Development of Enzyme Immobilization Technique

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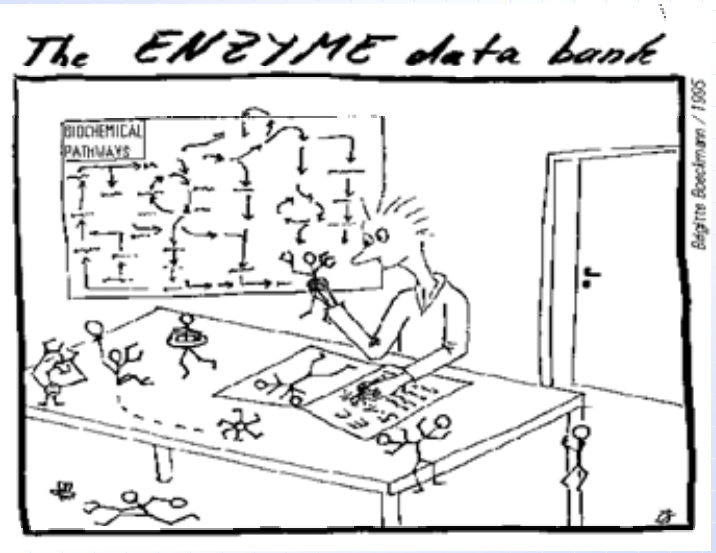
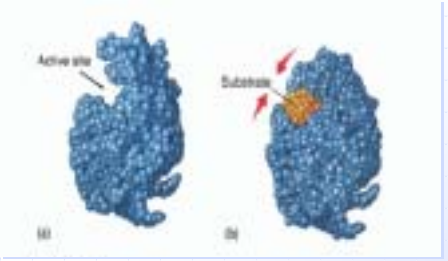
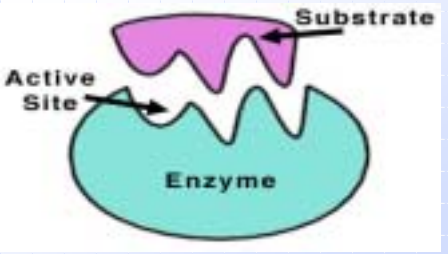
In this presentation…



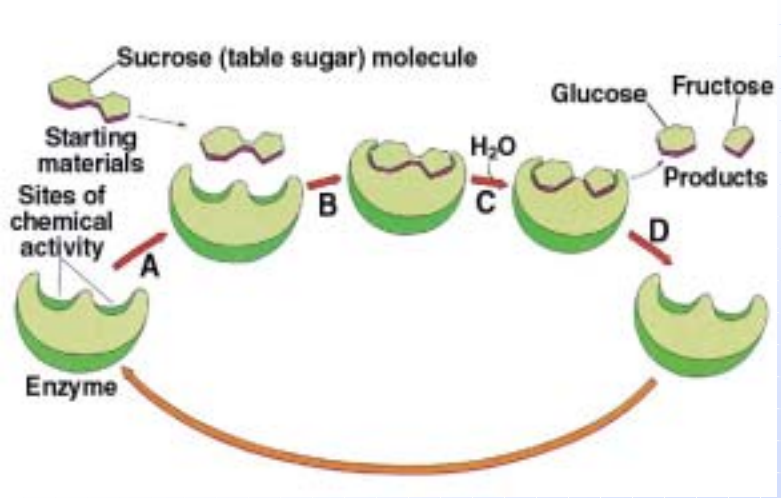
What is an Enzyme ?

- Enzymes are biological catalysts.
- They increase the rate of chemical reactions taking place within living cells without themselves suffering any overall change.
- The reactants of enzyme-catalysed reactions are termed substrates and each enzyme on a particular substrate to produce a particular product.
- Enzymes are protein molecules





Enzyme Action Model



Application of Enzyme

Advantages

- Enzyme of high or low specificity can be selected to desired function.
- Little (or No) by-product formation is observed.
- Optimal activity occurs under very mild reaction condition.

Problems

- The cost of enzyme preparation is often high
- Enzyme are intrinsically unstable
- Enzyme are easily inhibited
- Substrate or products which has low solubility in aqueous solution can pose difficulties

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What is an Immobilized Enzyme?

- An immobilized enzyme is one whose movement in space has been restricted either completely or to a small limited region.
- Attachment to solid structure, incorporation in gels etc for use.

