Unit-1

Short Q/Ans

→ **Define Biotechnology?**

Biotechnology offen abbreviated to biotech, is the area of biology that uses living process organism system to manufacture product or technology intended the quality of human life.

→ **Define enzyme immobilization & its application?**

Enzyme immobilization is a technique of restraining the enzyme in or on an inert support for their stability & functional use.

Application:

(1) **Industrial production** – Industrial prodn of antibiotics beverages, amino acid, etc use immobilized enzymes or whole cells.

(2) **Biomedical Application** – Immobilized enzymes are widely used in the diagnosis & treatment of many diseases. Immobilization techniques are effectively used in drug delivery system.

(3) **Production of bio-diesel** from vegetable oils.

(4) **Food industry** – Enzymes like pectinases & cellulases immobilized on suitable carrier are successfully used in the prodn of gems, jellies, syrups from food & vegetables

(5) Research - A research activity extensively uses many enzymes. The use of immobilized enzyme allow researches to increase the efficiency of different enzymes such as Horse Radish Peroxidase (HRP) in blotting experiment & different proteases from cell or organelles.

(6) Waste-water management -
- Treatment of sewage & industrial effluents.
- Textiles, industry spowing, biopolishing & designing of fabrics.
- Immobilization of lipase enzyme for effective dirt removal from clothes.
- Isocitrate dehydrogenase is used in the diagnosis of acute hepatitis.
- To produce medicinal plant derivative (like anthraquinone (glycoside) to relieve constipation).

→ What do you understand by protein engineering?
Protein engineering is the design of new protein with desirable function.
It is based on the use of r-DNA technology to change Amino-Acid sequence.

Approaches:
This can be done by taking two main approaches:
(1) Rational Design -
In the former case, knowledge of the protein structure & function is considered & a rational gene mutation is planned. This is done by making rationally designed changes in the gene of the protein cloned in the expression vector of heterologous expression.

The production of protein molecules is changed by site directed or site specific mutagenesis of the genes.

Knowledge based engineering of protein with desired characteristic
↓
Design modelling
↓
Generate required changes in the cloned DNA construct
↓
Express protein
↓
Purify protein
↓
Assess protein for desired changes.

(2) Irrational Design-
There are cases in which protein structure is not available & thus irrational design is required in which random changes (mutation) are made in the protein & a mutant with desired properties is selected.

High throughput protein engineering
↓
Generate library of DNA constructs
↓
Express all constructs
↓
Screen protein with designed characteristics (hit) by high throughput
↓
Get DNA construct for the hit for large scale expression

Objective:

- To create a superior enzyme to catalysis the prodⁿ of high value specific resistance.
- To produce enzyme in large quantities
- Eliminate the need for co-factor in enzymatic rxⁿ.
- Changing substrate binding site to ↑se specificity.
- Change the thermal tolerance & pH stability.
- Increase protein resistance to proteases.
- Rare codon changes.
- To produce biological compound.
- Investigate how desired mutation can be introduced into a clone gene.

Application:

(1) In Medical -

- It is used to produce recombinant protein vaccine, diagnostic reagent & therapeutic antibodies.
- It is used to produce growth factors, interferons hormones & biological responses.

(2) In Industrial -

At industrial level, it is used to produce:

- Bromelain (Meat tendrize juice clarification).
- Glucomylase (Beer making alcohol prodⁿ).
- Glucose isomerase (Manufacture of high fructose syrup)

→ Write a note on uses of microbes in Industry?

(1) Microorganism in food industry -

Microorganism are used in the prodⁿ of fermented food & beverages.

eg - Yeast, Penicillium, lactobacillus.

(2) Microorganism in ph. industry-
They have immense potential in the field of medicine. They are used industrially for the prodn of antibiotics, vaccines, insulin, growth hormone, diagnostic kit.
eg- Polio virus, E.coli.

(3) Microorganism in Biotech industry-
- Industrially important acids, enzymes, pigments are produced with the help of microorganism.
eg- Aspergillus niger & Bacillus subtilis.
- Microorganism have evolved as a potential alternate source of energy.
- Microorganism are used to produce biofuels like biodiesel, bioalcohol & also microbial fuel cell.
eg- Algae.

(4) Microorganism in waste treatment-
Microorganism play a crucial role in managing & treating domestic & industrial waste.

(5) Microorganism in Agriculture industry-
Microorganism have been ruling in agriculture from the past century. They are used as biofertilizers & biopesticides.
eg- Rhizobium, Bacillus, Azotobacter.

