CELL AND ENZYME IMMOBILIZATION TECHNIQUES

MODULE 8

WHAT IS IMMOBILIZATION?

- Imprisonment of cell/enzyme in a distinct support or matrix.
- The support or matrix allows exchange of medium.
- The medium contains substrate or effector or inhibitor molecules.
- First immobilization technology: amino acylases by *Aspergillus oryzae* for the production of L-amino acids in japan.
- Two main advantages of cell immobilization technology includes;
  - Increased functional efficiency
  - Enhanced reproducibility`

SUPPORTS OR MATRIX USED IN IMMOBILIZATION TECHNOLOGY

- The matrix holds the enzyme/cells.
- The matrix used should be cheap and easily available.
- Their reaction with the product should be minimum
- Various types of matrix are used and the major category includes;
  - Natural polymers
  - Synthetic polymers
  - Inorganic materials

(1) NATURAL POLYMERS

1. ALGINATE: Derived from algal cell wall (calcium or magnesium alginate)
2. CHITOSAN AND CHITIN: Used for enzyme attachment; binds to the free oh group
3. COLLAGEN: Protein derived
4. CARRAGEENAN: A sulphated polysaccharide obtained from algae
5. GELATIN: Partially hydrolised collagen with high water holding capasity.
6. CELLULOSE: Cheapest support available
7. STARCH: Good water holding capacity
8. PECTIN: Good water holding capacity

(2) SYNTHETIC POLYMERS

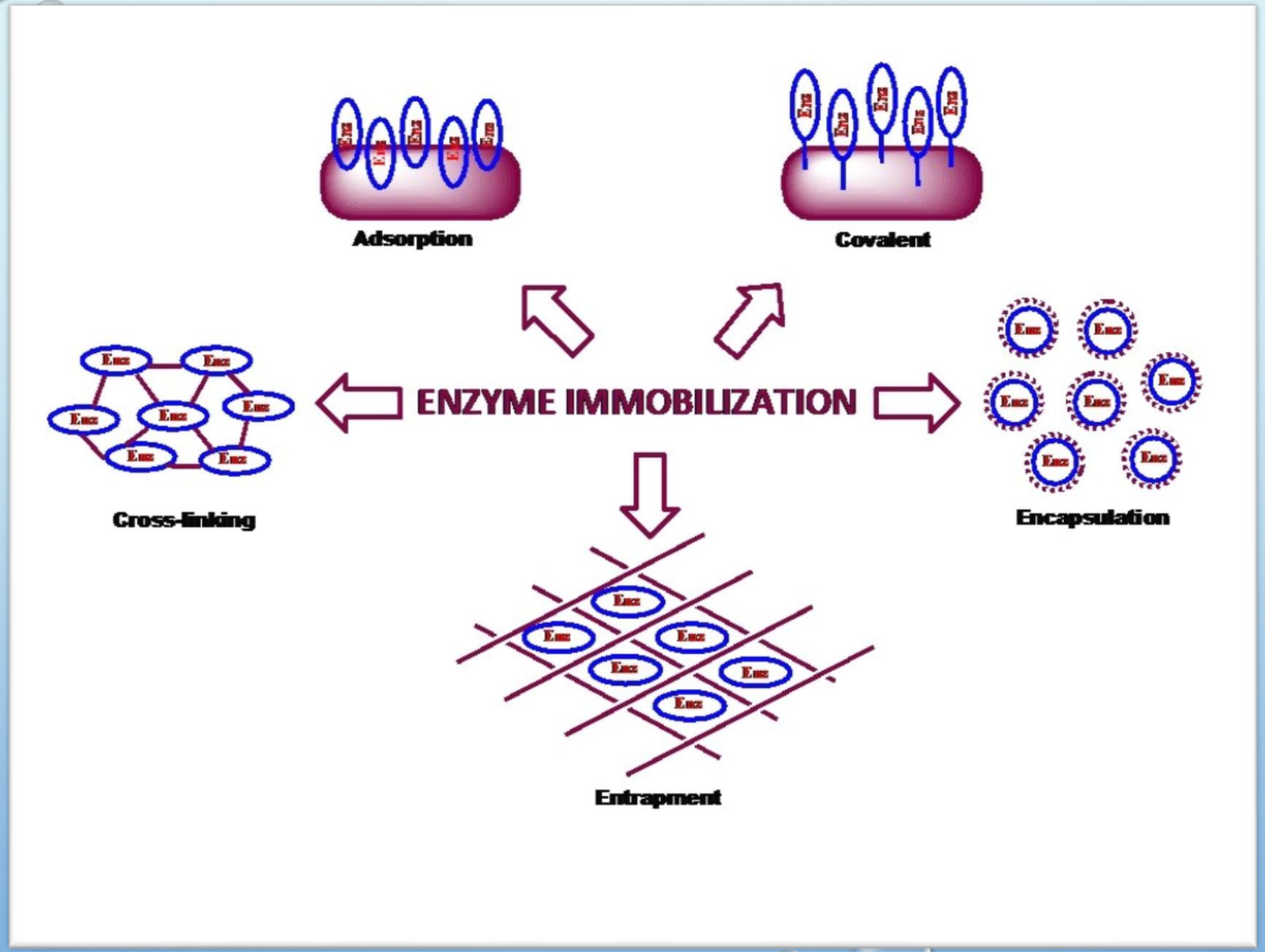
- They are ion exchange resins or polymers
- They are insoluble supports with porous surface
- The porous surface trap and hold the enzymes/cells
- Examples;
    1. DEAE CELLULOSE
    2. POLYVINYL CHLORIDE
    3. UV ACTIVATED POLYETHYLENE GLYCOL

