UNIT-1

Brief Introduction to Biotechnology

→ Biotechnology is a science that utilises biological system, living organisms or parts of this to develop or create different products.
Baking bread is an example of concept of biotechnology.

→ The development of insulin and growth hormone, gene therapies, vaccines such as hepatitis B are some of the big achievements in the field of biotechnology.

Biotechnology examples in everyday life

- Biofuels (ethanol, biodiesel)
- Bioplastics
- Alcohol production
- Pest resistant crops (Bt cotton)
- cheese production
- Vaccines

Applications of Biotechnology

1) Cloning → producing genetically identical copies of biological entities.
2) Paternity or Maternity → This is the test used to find out who are the parents of the child.
3) Criminal Identification and Forensics → This is a field of DNA fingerprinting.
4) Diagnosis and cure of inherited diseases
DNA fingerprinting can also be used to detect and cure genetically inherited disease.

Enzyme Biotechnology

Enzyme is a substance that acts as a catalyst in living organisms, regulating the rate of chemical reactions. Enzyme does not have its own involvement in chemical reaction. It remains unaltered.

→ The molecules upon which the enzymes act are called substrates

→ If there is any change in temperature or pH, the enzyme is denatured and its enzymatic activity may be lost. Denaturation is sometimes, but not always, reversible.

→ **Coenzyme** is an organic molecule that binds to the active sites of certain enzymes to assist in the catalysis of a reaction.

Applications

1) Industrial applications
   - fermenting of wine
   - bread baking
   - curdling of cheese
   - brewing of beer

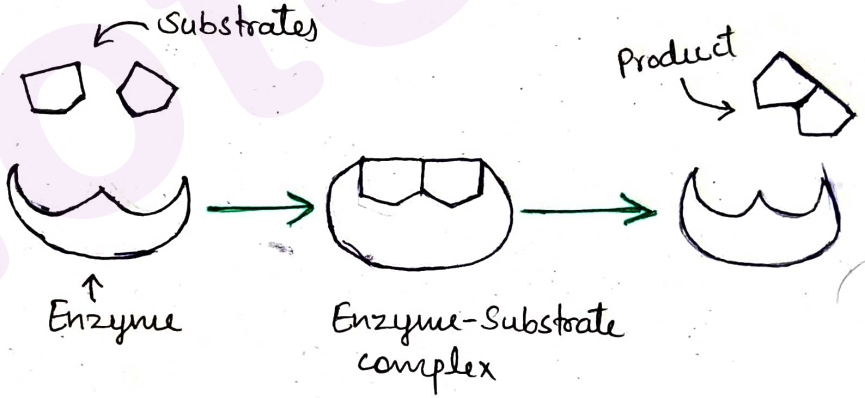
2) Medical applications
   - killing disease-causing microorganisms
   - promote wound healing
   - diagnosing certain diseases.

Mechanism of Enzyme Action

(i) The enzyme grabs onto the substrate at its active site. The combination of both is called enzyme-substrate complex.

(ii) A process called catalysis happens. During catalysis, the substrate will break or will build chemical bonds to form new products.

(iii) The enzyme releases the product. This enzyme is ready to work on another molecule.



Examples of enzymes

(i) Lipases → digest fats
(ii) Amylase → found in saliva, digest carbs
(iii) Trypsin → digest proteins
(iv) Acetylcholinestrase → breaks acetylcholine
(v) Helicase → opens up DNA
(vi) DNA polymerase → synthesise DNA from dioxyribonucleotides.

Enzyme Immobilisation

The enzymes are separated from the enzyme-substrate complex after the products are formed. This process of separation of enzymes is very difficult and expensive. Most of the enzymes are lost after the first use.

To overcome this problem, technology of enzyme Immobilisation was introduced.

Immobilised enzymes are the enzymes that are physically attached to specific solid supports and thus confined, and which can be used repeatedly and continuously while maintaining their catalytic abilities.

And this confining of enzymes is called enzyme immobilisation.

Why Immobilise Enzymes ?