→ What are the principles of genetic engineering?
Gene cloning involves inserting a specific piece of 'desired DNA' into a host cell in such a manner that the inserted DNA is replicated & handed onto the daughter cells during cell division.

→ Write a note on amylase & Catalase ?

Amylase :
It is an enzyme that breaksdown the starch into Sugar. It is present in human saliva, where it begins the process of chemical digestion.
All the amylases are Glycoside hydrolases & act on α-1,4-glycosidic bonds.

Types of Amylase -

Category	α-amylase	β-amylase	γ-amylase
Source	Animal, plants, microbe	Plants, microbes	Animal, microbes
Tissue	Saliva, Pancreas	Seeds, fruits	Small intestine
cleavage site	Random α-1,4-glycosidic bond	Second α-1,4-glucosidic bond.	last α-1,4-glycosidic bond.
Reaction Product	Maltose, Dextrin	Maltose	Glucose
optimum pH	6.7-7.0	4.5-5.0	3.0
Optimum temp. in brewing	63-70°C	55-65°C	

Sources of α-amylase -
- The bacterial Source of α-amylase is Bacillus species
- The fungus source of α-amylase is Aspergillus species & few Penicillium species.

Production method of α-amylase -
They are mainly 2 methods which are used for production of α-amylase on a commercial source:

(1) Submerged fermentation (SMF):-
SMF employee free flowing liquid substrate such as molasses & broths.
The product yielded in fermentation are secreted into the fermentation broth. The substrates are rapidly utilised.
- This fermentation technique is suitable for microorganism such as bacteria that required high moisture content for their growth.
- SMF is mainly used for the expression of secondary metabolite that need to be used in liquid form.

Advantage -
(i) It allowed the utilization of genetically modified organism to a greater extent.
(ii) The stabilisation of the medium & purification process & products can be done easily.
(iii) The control of process parameters like temperature, pH, oxygen transfer & moisture can be done conveniently.

(2) Solid state fermentation (SSF):-
It is a method used for microbes which require the less moisture content for their growth.
The solid substrate commonly used in this method are paper pulp.

Advantage-

(i) Nutrient base material can be easily decided & used as substrate in this method.

(ii) Same substrate can be utilised for a longer duration.

Importance of amylase:-

- It is used for the production of sweetness in food industries.
- It has a good heat stability.
- It does not undergo browning reactions.
- It is used for converting glucose into fructose.
- It is used commercially for the prepn of sizing agents, for the liquefaction of heavy starch pastes.

Application of amylases-

	Name of Industry	Sources	Application
(1)	Alcohol industry	Bacillus, Aspergillus	Liquefaction of starch before incorporation of 'malt' for saccharification
(2)	Baked Products	Aspergillus	Increase in the prodn of fermentable carbohydrates
(3)	Brewing	Aspergillus	Improved fermentability of grain & modification of beer characteristics.
		Bacillus	Barley prepn & liquefaction of additives.
(4)	Feed products	Bacillus	Improvement in utilization of enzymatically treated barley in poultry & cattle-up bringing.
(5)	Milling	Aspergillus	Supplementation of α-amylase deficient flour.

(6)	Laundry & detergent	Bacillus	Enhancement in 'cleansing power' for soiled clothing mixed with Starch & additive in 'dishwashing detergents'.
(7)	Paper	Bacillus	Liquefaction of Starch without sugar prodn of sizing of paper.
(8)	Starch products	Bacillus	Liquefaction of starch for prodn of glucose, fructose & maltose.
(9)	Sugar	Bacillus	Filterability improvement of Cane sugar juice via cleavage of Starch in juice.

Catalase:

Catalase is a common enzyme found in nearly all living organism exposed to oxygen.
It catalyse the decomposition of hydrogen peroxide to water & oxidation.
Catalases can be divided into 3 classes:
(1) Monofunctional catalase or typical catalase
(2) Catalase-peroxidase
(3) Pseudocatalase or Mn-catalase

Specificity -

The rxn of catalase occurs in two steps. A molecule of hydrogen oxidizes the heme to an oxyferryl species. A porphyrin cation radical is generated when one oxidation equivalent is removed from Iron & one from the porphyrin ring. A second hydrogen peroxide molecules act as a reducing agent to regenerate the resting state enzyme, producing a molecule of oxygen & water.