(3) INORGANIC MATERIALS

1. ZEOLITES
2. CERAMICS
3. DIATOMACEOUS EARTH
4. SILICA
5. GLASS
6. ACTIVATED CARBON
7. CHARCOAL

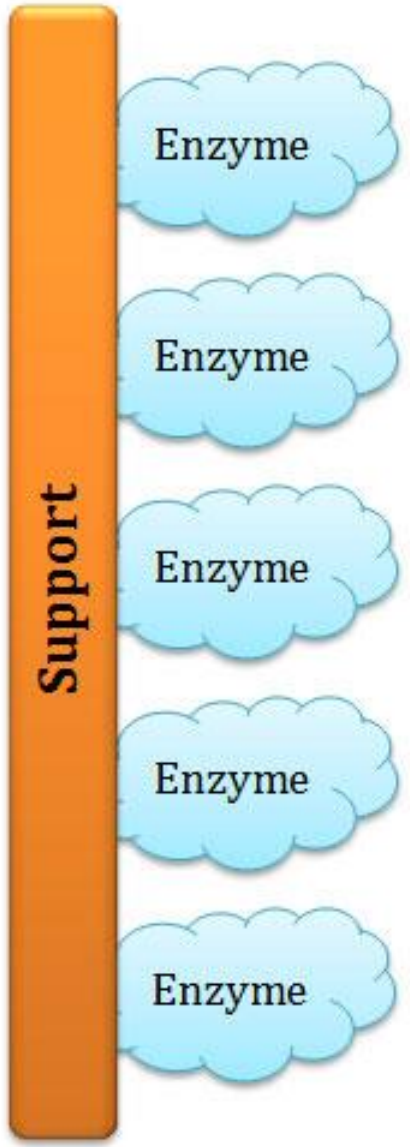
METHODS OF IMMOBILIZATION

1. ADSORPTION
2. COVALENT BONDING
3. ENTRAPMENT
4. COPOLYMERIZATION
5. ENCAPSULATION


Adsorption
Covalent
ENZYME IMMOBILIZATION
Cross-linking
Encapsulation
Entrapment

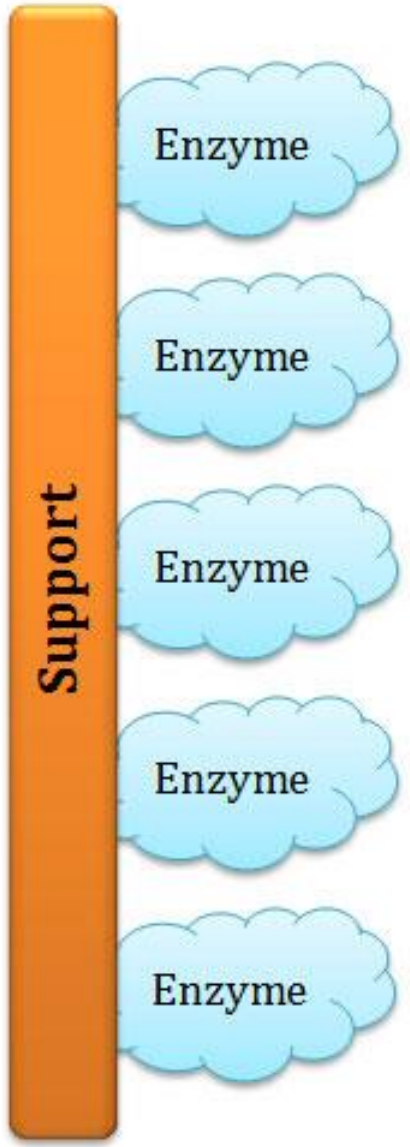
ADSORPTION

- Oldest and simplest method of immobilization
- Technique was developed by Nelson and Griffin
- Enzymes/cells are adsorbed to the surface of the support
- Support can be;
  - Mineral support
  - Organic support
  - Modified sepharose and iron exchange resins



Adsorption
(Enzyme Immobilization)

- Weak bonds stabilize enzymes to the support
- Bonds involved are low energy bonds such as;
  - Ionic interactions
  - Hydrogen bonds
  - Van der waal forces
- Support particle size must be small and should give maximum space for adsorption



Adsorption
(Enzyme Immobilization)

ADVANTAGES OF ADSORPTION METHOD

- Easy to carry out
- Minimum activation steps involved
- Comparatively cheap method

DISADVANTAGES OF ADSOPRPTION METHOD

- Desorption of enzymes from the support
