Enzyme immobilization

B.Pharmacy 6th sem

Pharmaceutical Biotechnology(BP605T)

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Process of enzyme immobilization

Supports/ Matrix used in immobilization technology:

- The matrix/supports hold the enzyme
- The matrix used should be cheap and easily available
- Their reaction with medium and enzyme should be minimums as possible
- A wide range of matrix are used in immobilization of enzyme/whole cells
- The matrix/supports are grouped into three major categories

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

Supports/ Matrix used in immobilization technology:

(1). Natural polymers

- **Alginate**: derived from algal cell wall (calcium or magnesium alginate)
- **Chitosan and chitin**: enzyme bins to the – OH groups
- **Collagen**: protenaceous support
- **Carrageenan**: a sulfated polysaccharide obtained from algae
- **Gelatin**: partially hydrolyzed collagen, good water holding capacity
- **Cellulose:** cheapest support available
- **Starch:** good water holding capacity
- **Pectin:** good water holding capacity

Supports/ Matrix used in immobilization technology:

(2). Synthetic polymers

- They are ion exchange resins/polymers
- They are insoluble supports with porous surface
- The porous surface trap and hold the enzymes/cells
- Example:
  - DEAE cellulose
  - Polyvinyl chloride (PVC)
  - UV activated Polyethylene glycol (PEG)

Supports/ Matrix used in immobilization technology:

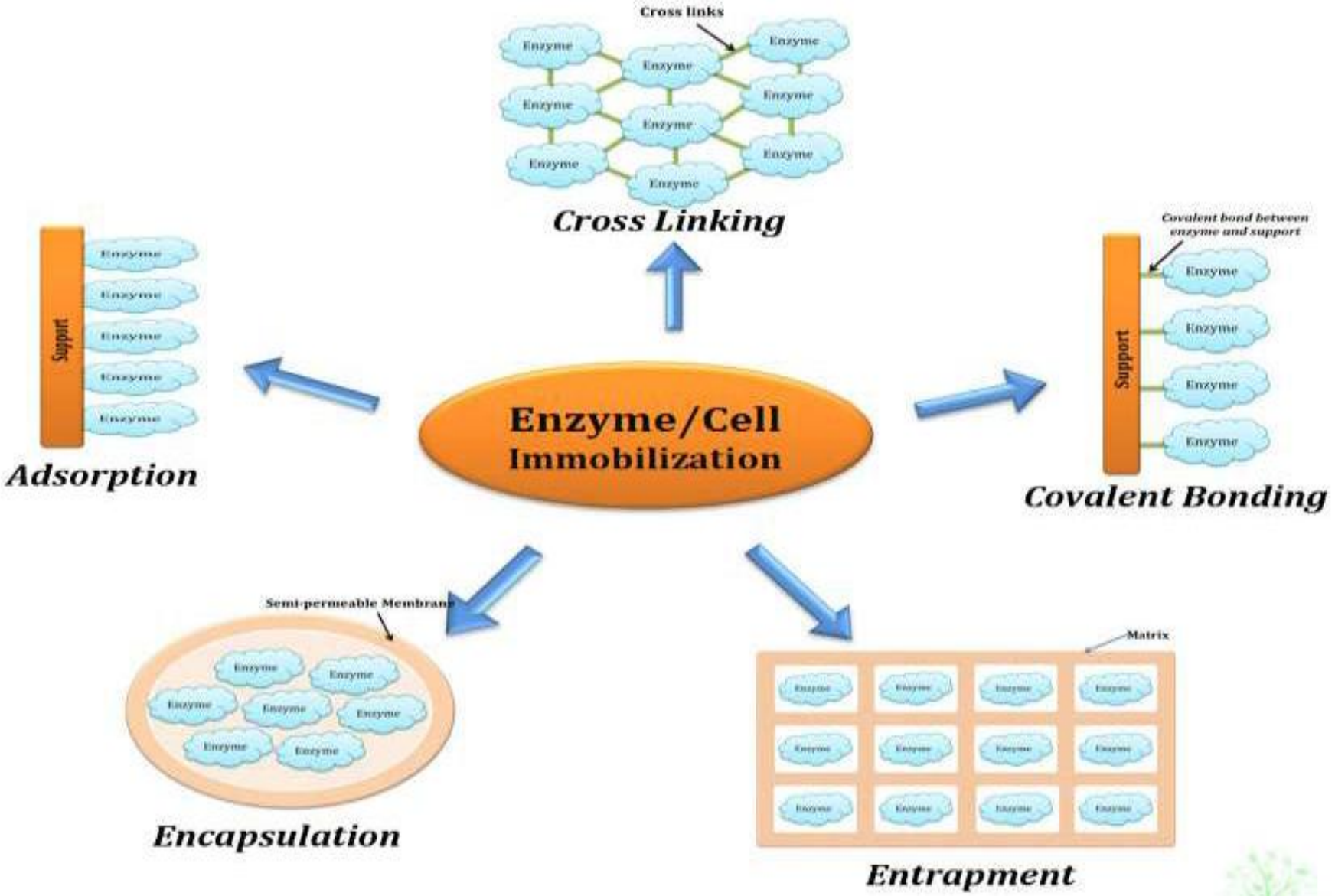
(3). Inorganic materials

- *Zeolites:*
- *Ceramics:*
- *Diatomaceous earth (Trade name celite)*
- *Silica:*
- *Glass*
- *Activated carbon*
- *Charcoal*

Types/Methods of Immobilization

- Five different methods of immobilization of enzyme/cells

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

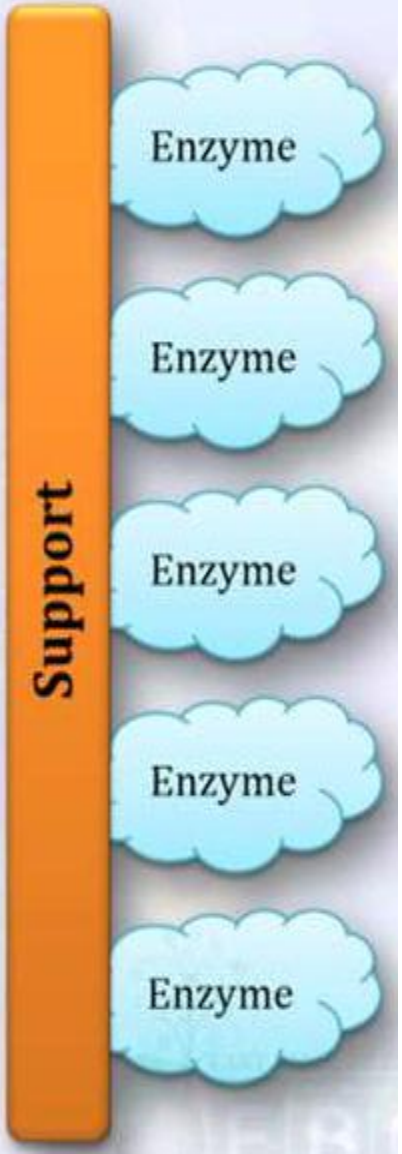


Enzyme/Cell Immobilization Methods

(1). Adsorption

- Oldest method of enzyme immobilization
- Simplest method of enzyme immobilization
- Nelson & Griffin used charcoal to adsorb ***invertase***
- Enzymes are adsorbed to external surface of support
- Support/carrier may be :

    1. Mineral support (aluminum oxide, clay)
    2. Organic support (starch)
    3. Modified sepharose and ion exchange resins



Adsorption

(1). Adsorption

- Weak bonds stabilize enzymes to the support/carrier
- Bonds involved are low energy bonds such as:
  - ❖ Ionic interaction
  - ❖ Hydrogen bonds
  - ❖ Van der Waal forces
- Carrier particle size must be small (for appreciable surface bonding)
- Particle size used: 500 Å to 1 mm diameter
- No pore diffusion limitations (since enzyme are immobilized externally)

Methods of adsorption:

1. **Static process:** Immobilization to carrier by allowing the solution containing enzyme to contact the carrier (without stirring)
2. **Dynamic batch process:** Carrier is placed in the enzyme solution and mixed by stirring or agitation
3. **Reactor loading process:** Carrier is placed in the reactor, then enzyme solution is transferred to reactor
4. **Electrode position process:** Carrier is placed proximal to an electrode in an enzyme bath and the current is put on, the enzyme migrates to the carrier and deposited on the surface

(1). Adsorption

Advantages of adsorption method

- Easy to carry out
- No reagents are required
- Minimum activation steps involved
- Comparatively cheap method
- Less disruptive to protein than chemical methods

Disadvantages of adsorption method

- Desorption of enzymes from the carrier
- Efficiency is less

(2). Covalent bonding:

- Involves the formation of **covalent** bonds between enzyme and support
- Widely used method of enzyme immobilization
- Chemical groups in enzymes that forms covalent bonds with support are:
  - *Amino groups, Imino groups*
  - *Hydroxyl groups*
  - *Carboxyl groups*
  - *Thiol groups and Methylthiol groups*
  - *Guanidyl groups and Imidazole groups*
  - *Phenol rings*

(2). Covalent bonding:

- Important functional groups of enzyme that provide chemical groups to form covalent bonds with support/carrier are:

1. Alpha carboxyl group at 'C' terminal
2. Alpha amino group at 'N' terminal
3. Epsilon amino groups of Lysine and Arginine
4. Beta and gamma carboxyl groups of Aspartate and Glutamate
5. Phenol ring of Tyrosine
6. Thiol group of Cysteine
7. Hydroxyl groups of Serine and Threonine
8. Imidazole group of Histidine
9. Indole ring of Tryptophan

(2). Covalent bonding:

- Carriers/supports used for covalent bonding:
  - *Carbohydrates*: Eg. Cellulose, DEAE cellulose, Agarose
  - *Synthetic agents*: Eg. Polyacrylamide
  - *Protein carriers*
  - *Amino group bearing carriers*: Eg. amino benzyl cellulose
  - *Inorganic carriers*: Porous glass, silica
  - *Cyanogen bromide* (CNBr)-agarose and CNBr Sepharose
- Hydroxyl and Amino groups form covalent bonds more easily

Methods of covalent bonding

1. ***Diazoation:*** Bonding between amino group of support and thyrosil or histidyl group of enzyme

2. ***Peptide bond:*** between amino/carboxyl groups of support and enzyme

3. ***Poly functional reagents:*** Use of a bi-functional or multifunctional reagent (glutaraldehyde) which forms bonding between the amino group of the support and amino group of the enzyme

(2). Covalent bonding:

Advantages

- Strong linkage of enzyme to the support
- No leakage or desorption problem
- Comparatively simple method
- A variety of support with different functional groups available
- Wide applicability