Principle -
Catalase is the another dismutase enzyme. It contains a heme moiety at the active site & converts two hydrogen peroxide molecules oxygen & water.

Importance -
- Recent scientific studies indicate that low catalase levels could be a factor in the graying process of human hair.
- Hydrogen peroxide is produced naturally in human body.
- It is a strong oxidizing & bleaching agent.
- Catalase is widely used in food industry.
- Catalase remove hydrogen peroxide from milk thus its aids in cheese production.
- Catalase prevents food from oxidizing therefore, it can be used in food wrapping.
- Catalase is used to disinfect contact lenses.
- Catalase is used in aesthetics industry in several mask treatment because it helps increase cellular oxygenation in the upper layers of epidermis.

→ Write a note on Peroxidase?
Peroxidase is an enzyme that decomposes hydrogen peroxide by the oxidation of wide range of phenolic & non-phenolic substrates.
They are found in bacteria, fungi, algae, plants & animals.
Peroxidase enzyme is classified into heme & non-heme peroxidase based on the presence or absence of heme.

more than 80% of known peroxidase genes can code for heme peroxidase, while the non-heme perioxidases.
eg- Thiol peroxidase, alkylhydroperoxidase, NADH peroxidase constitute only a small proportion.

Application -
- Peroxidase can be used for treatment of industrial waste water.
- It can be used to convert toxic materials into less harmful substances.
- Peroxidases are used as histological markers.
- Cytochrome c peroxidase is used as a soluble, easily purified model for cytochrome c oxidase.
- Other studies have shown that peroxidase may be used successfully to polymerize anilines & phenols in organic solvent matrices.

→ What are the importance of Penicillinase?

(1) Penicillinase is a narrow spectrum β-lactamase which opens the β-lactam ring & inactivates Penicillin G & some closely related congeners.
(2) Majority of Staphylococci & some strains of Gonococci, B. subtilis, E. coli, H. influenza & few other bacteria produce penicillinase.
(3) The gram (+ve) penicillinase produces large quantities of the enzyme which diffuses into the surroundings & protects other inherently sensitive bacteria.
(4) The gram (-ve) penicillinase produces small quantities of the enzyme found b/w the lipoprotein & peptidoglycan layers of the cell wall.
(5) The Methicillin Resistant S. aureus (MRSA) has acquired a Penicillin Binding Protein (PBP) which has a very low affinity for β-lactam antibiotics.