(i) Protection from degradation and deactivation
(ii) Re-use of enzymes for many reaction cycles
(iii) Ability to stop the reaction rapidly
(iv) Enhanced stability
(v) Easy separation of enzyme from the product.
(vi) cost efficiency

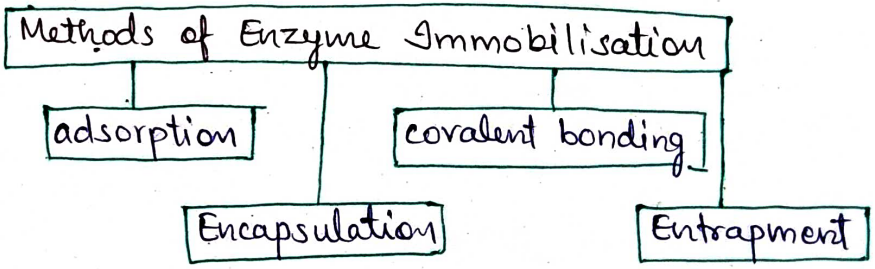
Carrier Matrices

These are the substances that are employed for the immobilisation of enzymes.

e.g. inorganic materials (salts), inert polymers

An ideal carrier matrix should be:

- inert
- physically strong and stable
- cost effective
- Regenerable
- reduction in product inhibition



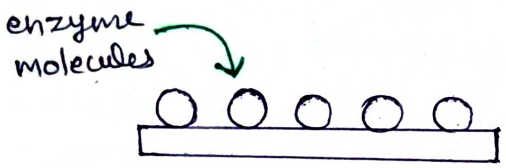
1) Adsorption

→ Involves the physical binding of the enzyme on the surface of carrier matrix.

→ Carrier may be organic or inorganic

→ adsorption involves weak forces, mostly electrostatic like Van der Waals forces, ionic bond, hydrogen bond.

Carriers used are silica, bentonite, cellulose, etc.



Advantages	Disadvantages
- simple/economical - Limited loss of activity - can be recycled, regenerated & Reused	- low surface area for binding - Exposure of enzyme to microbial attack - Yield are often low

2) Covalent Bonding

Enzymes bind to water-insoluble carriers by covalent bonds.

The functional groups that may take part in this binding are, amino group, carboxyl group, sulfhydryl group, hydroxyl, imidazole, phenolic, etc.

strength of reactivity to enzymes:

$-S^- > -SH > -O^- > -NH_2 > -COO^- > -OH$

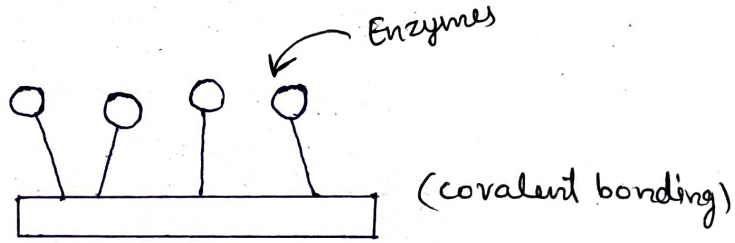
Advantages of covalent bonding

(i) easy and convenient

(ii) binding force is so strong that no leakage of enzymes occur, even in the presence of substrate or a solution of high ionic strength.

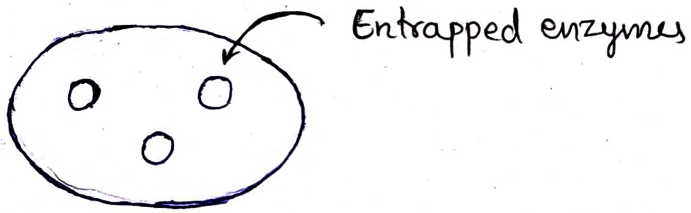
Disadvantages

(i) loss in enzyme activity



3) Entrapment

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



Entrapment immobilization process is conducted through two steps:

(i) mixing enzyme into a monomer solution
(ii) polymerisation of monomer solution by a chemical reaction.

→ Enzymes are held or entrapped within the suitable gels and fibres.
It is done in such a way as to retain enzyme while allowing penetration of substrate.

Polymers used:
- cellulose triacetate, alginate, agar, gelatin

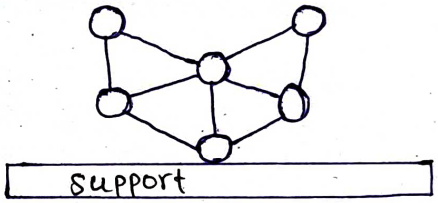
Advantages
- No chemical modification
- Relatively stable forms
- Easy handling and reusage

Disadvantages
The enzyme may leak from the pores.