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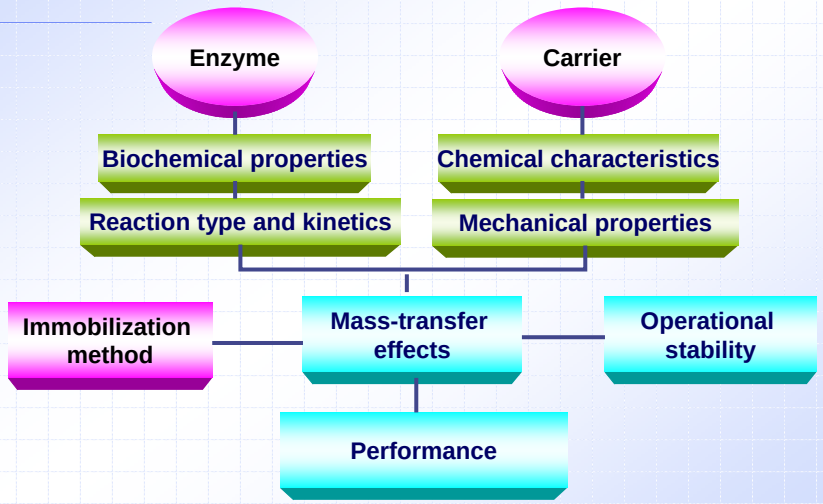
Benefits of Immobilizing an Enzyme

There are a number of advantages to attaching enzymes to a solid support and several major reasons are listed below:

- Multiple or repetitive use of a single batch of enzymes
- The ability to stop the reaction rapidly by removing the enzyme from the reaction solution (or vice versa)
- Enzymes are usually stabilized by binding
- Product is not contaminated with the enzyme (especially useful in the food and pharmaceutical industries)
- Easy seperation of enzyme from the product
- Allows development of a multienzyme reaction system
- Reduces effluent disposal problems

The properties of immobilized enzymes



Selected characteristic parameters of immobilized enzymes

Biochemical properties

- Molecular weight,
- Functional groups on protein surface,
- Purity (inactivating/protective function of impurities)

Enzyme kinetic parameters

- Specific activity,
- pH-, temperature profiles,
- Kinetic parameters for activity and inhibition,
- Enzyme stability against pH, temp., solvents, contaminants, impurities.

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Chemical characteristics

- Chemical basis and composition,
- Functional groups, swelling behavior,
- Accessible volume of matrix and pore size,
- Chemical stability of carrier

Mechanical properties

- Particle diameter,
- Single-particle compression behavior,
- Flow resistance (for fixed bed application),
- Sedimentation velocity (for fluidized bed),
- Abrasion(for stirred tanks)