- Less efficiency

COVALENT BONDING

- Involves the formation of covalent bonds between enzyme/cell and support
- Widely used method for enzyme immobilization
- Chemical bonds in enzymes that forms covalent bonds with support are;
    1. Amino groups and imino groups
    2. Hydroxyl groups
    3. Carboxyl groups
    4. Thiol groups and methylthiol groups
    5. Guanidyl groups and imidazole groups
    6. Phenol rings

CARRIERS OR SUPPORTS USED FOR COVALENT BINDING

I. Carbohydrates: Cellulose, DEAE cellulose, Agarose

II. Synthetic agents: Polyacrylamide

III. Amino group bearing carriers: Amino benzyl cellulose

IV. Inorganic carriers: Porous glass, Silica

V. Cyanogen bromide: CnBr-agarose and CnBr-sepharose

VI. Protein carriers: Collagen and Gelatin

METHODS OF COVALENT BONDING

1. DIAZOATION: Bonding between amino group of support and tyrosil or histidyl group of enzyme

2. PEPTIDE BOND: Between amino and caboxyl groups of support and enzyme

3. POLY FUNCTIONAL REAGENTS: Use of a bi-functional or multifunctional reagent like glutaraldehyde which forms a bonding between the amino group of the support and the amino group of the enzyme

