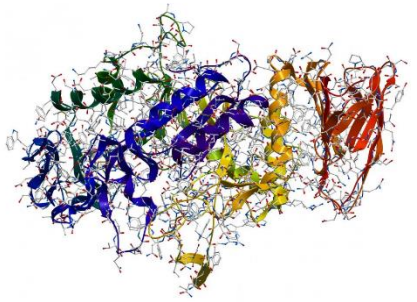


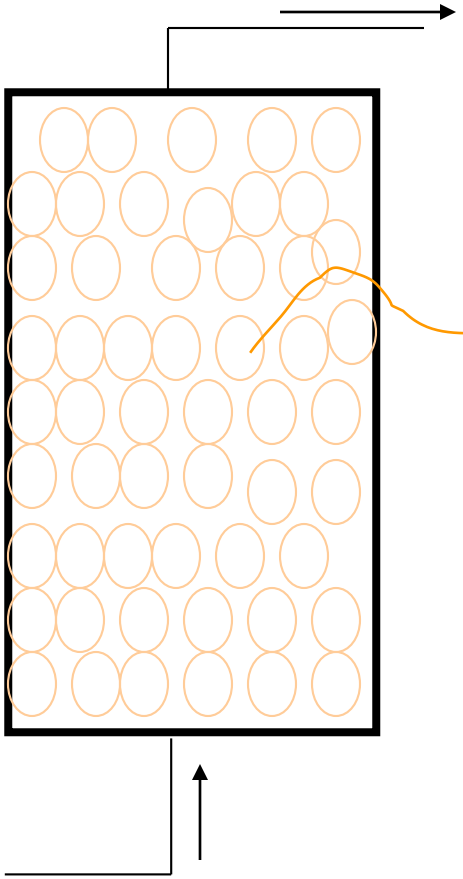
CEN-CHE 422
ENZYME ENGINEERING

IMMOBILIZED ENZYMES

IMMOBILIZED ENZYME

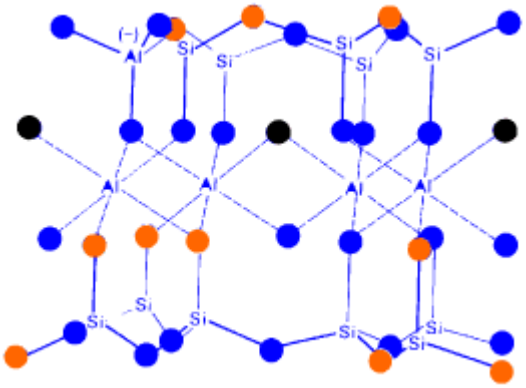
Enzyme physically localized in a certain defined region of space with retention of their catalytic activities, and which can be used repeatedly and continuously."

free enzyme ✗ ***immobilized enzyme***

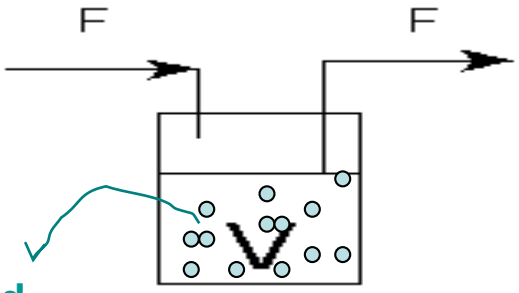


Immobilized biocatalyst

Continuous packed bed bioreactor



Porous structure of bentonite



Immobilized biocatalyst

Continuous stirred bioreactor

Advantages of immobilized enzymes over free enzymes

- They retain their activity more than in the free state *(high stability-operational, thermal, …)*
- They can be used repeatedly in batch reactors and for a long time in continuous reactors
- They are easy to separate from product solutions
- They are economical to use
- They can be used in sequential and parallel reactions by co-immobilization

Desired properties of immobilized enzymes

- ✓ High surface area
- ✓ Permeability
- ✓ Chemical, physical and thermal stability
- ✓ High mechanical strength
- ✓ Proper shape and pore diameter
- ✓ Resistance against microbial forces
- ✓ Renewability

Immobilization Supports (Carriers)

Organic

Natural polymers

Polysaccharides

Cellulose
Starch
Dextran
Agar
Alginate
Carragenan
…

Proteins

Collagen
Gelatin
Albumin
…

Synthetic Polymers

Polystyrene
Polyacrylate
…

Inorganic

Minerals

Bentonite
Kieselguhr
…

Processed Materials

Non-porous glass
Controlled-pore glass
Metals
Uncontrolled-pore metal oxides
…

Immobilization Methods

Bonding

- Bonding on a support
  - ✓*Physical adsorption*
  - ✓*Ionic bonding*
  - ✓*Covalent bonding*
- Cross-linking

Entrapment

- Gel-entrapment
- Fiber-entrapment
- Micro-encapsulation

Cross-linking

Cross-linking is one of the chemical methods of enzyme immobilization in which enzyme is attached to each other through covalent bond via bi- or multifunctional reagents.

bi- or multifunctional reagents:

Glutaraldehyde

Diazobenzidin

Hexamethylene diisocyanate

Physical Adsorption

Attachment of enzymes to stationary solids by weak physical forces (van der Waals or dispersion forces)

- ✓ Advantages: Easy, cheap, no reaction, active site is unaffected and nearly full activity is observed.

- ✓ Disadvantages: Desorption of enzymes is a common problem *(pH, ionic strengths etc.)*

Adsorbents:

• Alumina • Silica • Porous Glass • Ceramics • Diatomaceous Earth • Clay • Cellulose Materials • Activated Carbon • Ion Exchange Resin • Starch

Ionic Bonding

Tightly bonding of enyzme to solid support with ionic and van der Waals bonds

Suppports:
Ion exchange resins
Dextran
DEAE
Sephadex
CMC

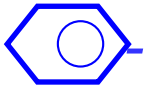
✓Electrostatic forces

✓Advantages: easy, stronger than adsorption

✓ *Disadvantages:*
enzyme leakage
(pH, ionic strengths, etc)
minor changes in the enzyme's structure

Covalent Bonding

The retention of enzyme on support surfaces by covalent bonding between functional groups on the enzyme and those on the support surface

Functional groups on enzyme: *-NH_2, -SH, -OH, -COOH,*  *-OH*

Functional groups on support: *triazinyl, imidoester, amine,*

diazonium isocyanate

Bonds: *diazo bonds, peptide bonds,....,*

✓Advantages : no enzyme leakage

✓Disadvantages: Active site of enzyme must not participate in covalent bonding