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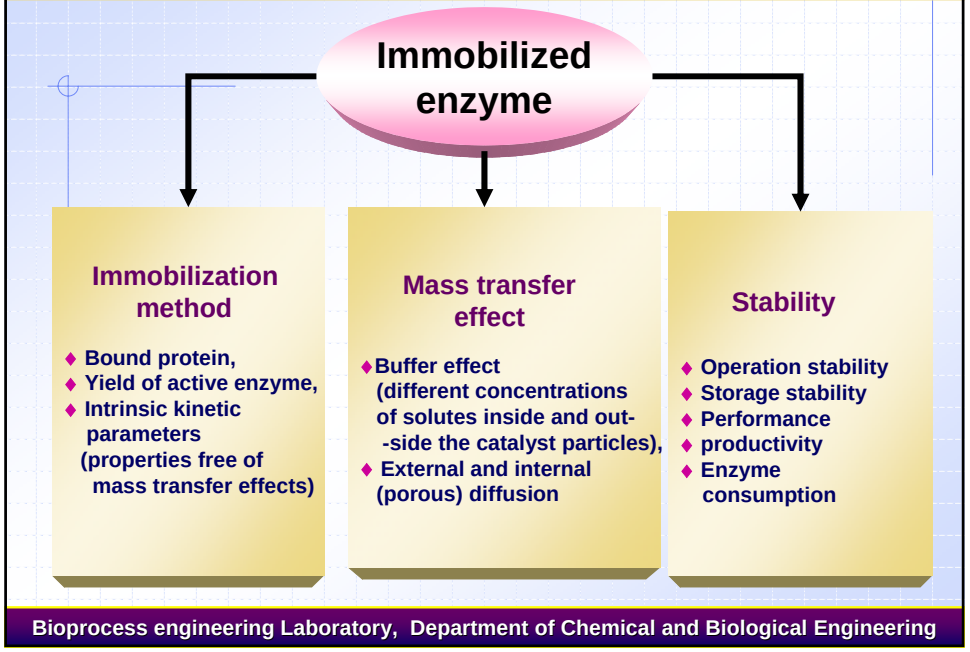
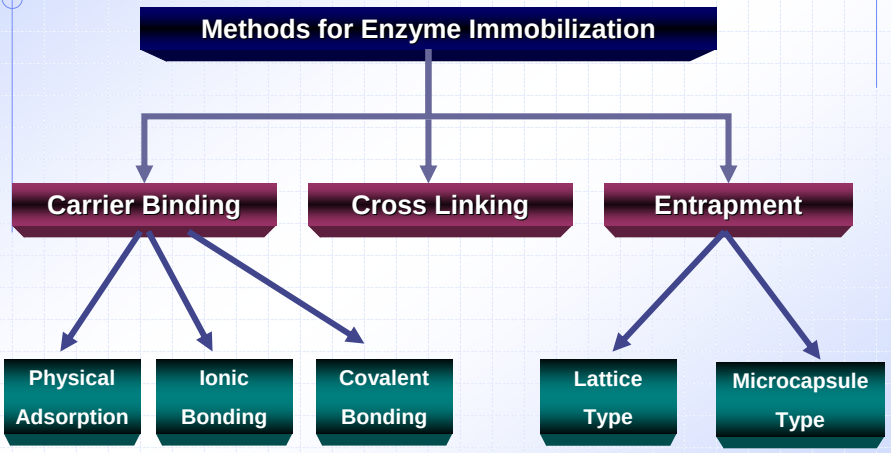
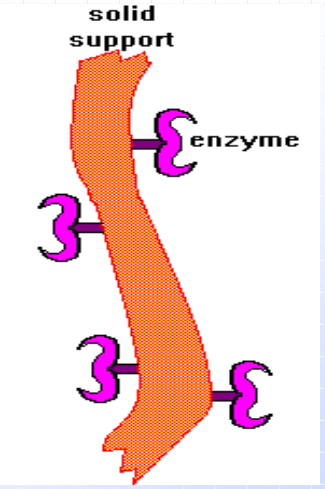


Methods for Enzyme Immobilization



Carrier-Binding

- The carrier-binding method is the oldest immobilization technique for enzymes.
- In this method, the amount of enzyme bound to the carrier and the activity after immobilization depend on the nature of the carrier.



- The carrier-binding method can be further sub-classified into:

  Physical Adsorption
  Ionic Binding
  Covalent Binding

Physical Adsorption Mode

This method is based on the physical adsorption of enzyme protein on the surface of water-insoluble carriers.

Advantages

- Little or no conformational change of the enzyme or destruction of its active center
- Usually no reagents and only a minimum of activation steps are required
- Simple and cheap

Disadvantages

- Desorption of the protein resulting from changes in temperature, pH, ionic strength
- Non-specific

Ionic Binding Mode

This method relies on the ionic binding of the enzyme protein to water-insoluble carriers containing ion-exchange residues.

Advantages

- The conditions are much milder than those needed for the covalent binding method.
- Little changes in the conformation and the active site of the enzyme.
- High activities in most cases.

Disadvantages

- Leakage of enzymes from the carrier may occur in substrate solution of high ionic strength or upon variation of pH

Covalent Binding Mode

The covalent binding method is based on the binding of enzymes and water-insoluble carriers by covalent bonds.

. The functional groups

Amino group	Carboxyl group	Sulfhydryl group,
Hydroxyl group	Imidazole group	Phenolic group
Thiol group	Threonine group	Indole group

When trying to select the type of reaction by which a given protein should be immobilized, the choice is limited by two characteristics:
(1) the binding reaction must be performed under conditions that do not cause loss of enzyme activity, and
(2) the active site of the enzyme must be unaffected by the reagents used.