4) 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 (CLEA) by means of a multifunctional reagent that links covalently to the enzyme molecules.

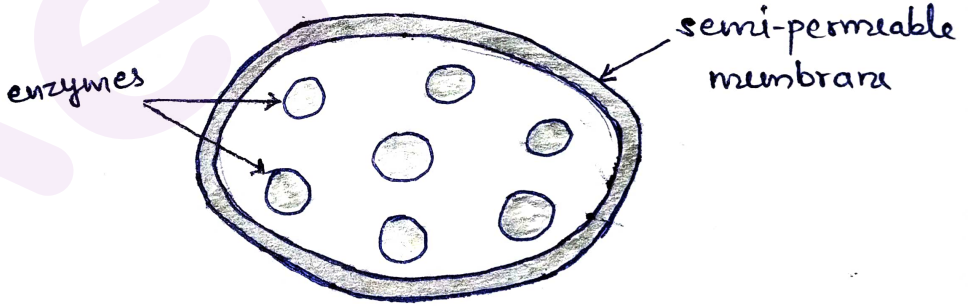


Advantages
(i) enzyme strongly bound
(ii) Higher stability

Disadvantages
- cross linking may cause significant changes in the active site.
- not so cost effective.

5. Encapsulation

Encapsulation or microencapsulation is a type of entrapment which is done by enclosing the enzymes in a membrane capsule. The capsule will be made up of semi-permeable membrane like nitro-cellulose or nylon. In this method, the effectiveness depends upon the stability of enzymes inside the capsule.



Advantages

a) cheap and simple method
b) large quantity of enzymes can be immobilized.

Disadvantages

a) Pore size limitation
b) only small substrate molecule is able to cross the membrane.

Advantages of Enzyme Immobilisation

- Retain almost all activity of enzyme
- Support is chemically or mechanically stable and resistant to microbial attack
- Relatively stable to hydrolysis at neutral pH
- Immobilised enzymes may exhibit thermo-stability of the highest order.
- Recovery of immobilised enzymes after use would minimise the disposable problems of free enzymes.

Disadvantages

- Diffusion of substrate to the enzyme is restricted
- Enzyme immobilisation affects the stability and activity of enzyme.
- variable pore size preparation in harsh conditions causing denaturation of enzyme (this is in context of adsorption on mesoporous silicates)

Applications of Enzyme Immobilisation

(i) Food industry
(ii) Dairy industry
(iii) Protein purification
(iv) Biodiesel production
(v) Textile industry
(vi) Wastewater treatment
(vii) decolourisation of dyes
(viii) Antibiotic production

Biosensors

→ Biosensors are the analytical devices that convert a biological response (BP, temperature, heart rate) into an electrical signal

→ Biosensors started with development of 'enzyme electrodes'.

→ Biosensors must be highly specific, independent of physical parameters such as pH, temperature, etc and should be reusable.

Working of Biosensor

A biosensor consists of following components:

- bioreceptor
- Tranducer element
- Electronic circuit and display.

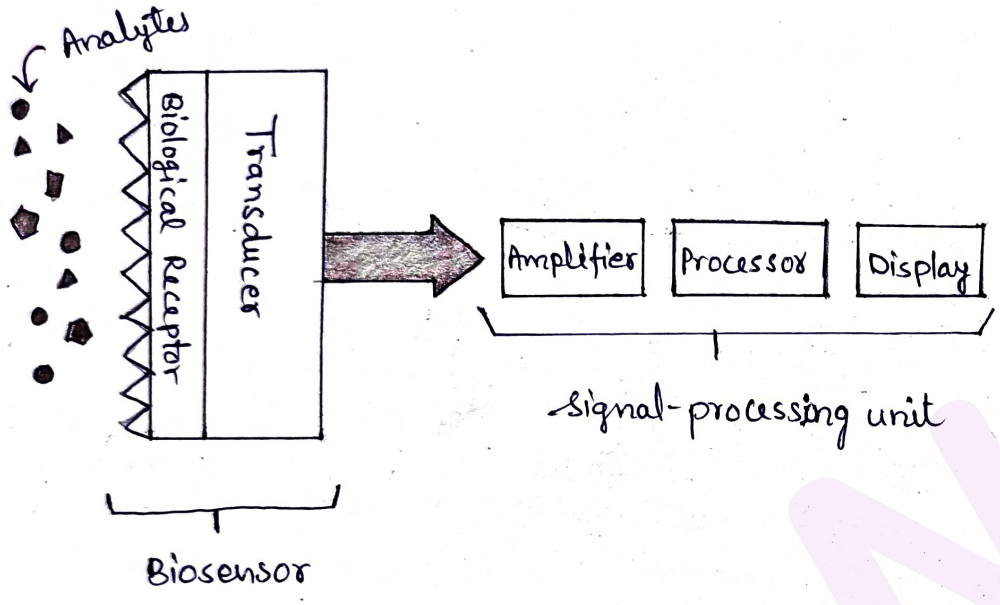
[Please refer to the diagram]

