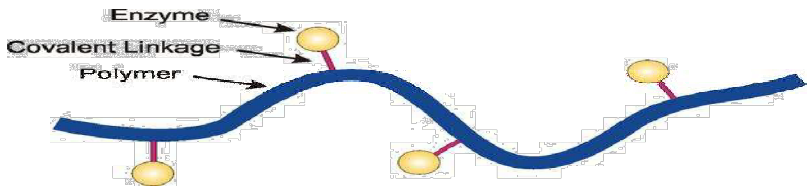
2. Chemical Methods For Immobilization

Covalent Binding

- Based on the binding of enzymes and water-insoluble carriers by covalent bonds
- The functional groups that may take part in this binding are **Amino group, Carboxyl group, Sulfhydryl group, Hydroxyl group, Imidazole group, Phenolic group, Thiol group** etc.
- **Advantages:** The binding force between enzyme and carrier is so strong that no leakage of the enzymes occurs, even in the presence of substrate or solution of high ionic strength.
- **Disadvantages:** Covalent binding may alter the conformational structure and active center of the enzyme, resulting in major loss of activity and/or changes of the substrate



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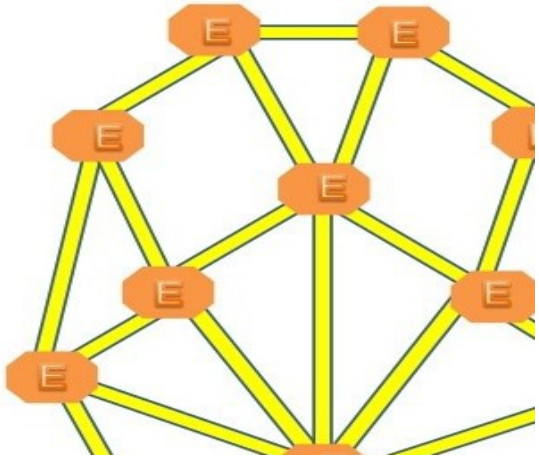
Cross Linking

- Cross linking involves intermolecular cross linking of enzyme molecules in the presence/absence of solid support.
- The method produces a 3 dimensional cross linked enzyme aggregate (insoluble in water) by means of a multifunctional reagent that links covalently to the enzym



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- **Advantages of cross linking:-**
- 1. Very little desorption(enzyme strongly bound)
- 2. Higher stability (i.e. p^H, ionic & substrate concentration)
- **Disadvantages of cross linking:-**
- 1. Cross linking may cause significant changes in the active site.
- 2. Not cost effective.