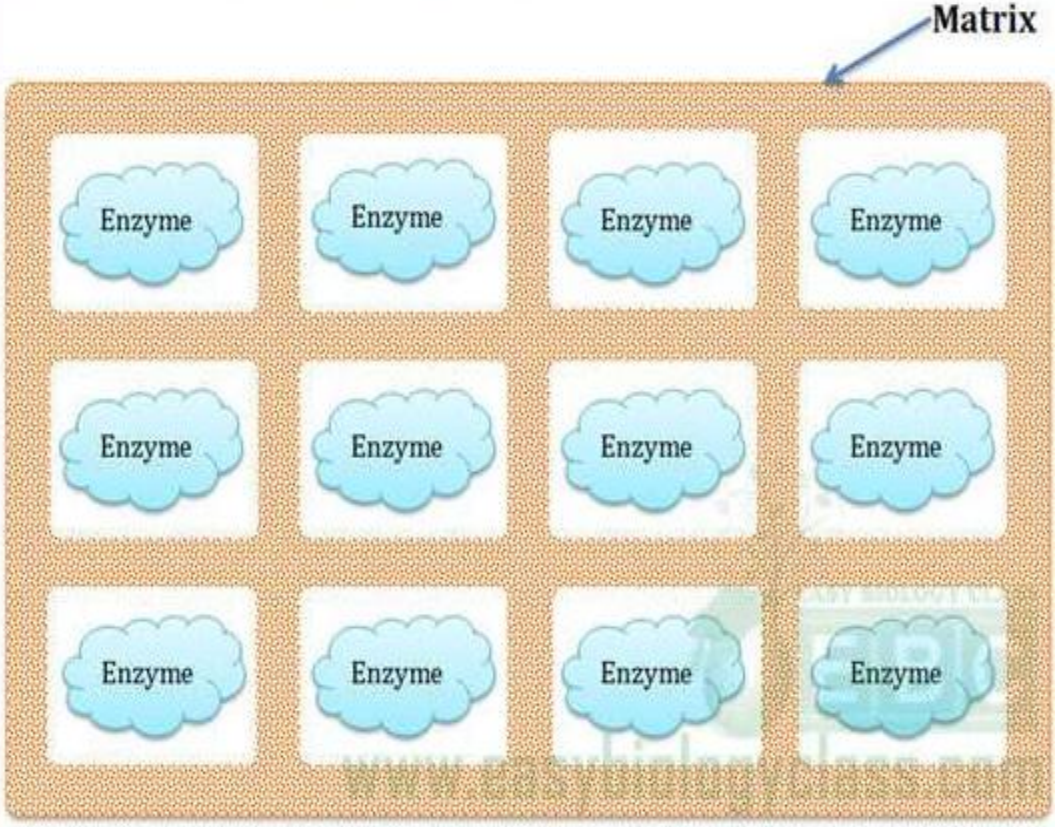
(2). Covalent bonding:

Disadvantages: (major problem with covalent bonding):

- Chemical modification of enzyme leading to functional conformation loss
- Enzyme inactivation by changes in the conformation when undergoes reactions at active sites
- This can be overcome through immobilization in the presence of enzyme substrate or a competitive inhibitor

(3). Entrapment:

- Enzymes are physically entrapped inside a matrix
- Bonds involved may be covalent or non-covalent
- Matrix used will be water soluble polymer
- Examples of matrix:
  - ❖ polyacrylamide gels
  - ❖ Cellulose triacetate
  - ❖ Agar
  - ❖ Gelatin
  - ❖ Carrageenan
  - ❖ Alginate



(3). Entrapment:

- Form and nature of matrix varies
- Pore size of matrix is adjusted to prevent loss of enzyme
- Possibility of leakage of low molecular weight enzymes
- Agar and carrageenan have large pore sizes
- Pore size can be adjusted with the concentration of the polymer
- Entrapment of enzyme can be used for sensing application
- Not much success in industrial process
- Easy to practice at small scale

Methods of entrapment:

1. **Inclusion in the gels:** enzymes trapped in gels
2. **Inclusion in fibers:** enzymes supported on fiber format
3. **Inclusion in microcapsules:** Enzymes entrapped in microcapsules formed by monomer mixtures such as polyamine, calcium alginate

(3). Entrapment:

Advantages:

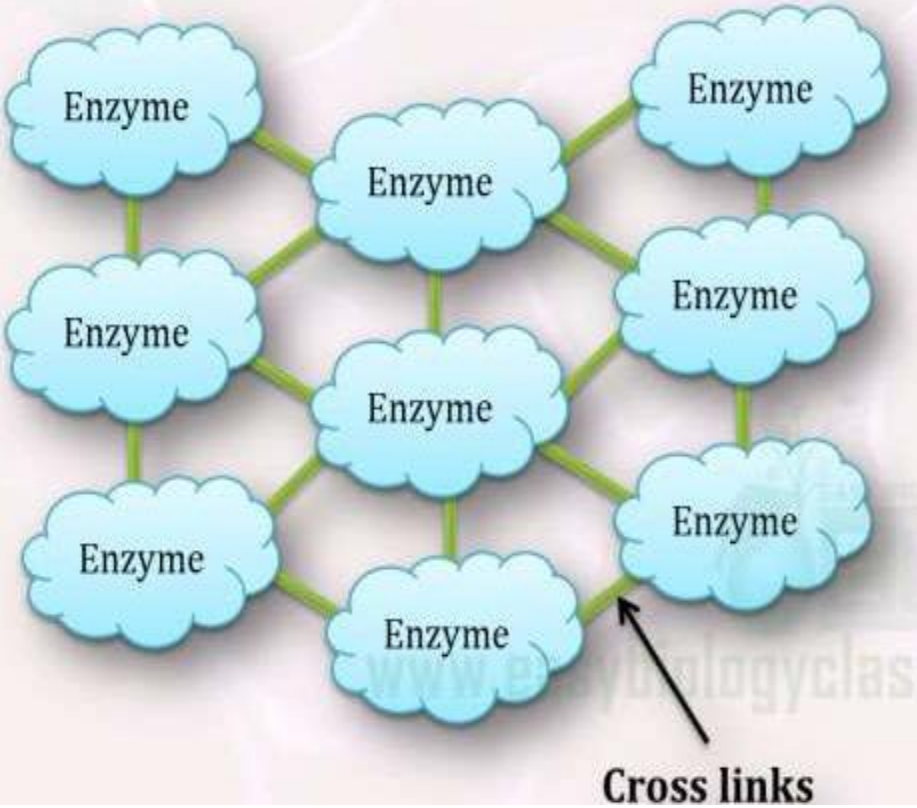
- Fast
- Cheap (low cost matrix available)
- Mild conditions are required
- Less chance of conformational changes in enzyme

Disadvantages:

- Leakage of enzyme
- Pore diffusion limitation
- Chance of microbial contamination

(4). Cross linking (copolymerization):

- Cross linking: covalent bonding between various groups of enzymes via polyfunctional reagents
- No matrix or support are involved

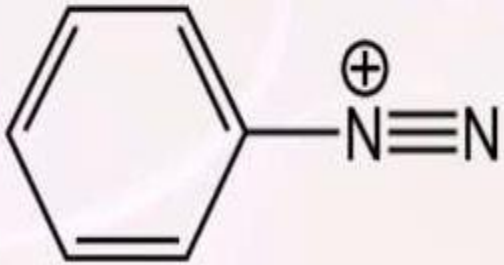


(4). Cross linking (copolymerization):

- Commonly used polyfunctional reagents: Glutaraldehyde, Diazonium salt
- Technique is cheap and simple but not often used with pure proteins
- It is widely used in commercial preparations

Demerit:

- Polyfunctional reagents can denature the enzyme

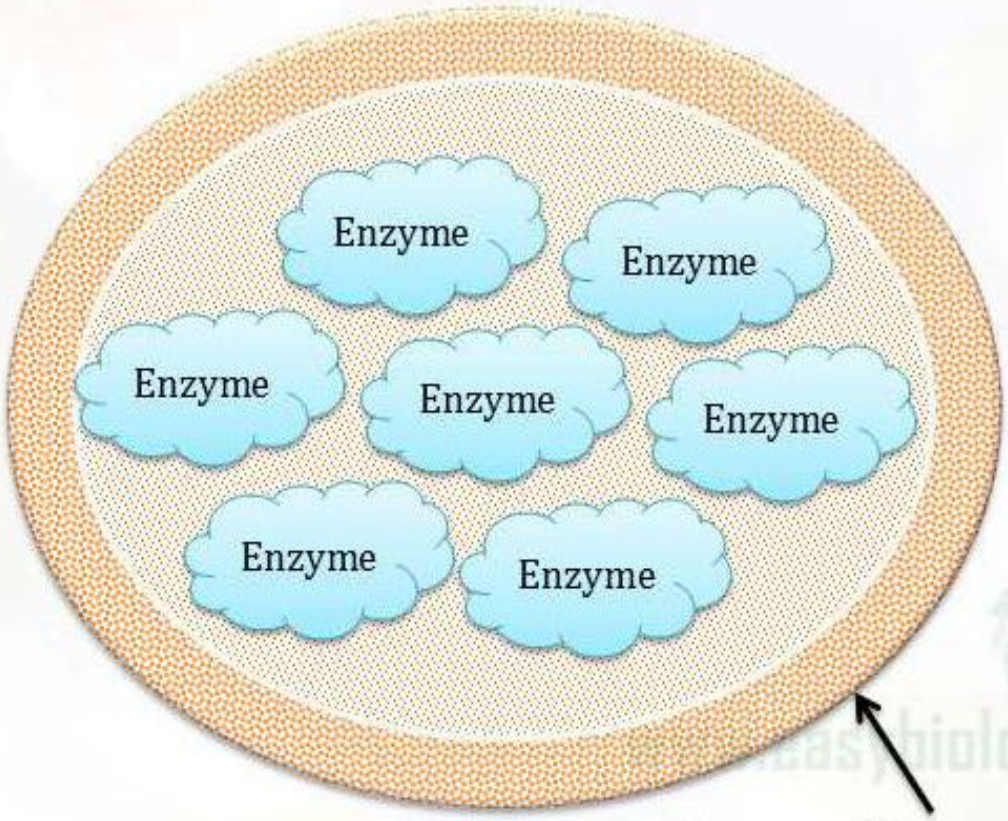


Diazonium

Glutaraldehyde

(5). Encapsulation:

- Enclosing enzymes in a semi permeable membrane capsule
- Capsule is made up of nitro cellulose or nylon
- Effectiveness depends upon the stability of enzymes



(5). Encapsulation:

Advantages:

- Cheap and simple method
- Large quantity of enzymes can be immobilized by encapsulation

Disadvantages:

- Pore size limitation
- Only small substrate molecule is able to cross the membrane

Immobilization of cells:

- An alternative to enzyme immobilization
- Well developed method for the utilization of enzymes from microbes
- Effective method when:
  - Individual enzymes become inactive during immobilization
  - Isolation and purification of enzyme is not cost effective
- Here enzymes will be active and stable for a long period of time
- Method of cell immobilization are same as described for enzyme immobilization
- Adsorption method is the oldest method (use of woodchips as a carrier)

Advantages of whole cell immobilization:

- Multiple enzymes can be introduced to a single step
- Extraction and purification of enzymes are not required
- Enzymes are stable for long time
- Native conformation of enzyme is best maintained
- Cell organelles like mitochondria and chloroplasts can be immobilized

Disadvantages of whole cell immobilization:

- Concentration of enzymes will be less
- Production of unwanted enzymes and unwanted products
- Modification of end products by other enzymes

Immobilization of cells:

- Methods of whole cell immobilization are similar to enzyme immobilization
- Methods of whole cell immobilization:
  - ❑ ***Adsorption***
  - ❑ ***Covalent bonding***
  - ❑ ***Cell to cell cross linking***
  - ❑ ***Encapsulation***
  - ❑ ***Entrapment***

Immobilization of cells: Methods, Support materials, Cells and Reaction

Method	Support Material	Cells	Reaction
Adsorption	Gelatin	*Lactobacilli*	Lactose ⟹ lactic acid
	Porous glass	*Saccharomyces*	Glucose ⟹ ethanol
	Cotton fibers	*Zymomonas*	Glucose ⟹ ethanol
	DEAE Cellulose	*Nocardia*	Steroid conversion
Covalent bonding	Cellulose + cyanuric chloride	*S. cerevisiae*	Glucose ⟹ ethanol
	Titanium oxide	*Acetobacter*	Vinegar
Cross linking	Glutaraldehyde	*E. coli*	Fumaric acid
Entrapment	Aluminium alginate	*Candida tropicalis*	Phenol degradation
	Calcium alginate	*S. cervisiae*	Glucose ⟹ ethanol
Encapsulation	Polyester	*Streptomyces sps.*	Glucose ⟹ fructose
	Alginate polylysine	Hybridoma cells	Monoclonal antibodies