Steps in working of bioreceptors:

(i) Bioreceptors detect the input physical change due to biological element.

(ii) The transducer transforms the physical change into electrical signal.

(iii) The electrical signal is amplified, processed and given to display (on screen).



Main components of a Biosensor

Biosensor consists of two main parts:

(i) A bioreceptor
(ii) A transducer

Bioreceptor is a biological component (tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, etc.) that recognises the target analyte.

Transducer is a physicochemical detector component that converts the recognition event into a measurable signal.

Types of Biosensors

1) Resonant Biosensor → an acoustic wave transducer is used.

2) Optical biosensors → These biosensors are made based on optical diffraction or electro-chemiluminescence.

3) Thermal Biosensors → These biosensors exploit one of the fundamental properties of biological reactions, i.e., absorption or production of heat. Biosensors detect the temperature change of the medium in which the reaction take place and regulate it.

4) Electrochemical Biosensors → are used for the detection of hybridized DNA, DNA-binding drugs, glucose concentration, etc.
   a) conductimetric biosensors
   b) amperometric biosensors
   c) potentiometric biosensors

5) Bioluminescence Biosensors → These sensors exploit the photons emitted by certain enzymes as a by-product of their chemical reactions.

6) Nanobiosensors → based on nanotechnology.

7) Microbial Biosensors → Microbes are used as biological sensing materials.

Biosensors Applications

- common healthcare checking
- screening for sickness
- Agricultural and Veterinary Applications
- Drug improvement, offense detection
- Processing and monitoring in industry
- Ecological pollution control
- Monitoring of pollutants in water
- In monitoring of toxic gases (e.g. in chemical industries, in wars)
- Medical diagnosis of enzymes

Protein Engineering

Protein engineering is defined as the techniques which are used to manipulate the structure and function of a protein or enzyme so that it acquires specific desired properties.
It is based on the use of recombinant DNA technology to change amino acid sequences.

Objectives of protein engineering

- to create a superior enzyme to catalyse the production of high value specific chemicals.
- to produce enzymes in large quantities.
- to produce biological compounds

Rationale of Protein Engineering (Reason)

For industrial application, an enzyme should possess some characteristics in addition to those of normal enzyme. These are:

(i) enzyme should be robust with long-life.
(ii) enzyme should be able to use substrate supplied in the industry.
(iii) enzyme should be able to work under extreme conditions, e.g., pH, temperature

The characters that have to be changed in protein engineering to get the desired functions are:

- Kinetic properties of enzyme
- Thermostability
- Optimum temperature for the enzyme
- stability and activity in non-aqueous solutions
- Coenzyme (cofactor) requirements
- optimum pH

Basic assumptions

While doing protein engineering, one should recognise the following properties of enzymes.

(i) many amino acid substitution, deletions or additions lead to no changes in enzyme activity.
(ii) Protein have limited number of basic structures, so only minor changes are allowed for variation.
(iii) Similar patterns of chain folding and domain structure can arise from different amino acid sequences with little or no homology.

Methods for protein engineering

- Rational design
- Random and site-directed mutagenesis
  → DNA shuffling
  → X-ray crystallography
  → Molecular dynamics
  → Homology modeling

Mutagenesis

Mutagenesis is the process by which an organism's DNA change, resulting in gene mutation.

Types

1) Random mutagenesis → is a tool for generating enzymes, proteins with desired properties.
2) Focused mutagenesis → It has been developed that involves producing mutations at specific sites of proteins.