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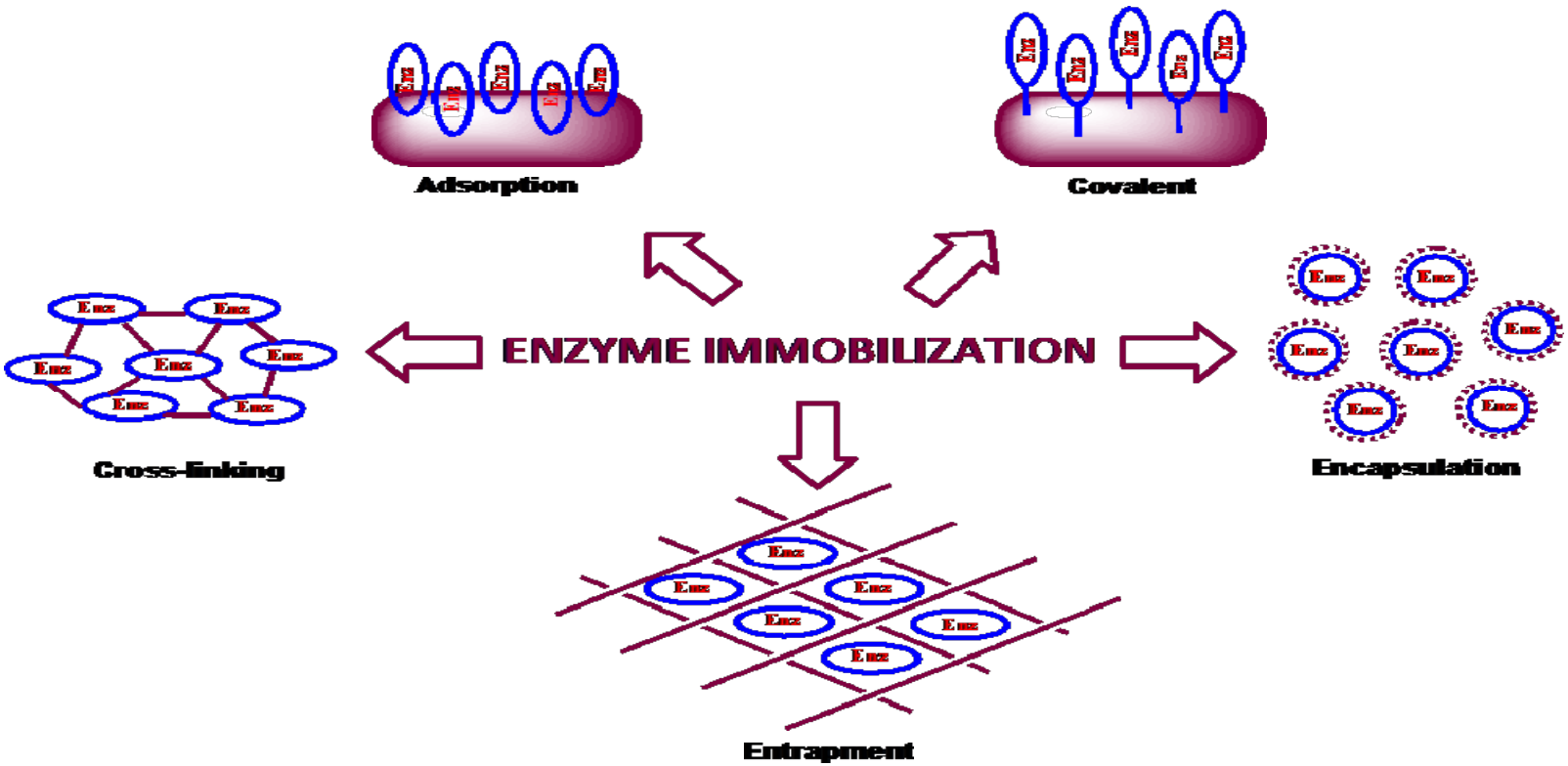
METHOD SUMMARY



Pictorial representation of different immobilization methods.

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Comparison Between The Methods

Characteristics	Adsorption	Covalent binding	Entrapment	Membrane confinement
Preparation	Simple	Difficult	Difficult	Simple
Cost	Low	High	Moderate	High
Binding force	Variable	Strong	Weak	Strong
Enzyme leakage	Yes	No	Yes	No
Applicability	Wide	Selective	Wide	Very wide
Running Problems	High	Low	High	High
Matrix effects	Yes	Yes	Yes	No
Large diffusional barriers	No	No	Yes	Yes
Microbial protection	No	No	Yes	Yes

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OVERALL ADVANTAGE

- 1. Suitable for industrial and medical use
- 2. Held in place during Reactions.
- 3. Temperature and P^H Resistance.
- 4. Recovered for reuse after reactions.
- 5. Enzyme become more efficient.

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OVERALL DISADVANTAGES

- There are however certain disadvantages also associated with immobilization :

1. The possibility of loss of biological activity of an enzyme.
2. Immobilization is an expensive affair often requiring sophisticated equipment .
3. Some time native structure of enzyme is disrupted due to immobilization.
4. All the enzymes are not immobilized by immobilization.
5. Cost of carriers .