Covalent binding can be brought about by the following ;

- •Diazotization : SUPPORT--N=N--ENZYME.
- •Amide bond formation : SUPPORT--CO-NH--ENZYME
- •Alkylation and Arylation: SUPPORT--CH_2-NH-ENZYME
  SUPPORT--CH_2-S--ENZYME
- •Schiff's base formation : SUPPORT--CH=N--ENZYME
- •Amidation reaction : SUPPORT--CNH-NH--ENZYME
- •Thiol-Disulfide interchange : SUPPORT--S-S--ENZYME
- •UGI reaction
- •Mercury-Enzyme interchange
- •Gamma-Irradiation induced coupling
- •Carrier binding with bifunctional reagents :

SUPPORT-$O(CH_2)_2$ $N=CH(CH_2)_3$ CH=N-ENZYME

Advantages and Disadvantages of Covalent Binding Method

Advantages

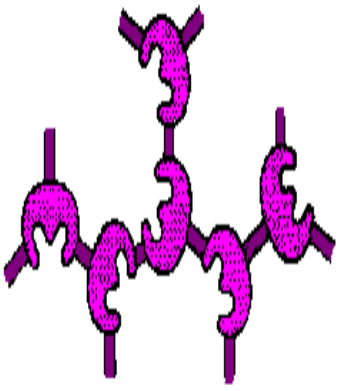
- Covalent binding is usually thought to be ***stable method*** by the enzyme carrier bond, which ***prevents elution of protein*** into the production stream.
- ***The wide range of choices*** is possible by selecting carrier materials and binding method. This allows a great deal of flexibility in designing an immobilized enzyme with specific physical and chemical properties, such as charge distribution, hydrophobe/hydrophile ration, spacer arm separation, partitioning capabilities, etc.

Disadvantages

- Covalent methods are *the relatively expensive and complicated in procedures* which are involved. Also, activity yields may be low due to exposure of the enzyme to harsh environments or toxic reagent.
- *Active site may be modified* through the chemical reactions used to create covalent bonding.

Cross-Linking

This method is based on the formation of *covalent bonds between enzyme molecules*, by means of bi- or multi-functional reagent, leading to three-dimensional crosslinked aggregates.



Advantages and Disadvantages

Advantages

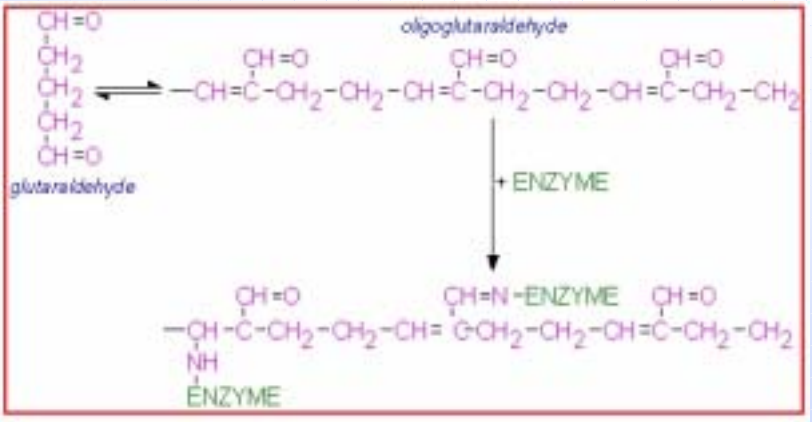
- Very *little desorption* (Enzyme strongly bound)
- Cross-linking is best used in *conjunction* with one of the other methods. It is used mostly as a means of *stabilizing adsorbed enzymes* and also for *preventing leakage.*

Disadvantages

- Cross-linking may cause *significant changes in the active site* of enzymes, and also severe *diffusion limitation* may lead to significant loss of activity.
- *Loss of enzyme activity* during preparation.

The most common reagent used for cross-linking is

glutaraldehyde

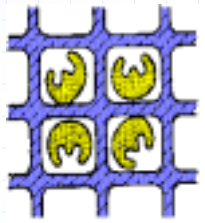


Entrapping Enzymes

♦ The entrapment method of immobilization is based on the *localization of an enzyme* within the *lattice* of a polymer matrix or *membrane.*

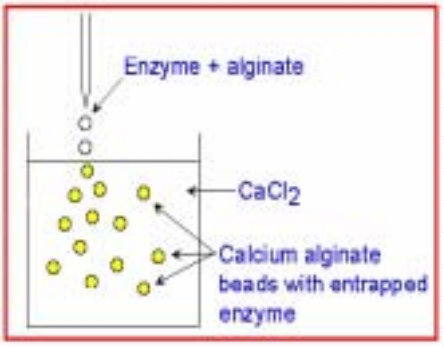
♦ It can be classified into *lattice and micro capsule types.*