ADVANTAGES OF COVALENT BONDING METHOD

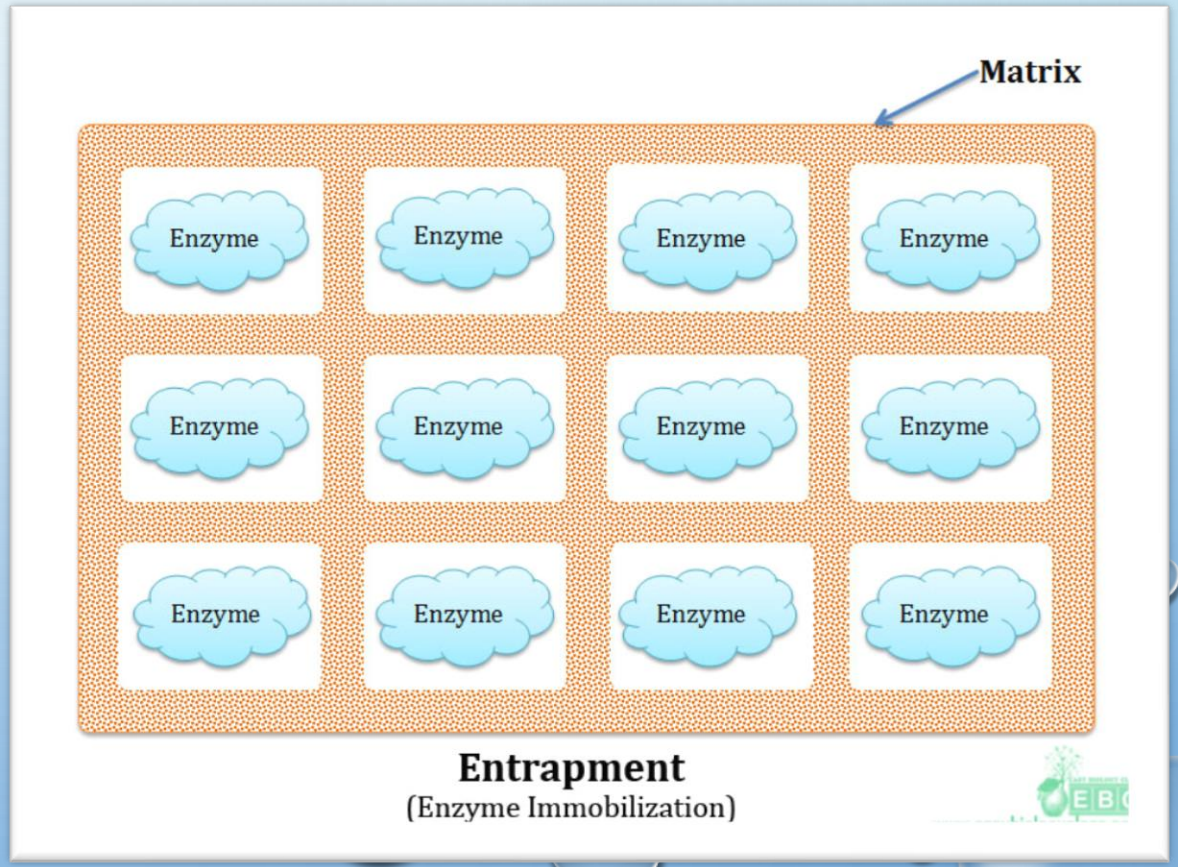
- Stong linkage of enzyme to the support
- No leakage of desorption problem
- Comapritively simple mehod
- A variety of support with different functional groups

DISADVANTAGES OF COVALENT BONDING METHOD

- Chemical modification of enzyme leading to functional conformation loss
- Enzyme inactivation can occur due to conformation changes in the enzyme

ENTRAPMENT

- Cells/ enzymes are physically entrapped in a matrix
- Bonds involved can be covalent or non covalent
- Matrix used will be a water soluble one
- Examples of matrix;
    1. Polyacrylamide gels
    2. Cellulose triacetate
    3. Agar
    4. Gelatin
    5. Carageenan
    6. Alginate



- Form and nature of matrix varies
- Pore size of matrix is adjusted to prevent loss of cell/enzyme
- Pore size can be adjusted with the concentration of polymer
- Agar and carrageenan has high pore size
- It has not much application in industrial level
- Easy to practice at small scale level

METHODS OF ENTRAPMENT

1. INCLUSIONS IN GEL: Cells/enzymes are trapped in GEL

2. INCLUSIONS IN FIBRES: Cells/enzymes are supported on fibre matrix

3. INCLUSION IN MICROCAPSULES: Cells/enzymes are trapped in microcapsules formed by monomer mixtures such as polyamine, calcium alginate etc.