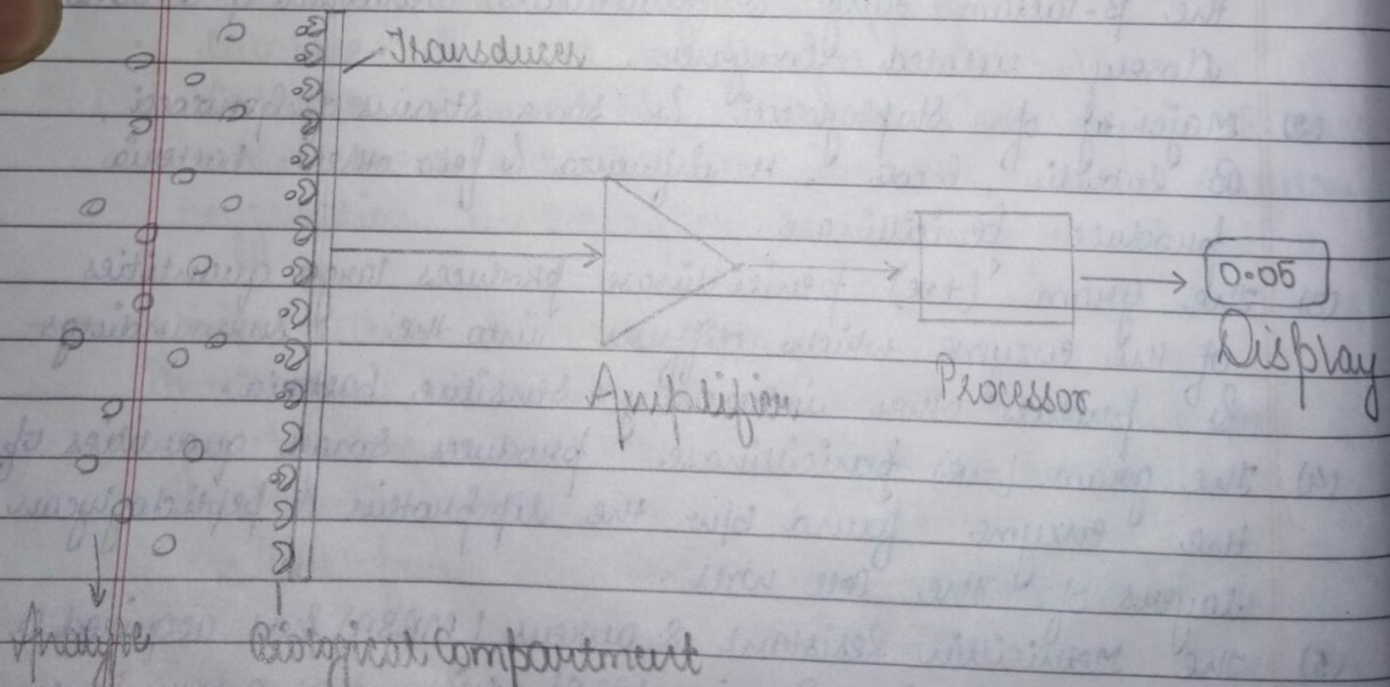
Long Q/Ans

→ Write a note on Biosensor? Write its application on Biosensor?

An analytical device used to change in biological response into a electrical signal is called biosensor.

Working :

- These are analytical devices, which measures concⁿ of an analyte.
- In biosensors, a biological material (such as enzyme, antibody, whole cell, nucleic acid) is used to interact with the analyte.
- This interaction produces a physical or chemical change, which is detected by the transducer & converted to an electric signal.
- This signal is interpreted & converted to analyte concⁿ present in the sample.



Diagrammatic Representation of Biosensor

Types of Biosensor :

(1) Calorimetric Biosensor -
They measure change in temp. due to either release (exothermic) or absorption (endothermic) of heat.
eg- Temperature Biosensor.

(2) Potentiometric Biosensor -
They measure potential difference arising during a redox reaction.
eg. Urea Biosensor.

(3) Amperometric Biosensor -
They measure current (the flow of e^-) arising during a rx^n.
eg- Glucose Biosensor.

(4) Acoustic wave Biosensor -
They measure electric field developed by piezoelectric effect.
eg- Cocaine Biosensor

(5) Conductometric Biosensor -
They measure changes in electrical conductivity arising during a rx^n.
eg Urea Biosensor.

(6) Optical Biosensor -
They measure light arising from the action of enzyme luciferase (firefly)
eg- Detection of bacteria.

Properties of a Good Biosensor:

- Highly specific for the analyte.
- The response should be linear over a broad substrate range.
- The device should be small & biocompatible.
- It should be low cost, & easy to use.
- Assay cost should be lower than conventional test.
- Assay should be fast, reliable & repeatable.

Application of Biosensor in Medicine:

(1) Pregnancy test -
Detects the HCG protein in urine. Interpretation & data analysis performed by user.

(2) Glucose Monitoring device -
Monitors the glucose level in the blood. Interpretation & data analysis performed by a microprocessor.

(3) Bacteria in Blood platelets -
A real tym biosensor has been developed for detection of Bacteria (B. cereus, E.coli, P.aeruginosa) in platelet concentrates.

(4) T.B. Breathalyzer -

- Rapid biosensor system is a UK based development company.
- Technology for rapid screening of infectious agent.
- TB Breathalyzer device for screening with in a few minutes
- Analyses the sample by a displacement assay utilising the evanescent wave & bio-optical sensing technologies.

(5) Cancer diagnosis -
Different biosensors are available for early lung diagnosis of cancer based on detection of tumor associated antigens & its corresponding antibodies.

(6) Lie Detection test -
The polygraph analyzes person's involuntary psycho-physiological responses to question. The sensor are placed on person's arm, fingers, abdomen & chest. The signal produced from sensors detect changes in variables such as breathing, pulse rates, blood pressure & stored digitally.

→ Write a detail note on Scope of biotechnology?

(1) Agricultural Biotechnology - The field of agricultural sciences in which the prodn of newer variety of high yielding, pest & disease resistant & cost effective agricultural plants are developed by using biotechnology based concept of r-DNA technology & gene cloning.

(2) Medical Biotechnology - The area of medical sciences which utilises the diagnostic aids like the AIDS detection kits, Glucose measuring kits, & attempts for correction of some hereditary disorders by gene incorporation utilising the basic concept of biotechnology is k/n as medical biotechnology. The prodn of artificial organ like liver & kidney are the emerging field of this subject.