It is possible to confine the enzyme in *a semi-permeable membrane* which allows *free passage of low molecular weight substrates and products* but *retains the high molecular weight enzyme.*

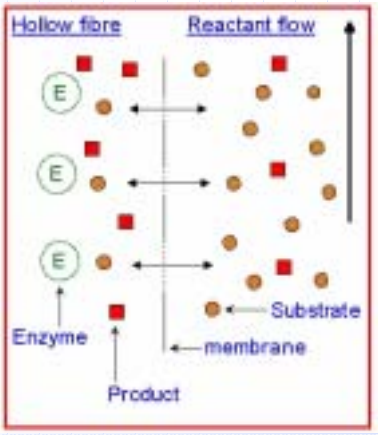


Entrapped in a matrix

Entrapped in droplets



The formation of calcium alginate beads.

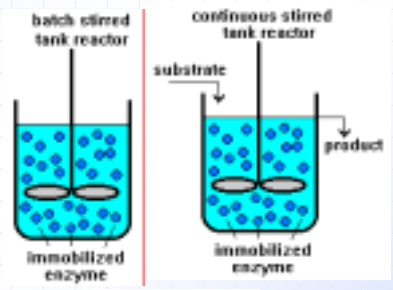


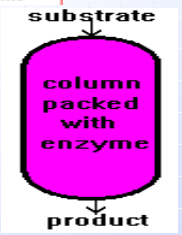
Membrane Incorporation

Types of Reactors

Batch reactors may include:

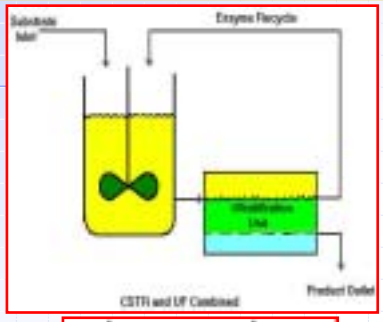
- Stirred Tank for Soluble enzymes
- Stirred Tank for Immobilized enzymes
- Stirred Tank with Immobilized enzyme Basket Paddles
- Stirred Tank with Immobilized enzyme Basket Baffles
- Total Recycle Packed Bed Reactor
- Total Recycle Fluidized Bed Reactor

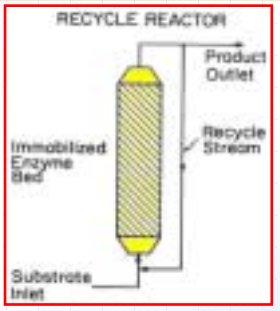


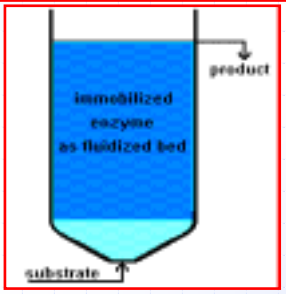


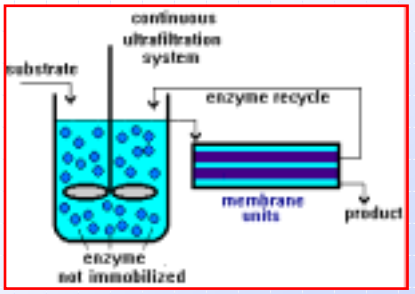
Continuous reactors may include:

- Stirred Tank Reactor with Filtration Recovery
- Stirred Tank Reactor with Settling Tank Recovery
- Stirred Tank Reactor with Immobilized Enzyme Basket Paddles
- Stirred Tank Reactor with Ultra filtration Recovery
- Packed Bed Reactor
- Packed Bed with recycle
- Membrane Reactor using Hollow Fibers
- Flat Bed Reactor
- Filter Bed Reactor
- Fluidized Bed Reactor, same but better design(expanded top section)


Enzyme Recycle
Product Outlet
CSTR and UF Combined


RECYCLE REACTOR
Product Outlet
Recycle Stream
Immobilized Enzyme Bed
Substrate Inlet


product
immobilized enzyme as fluidized bed
substrate


continuous ultrafiltration system
substrate
enzyme recycle
membrane units
product
enzyme not immobilized