ADVANTAGES OF ENTRAPMENT METHOD

- Fast
- Cheap (Low cost matrix available)
- Less chance of conformational change to the enzyme

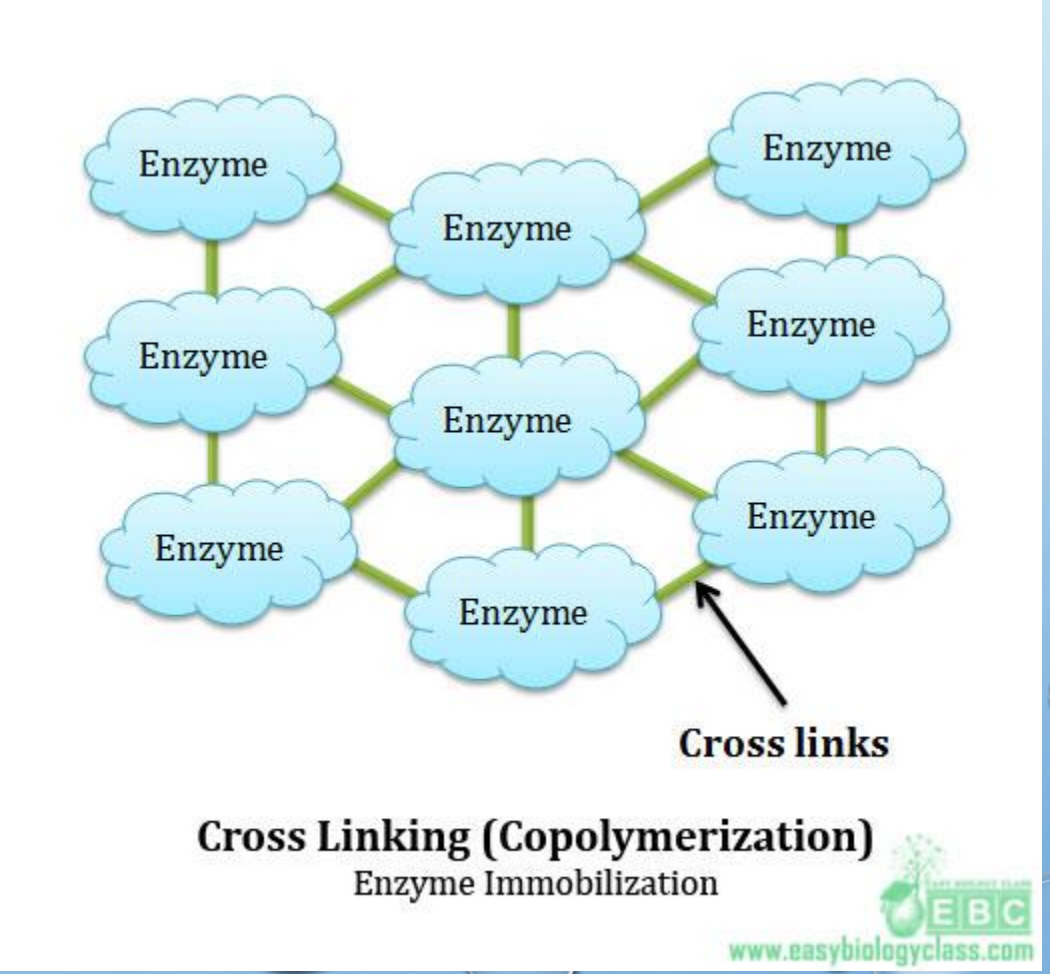
DISADVANTAGES OF ENTRAPMENT METHOD

- Leakage of enzyme
- Chances of microbial contamination

COPOLYMERIZATION (CROSS-LINKING)