(3) Engineering Biotechnology - The utilisation of biotech based enzyme sensor for chemical process monitoring utilisation of some genetically modified strain of microbes as an aid in the chemical synthesis degeneration of industrial

wastage by some class bacteria are group together & referred as engineering biotechnology.

(4) Biomedical Engineering - Prepⁿ of some artificial organ as a replacement alternative for the vital organ lost due to accident or birth disorder utilising the biotechnological concept like polymer engineering, enzyme immobilization is known as biomedical engineering.

(5) Textile & Paper Biotechnology - The application of the concept of biotechnology as the polymer engineering, prodⁿ of some special microbes strain as well as process monitoring using biotech based sensor in the prodⁿ of special fibres, paper & pulp is k/n as textile & paper biotechnology.

(6) Environment Biotechnology -
It is used in the treatment of purification of the waste water as well as the industrial wastage, safe to discharged into the rivers or sea.

(7) Mining & Metal Biotechnology - This branch deals with the isolation of micro-organism which are capable of affecting these types of degradation & utilises them in the isolation & purification of the metal & minerals.
Thiobacillus ferroxidans oxidises sulphur & iron present in the soil, leptospirillum ferroxidans T. organoparpus degrade pyrates (FeS_2) & chalcopyrite ($CuFeS_2$).

(8) Leather biotechnology- Leather industry is based on the tanning of the skin from the animals using various enzymes system. The development in the field of enzyme immobilization technology has offered the advantage for the automation of the technology & more cost effective treatment of the leather.

(9) Pharmaceutical biotechnology-

- It is a major branch of biotechnology undergoing fast development. The concept is based on biotechnology in the prodn of the therapeutic protein & hormones, fermentation products like the antibiotics, especially designed vaccines or drug design using the receptor hypothesis, gene correction, drug delivery to specific tissues (targeted delivery), prodn control using the biosensors, standardization of chemotherapeutic agents, r-DNA technology, enzyme immobilization, monoclonal antibiotics & mutagenesis.

- In 10 years now after FDA approval was granted for the first biotechnology product recombinant human insulin, over 100 products derived from biotechnological principles have progressed to the stage of clinical use.
- The case of insulin since its discovery in 1921 by Banting & Best, many animals have been sacrificed so as to achieve very small quantity of the protein.
- The human genetic coding for pro-insulin is inserted in E.coli cells, which were then grown by fermentation to produce pro-insulin. Currently the product is being manufactured by Eli-Lilly & Company.

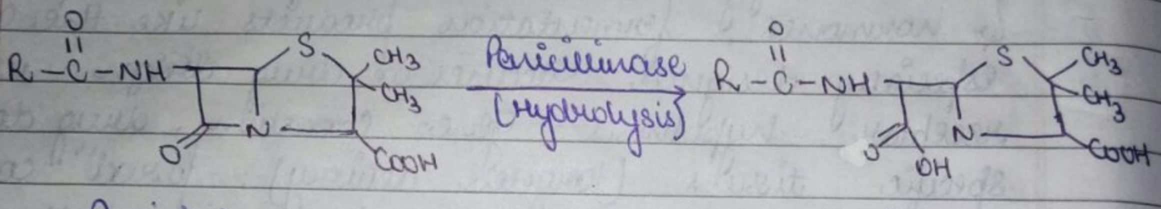
- The r-DNA technology offered many advantages like prodn of non-immunogenic & more effective cheaper products & sparing animals life too.

→ Give a detail note on Penicillinase, Lipase & Protease?

Penicillinase :

Penicillinase is a bacterial enzyme that inactivates most of the penicillins. It is an extracellular enzyme produced by the members of the coliform group of bacteria by the Bacillus species & some of the strains of Staphylococcus.

The enzyme hydrolyses the penicillin into penicilloic acid.



Penicillin Penicilloic acid

Penicillinase obtained from B. subtilis & B. cereus is industrially produced & exerts action to some extent in the removal of penicillin.

Penicillinase has been categorised into two major classes based on its activity :

(1) Penicillin amidase or penicillin acylase
(2) β-lactamase or penicillinase.

Penicillin inhibits the therapeutic activity of penicillin. Penicillin amidase enzyme specifically attacks the acyl group attached to the basic nucleus thus it also named as Penicillin acylase. This enzyme is more specific against Penicillin V & K.