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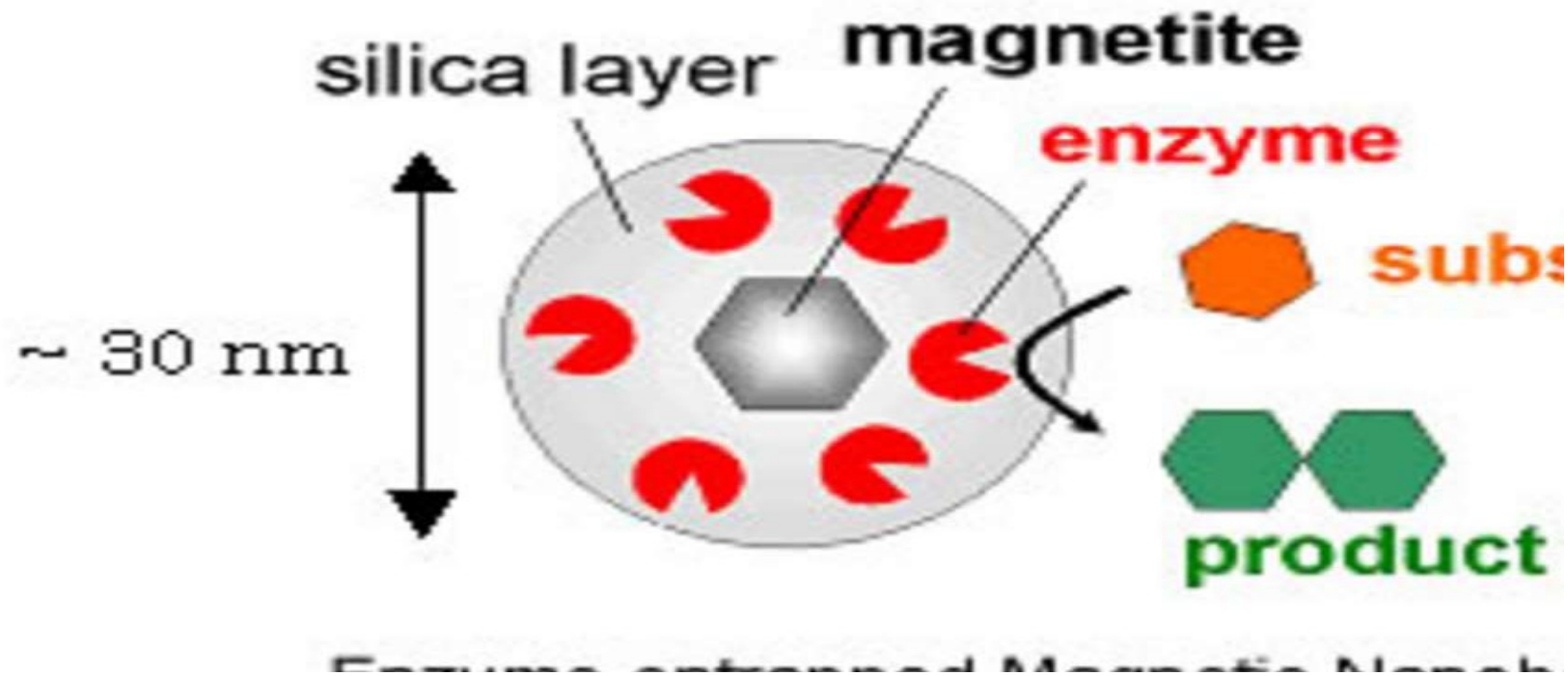
NOVEL METHOD OF IMMOBILISING ENZYMES



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APPLICATION OF IMMOBILISING ENZYMES

The enzyme applications are broadly classified into four categories-

- **1. Medical use**
- **2. Analytical use**
- **3. Manipulative use**
- **4. Industrial use**

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1. Medical use:

- Treatment of leukemia can be done by administering asparaginase from bacteria. Leukemic cell require exogenous asparagine for their growth and this will be destroyed by the enzyme.
- Lysozyme, a protective body fluid e.g. nasal mucosal fluid, lacrimal fluid etc. is used as antibacterial agent.
- Hyaluronidase, an enzyme is used in dental traetment to facilitate drug penetration.
- Penicillinase is used to stop Penicillin hypersensitivity.
- Glucose oxidase is used to diagnose the presence of glucose in urine.

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2. Analytical use:

- Enzymes can be used to analyze the blood concentration of D-glucose, D-alanine, D-galactose, Urea etc.

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3. Manipulative use

- Restriction enzymes are used in recombinant DNA technology to cut DNA.
- DNA ligase is used to join foreign genes to plasmid DNA.
- Alkaline phosphatase is used to prevent the recirculation of cut plasmid.
- Reverse transcriptase is used to produce DNA from RNA.
- DNA Polymerase is used in the formation of DNA

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4. Industrial use

- Invertase is used to convert glucose in to fructose.
- Penicillin acylase is used to produce semisynthetic Penicillin.
- Proteolytic enzymes are used to soften skin in leather industry.

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CONCLUSION

- Enzyme immobilization is one of the most promising approaches which used in various fields such as biotransformation , diagnostic , pharmaceutical and also in food industries .

- Several hundred of enzymes have been immobilized including penicillin, amylase ,lipase , protease etc . And are being currently used as catalyst in various large scale process .

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THANK YOU

Happy to Answer if you have any question.....