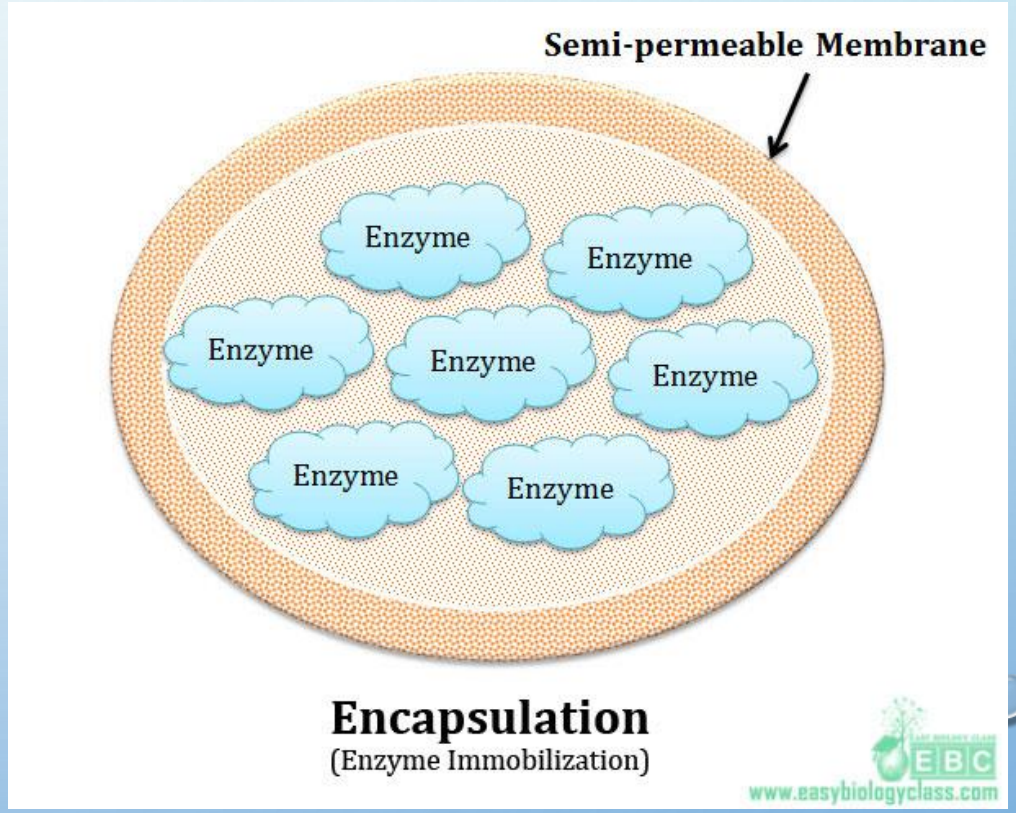
- Cross linking involves intermolecular cross linking of enzymes in the presence or absence of solid support
- Commonly used polyfunctional groups includes;
  - Glutaraldehyde
  - Diazonium salt
- It is widely used in commercial preparations
- A disadvantage of polyfunctional group is that,

  they may denature the enzyme



ENCAPSULATION

- ENCLOSING ENZYMES/CELLS IN A PERMEABLE MEMBRANE CAPSULE
- CAPSULE MADE UP OF NYLON OR NITRO CELLULOSE
- EFFECTIVENESS DEPENDS ON THE STABILITY OF ENZYMES



ADVANTAGES OF ENCAPSULATION METHOD

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

DISADVANTAGES OF ENCAPSULATION METHOD

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

IMMOBILIZATION TECHNIQUES

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

DISADVANTAGES OF USING NORMAL CELL CULTURES WHICH COLD BE OVERCOME WITH IMMOBILIZATION TECHNIQUES;

- SLOW GROWTH OF CELLS
- LOW SHEAR RESISTANCE OF CELLS
- INTRACELLULAR PRODUCTS
- LOW YIELD OF PRODUCT
- CELL AGGREGATIONS
- GENETIC INSTABILITY OF CELL LINE

POTENTIAL BENEFITS OF USING PLANT CELL IMMOBILIZATION

- INCREASED PRODUCTION OF SECONDARY METABOLITES AND RECOMBINANT PROTEINS
- IMMOBILIZATION REPRESSES CELL REPLICATION, THUS PROBLEMS DUE TO GENETIC INSTABILITY CAN BE SIGNIFICANTLY REDUCED.
- THE SIZE OF CELL AGGREGATES CAN BE CONTROLLED.
- CAN REGULATE PRODUCTION OF SECONDARY METABOLITE YIELDS AND ELICIT THE EXCRETION OF THESE METABOLITES TO THE SURROUNDING MEDIUM.