β-lactamase enzyme acts on the basic nucleus by breaking the β-lactam bond & producing penicilloic acid. This enzyme is more specific against Penicillin G & X & to a lesser extent against Penicillin V.

Importance -

(1) Penicillinase is a narrow spectrum β-lactamase which opens the β-lactam ring & inactivates Penicillin G & some closely related congeners.

(2) Majority of Staphylococci & some strains of Gonococci, B. subtilis, E. coli, H. influenza & few other bacteria produce penicillinase.

(3) The gram (+ve) penicillinase produces large quantities of the enzyme which diffuses into the surrounding & protect other inherently sensitive bacteria.

(4) The gram (-ve) penicillinase produces small quantities of the enzyme found b/w the lipoprotein & peptidoglycan layers of the cell wall.

(5) The Methicillin Resistant S. aureus (MRSA) has required a PBP which has a very low affinity for β-lactam antibiotics.

Lipase :

Lipase is an enzyme that breakdown dietary fats into fatty acids & glycerol. It splits fats into mono or di-glycerides, glycerol & fatty acid. They are extracellular enzymes.

There are 4 different types of lipase :

(1) Gastric lipase - It is produced with in the stomach & its primary function is to digest fatty acids.

(2) Pharyngeal lipase - It is secreted by the human salivary glands & attacks fatty acids from the moment food is inside the mouth.

(3) Hepatic lipase - It is a digestive enzyme produced by the liver.

(4) Pancreatic lipase - It is produced with in the Pancreas that acts in small intestine.

Eg - • Fungi producing lipases including Aspergillus, Mucor, Rhizopus, Penicillium.
• Bacteria producing lipases including species of Pseudomonas, Achromobacter & Staphylococcus.
• Yeast like Torulopsis & Candida are also commercially used.

Importance -
(1) It mainly helps in the body processes & absorbs fat.
(2) It helps in maintaining the level of triglycerides in the body by breaking them down into smaller molecules to be used by the body for energy.
(3) Microbial lipase is used to obtain PUFAs from animal & plant lipids and its mono & di-acylglycerides are used to produce various pharmaceuticals.
(4) PUFAs have metabolic benefits thus are used as food additives, pharmaceutical & nutraceuticals.
(5) Some of the PUFAs are required for the normal synthesis of lipid membranes & prostaglandins.

Proteases :
Proteases is the mixture of peptidases & proteinases are enzymes that perform the hydrolysis of peptide bonds.
Peptide bonds links the amino acid to give the final structure of a protein.
Proteinases are extracellular & Peptidases are endocellular.
Second most important enzyme produced on a large scale after amylase.

Classification -
(1) Serine proteases.
(2) Threonine proteases.
(3) Cysteine proteases.
(4) Aspartate proteases.
(5) Metallo proteases.
(6) Glutamic acid proteases.

Importance -
- Protease enzymes are required in digestion as they breakdown the peptide bonds in the protein foods to release the amino acids which are utilised by the body.
- It is used in various forms of therapy as they are beneficial in oncology, inflammatory condition, blood rheology control & immune-regulation.
- Protease can hydrolyse most of the proteins as long as they are not components of living cells
- Heavy metals (such as lead & mercury) bind to ionisable or sulfydryl groups of protein & exert their poisoning effect.

→ Write the advantages & disadvantages of enzyme immobilization & Describe its methods in detail?

Advantage :-
- Reuse of enzyme.
- Less chances of contamination in process.
- More stability of products
- Improved process control.
- High enzyme substrate ratio.
- Less labour input in the process.
- Minimum reaction tym.

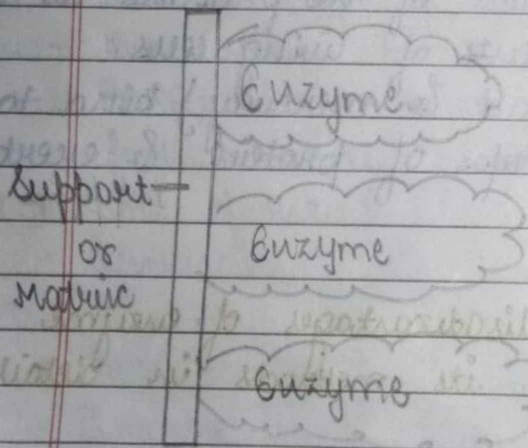
Disadvantages:

- High cost for isolation, purification & recovery of active enzyme.
- Industrial application are limited & only very few industries are using immobilized enzymes.
- Catalytic properties of some enzymes are reduced their immobilization on support.
- Some enzymes become unstable after immobilization.
- Enzymes are inactivated by heat generated in the system.