Use of Microbes in Industry

Microbes or microorganisms are microscopic organisms which may exist in its single-celled form or in a colony of cells.

The different types of microbes are:

- algae
- bacteria
- fungi
- protozoa
- viruses

Industrial Microbiology → Branch of microbiology which involves study of various microbes and its application in industry.

Microbes are widely used to synthesize a number of products such as:

- Food additives
- Alcoholic and non-alcoholic beverages
- Biofuels, metabolites, biofertilizers
- chemicals, enzymes and other bioactive molecs.
- Vaccines and other antibiotics
- dairy products (cheese, yoghurt)

Production of Enzymes

Process

(1) Screening (choosing an appropriate microbe for the desired enzyme)
↓
(2) Modification (possible application of gen. engineering to improve the microbial strain)
↓
(3) Lab. scale pilot (To determine the optimum conditions for growth of micro-organism)
↓
(4) Pilot plant (small scale production to clarify optimum conditions)
↓
(5) Industrial scale production

Methods of Enzyme Production

1) Semi-solid culture (Solid Surface Fermentation)

steps:

(i) The enzyme producing culture is grown on the surface of a suitable semi-solid substrate (Moistened wheat or rice bran with nutrients)

(ii) Preparation of Production medium – Bran is mixed with solution containing nutrient salts.

(iii) pH is maintained at a neutral level. Medium is steam sterilised in an autoclave while stirring.

(iv) The sterilised medium is spread on metal trays upto a depth of 1-10 centimeters.

(v) Culture is inoculated either in the autoclave after cooling or in trays.

(vi) High enzyme concentration in a crude fermented material.

Enzymes produced by semi-solid culture:

(i) α-amylase (Aspergillus oryzae)

(ii) Glucoamylase (Rhizopus species)

(iii) Pectinase (A. niger)

(iv) Protease (A. niger & A oryzae)

2) Submerged Culture Method

(i) Fermentation equipment used is the same as in the manufacture of antibiotics

(ii) Presence of inhibitors or inducers should be checked in the medium.

(iii) As the inducers are expensive, constitutive mutants are used which do not require an inducer.

(iv) Glucose repress the formation of some enzymes (α-amylases). So, the glucose conc. is kept low.

(v) Certain surfactants in the production medium increases the yield of certain enzymes.

(vi) Non-ionic detergents (Tween 80, Triton) are frequently used.

Amylase

→ Amylase is an enzyme that catalyses the hydrolysis of starch into sugars.

→ present in the saliva of humans

→ Types of amylases

(i) α-amylase (ii) β-amylase (iii) γ-amylase

→ Amylase producing strains:

(i) Bacteria (B. cereus, B. subtilis, B. polymyxa)

(ii) Fungi (A. oryzae, A. niger, Penicillium)

→ Applications

(i) Production of sweeteners for food industry.

(ii) Removal of starch sizing from woven cloth.

(iii) Liquefaction of starch pastes which are formed in the manufacturing of corn and chocolate syrups.

(iv) Production of bread and removal of food spots in the dry cleaning industry.

Lipases

→ Lipases are also called Glycerol ester hydrolases.
→ It breaks fats into mono or di-glycerides and fatty acids.
→ producing strains:
(i) Fungi (Aspergillus, mucor, Rhizopus)
(ii) Bacteria (species of pseudomonas, staphylococcus)
→ Applications
(i) Primarily marketed as digestive enzymes to supplement pancreatic lipases.
(ii) In the soap industry, lipases from Candida cylindraceal (a fungi) is used to hydrolyse oils.

Proteases

→ Proteases (mixture of peptidases and proteinases) are enzymes that perform the hydrolysis of peptide bonds of amino acids.
→ Classification of proteases based upon the pH upon which the proteases are active:
(i) Alkaline serine proteases
(ii) Acid proteases
(iii) Neutral proteases

Catalase

→ Catalase enzymes break down hydrogen peroxide to water and oxygen molecules, which potects cells from oxidative damage by reactive oxygen species.
→ catalase is produced by Aspergillus niger through a solid-state fermentation process.
→ Applications
(i) food preservation
(ii) egg processing.

Penicillinase

→ Penicillinase hydrolyses penicillin into penicilloic acid.
→ produced by Bacillus species of microorganisms and certain strains of staphylococcus.