ADVANTAGES OF ENZYME IMMOBILIZATION

- STABLE AND MORE EFFECTIVE FUNCTION
- CAN BE RESUSED AGAIN AND AGAIN
- PRODUCTS WILL BE FREE OF ENZYME
- IDEAL FOR MULTI-ENZYME REACTION SYSTEMS
- SUITABLE FOR INDUSTRIAL AND MEDICINAL USES

DISADVANTAGES OF ENZYME IMMOBILIZATION

- POSSIBILITY OF LOSS OF BIOLOGICAL ACTIVITY OF ENZYMES DURING IMMOBILIZATION PROCESS
- IMMOBILIZING ENZYME IS AN EXPENSIVE AFFAIR AND REQUIRE SOPHISTICATED EQUIPMENT'S
- REQUIRES OPTIMAL CONDITIONS FOR PROPER FUNCTIONING

APPLICATIONS OF CELL IMMOBILIZATION

- ANTIBIOTIC PRODUCTION BY IMMOBILIZED MICROBIAL CELLS

  - The most widely studied system is the production of penicillin G using immobilized cells of *Penicillium chrysogenum*. Gaucher group entrapped the conidia of the fungi in carrageenan and used for batch and continuous production of penicillin, and compared it with fungi adsorbed on celite. It has been reported that the adsorption on celite was five times more productive than entrapment in carrageenan. The adsorbed cells exhibited maximum specific reaction rates compared to the free-cells. This may be due to better oxygen availability to the cells.

➢ ORGANIC ACIDS PRODUCTION BY IMMOBILIZED CELLS

- Organic acids are important microbial products used in a variety of applications such as food and medicines. Among the various organic acids, citric acid occupies predominant position as a commercial biochemical. *Aspergillus niger* is the widely used microorganism for the synthesis of citric acid. In conventional practices, batch fermentation of *A. Nigeris* employed. The fungal fermentations have serious disadvantage of rising viscosity during growth, leading to poor oxygen supply to the cells. Therefore, it becomes necessary to aerate the cultures with large volumes of sterile air. In case of immobilized cells, since growth is restricted, it is possible to operate the fermentor without affecting the viscosity, thereby facilitating good oxygen transfer rates with minimal cause.

- The methods most widely used for immobilization of *A. niger* cells are the entrapment in alginate gels, agarose and polyacrylamide. In addition, adsorption on various supports, such as polyurethane foam and entrapment in hollow fibres.

➢ PRODUCTION OF ENZYMES BY IMMOBILIZED CELLS

- Microorganisms are the best sources for the production of useful enzymes. Cell immobilization technology is aptly suited to produce extracellular enzymes. Among the microbial enzymes, starch degrading enzymes a -amylase and glucoamylase have been studied extensively.

- Entrapment of cells in polyacrylamide, calcium alginate, agar, and several other polymer supports have been tried.

- *Aspergillus* strains and *Bacillus cereus* have been immobilized for the production of glucoamylase and a-amylase enzyme.

➢ PRODUCTION OF ALCOHOLS BY IMMOBILIZED CELLS

- Ethanol fermentation using immobilized cells of yeast, is one of the widely studied systems. In fact, almost all the methods of immobilization, namely, gel entrapment, adsorption on the surfaces of the various carriers, crosslinking were tried for alcohol fermentation.

- Gil have used ceramic-like matrix material constructed of aluminum silicate composition as a carrier for yeast immobilization. It has been reported that the continuous process was operated over a period of 2 years producing huge amount of alcohol compared to normal fermentation methods. Similarly, another research group used polyurethane foam to entrap *Z. Mobilis* to produce high concentrations of ethanol.

- PRODUCTION OF RECOMBINANT PROTEINS
  - Plant cells have been widely used to secrete biologically active mammalian proteins like human interleukin-2 and human interleukin-4. Human granulocyte macrophage colony stimulating factor have also been synthesized from plant cells using rDNA technology.
- PRODUCTION OF SECONDARY METABOLITES
  - Alkaloids, terpenoids, flavours and dyes are developed from plant cells with the help of immobilizing techniques.

- OTHER APPLICATIONS OF CELL/ENZYME IMMOBILIZATION INCLUDES;
  - PRODUCTION OF BIODIESEL
  - BIOCHEMICAL ANALYSIS
  - SEWAGE TREATMENT

THANK YOU