Methods -

(1) **Adsorption -**

Adsorption is the oldest & simplest method of enzyme immobilisation. In this method, enzyme is adsorbed to external surface of support.



The support or carrier used may be of different types such as:

(1) Mineral support (Al_2O_3, clay) (2) Ionic interaction
(3) Organic support (starch) (4) Hydrogen bond
(5) Vanderwaals forces (6) Modify cephalose & ion exchange basin.

There is no permanent bond formation b/w carrier & the enzyme in adsorption method.

only weak bonds stabilise the enzymes to the support or carrier. For significant bonding surface, the carrier particle size must be small [500Å - 1mm diameter].

Methods -

(a) Static process : In this process, the enzyme is immobilised on the carrier by bringing the enzyme-containing solⁿ in contact with the carrier without stirring.

(b) Dynamic process : In this process, the enzyme solⁿ is admixed with the carrier with constant stirring.

(c) Reactor loading process : In this process, the carrier is placed in the reactor & then enzyme solⁿ is transferred to the reactor with continuous agitation.

(d) Electro-deposition process : In this process, the carrier is placed near to an electrode in enzyme bath & then the current is put on under the electric field, the enzyme migrates to the carrier & deposit on its surface.

Advantages -

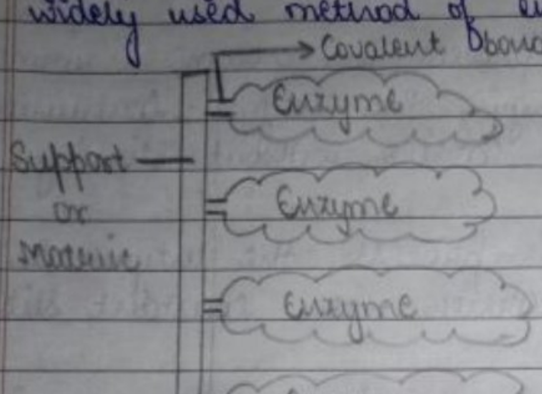
- No pore diffusion limitation
- Easy to carry out.
- No agents are required.
- Minimum activation step involved.
- Cheap method.

Disadvantages -

- Desorption of enzyme from carrier.
- Efficiency is less.

(2) Covalent Bonding -

This method involves the formation of covalent bonds b/w the chemical group of an enzyme to the chemical groups of the support or carrier. It is one of the widely used method of enzyme immobilization.



→ Enzyme commonly used for covalent bonding are:

(1) α-carboxyl group
(2) carboxyl group
(3) Phenol ring of tyrosine
(4) α-amino group
(5) Thiol group of cysteine
(6) Hydroxyl group of serine
(7) Indole ring of tryptophan

→ Carrier or Support commonly used for covalent bonding are:

(1) Carbohydrate (cellulose, agarose)
(2) Synthetic agents (Polyacrylamide)
(3) Protein carriers (Gelatin)
(4) Amino group bearing carrier (aminobenzyl cellulose)
(5) Inorganic carrier (porous glass & silica)

Methods -

(a) Diazotization: Bonding b/w amino group of support & tyrosine/histidyl group of enzyme.

(b) Peptide Bond: Bonding b/w amino (or carboxyl) group of the support & carboxyl (or amino) group of the enzyme.

(c) Polyfunctional reagents : The reagent (glutaraldehyde) is used to form bonds b/w amino groups of enzyme & amino groups of support.

Advantage -

- Strong linkage of enzyme to the support.
- No leakage or desorption problem.
- Simple method
- variety of support with different functional group available.

Disadvantage -

- May alter the conformational structure & active centre of the enzyme.
- Major loss of activity & changes of the substrate.

(3) Entrapment -

In this method, enzymes are physically entrapped inside a porous matrix. Bonds involved in stability the enzyme to the matrix may be covalent or non-covalent.

The matrix used will be water-soluble polymer. The pore size of matrix can be adjusted with the conc^n of the polymer used.

Agar-Agar & carrageenan have comparatively large pore size.

Matrix

Commonly used Matrices-

(1) Polyacrylamide gels (2) Gelatin
(3) Cellulose triacetate (4) Alginate
(5) Agar (6) Carrageenan.

Methods -

(a) Inclusion in the gels: Enzyme trapped inside the gel.

(b) Inclusion in fibres: Enzyme supported on fibres made of matrix material.

(C) Inclusion in microcapsule: Enzyme trap in microcapsule formed by monomer mixture such as polyamine & calcium alginate.

Advantages -

- Fast method of immobilisation.
- Cheap
- Easy to practice at small scale.
- Mild condition are required.

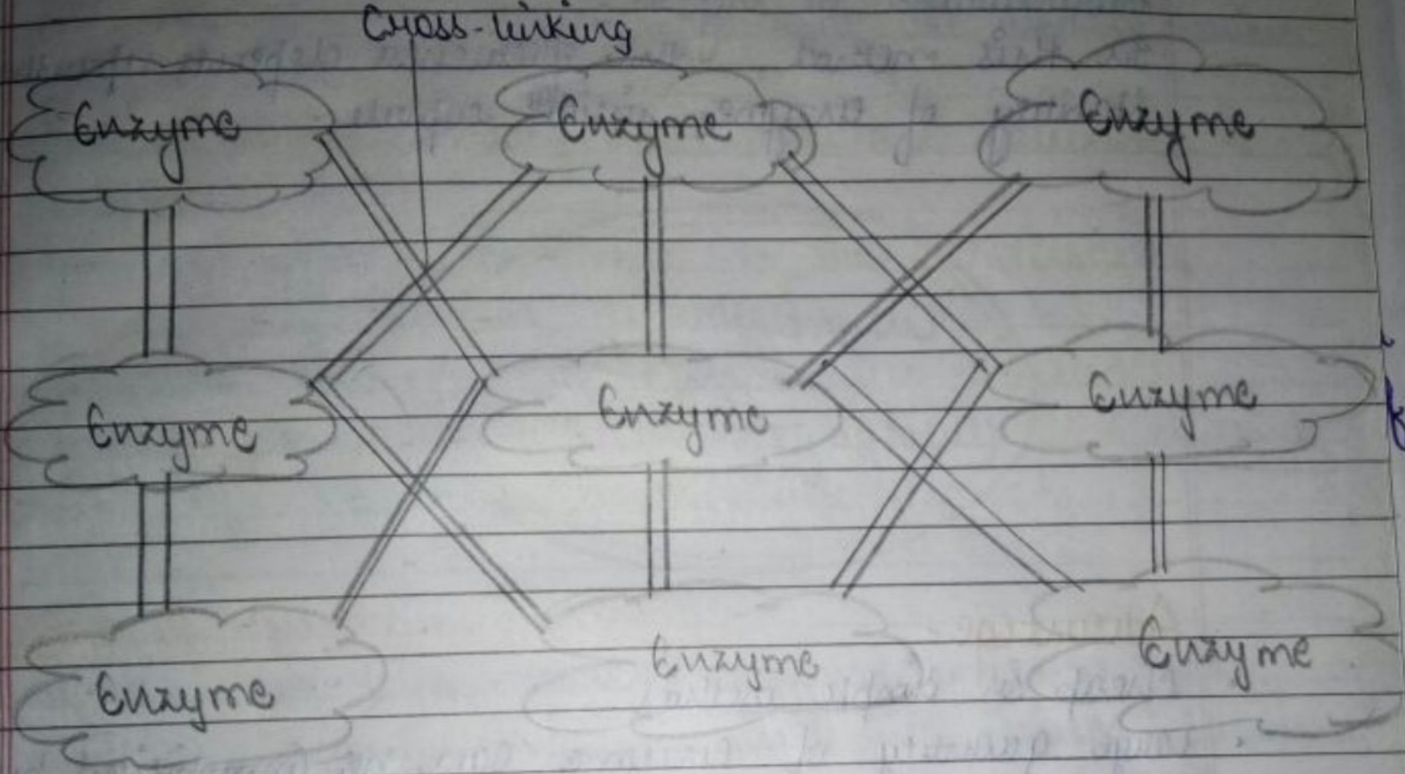
Disadvantages -

- leakage of enzyme.
- Pore diffusion limitation.
- Chance of microbial contamination
- Not

(4) Cross linking -

This method is also called co-polymerisation. In this method, enzymes are directly linked by covalent bond beginning various group of enzyme, polyfunctional reagent.

unlike other method, there is no matrix or support involve in this method.
Commonly used polyfunctional reagents are glutaraldehyde & diazonium salt.



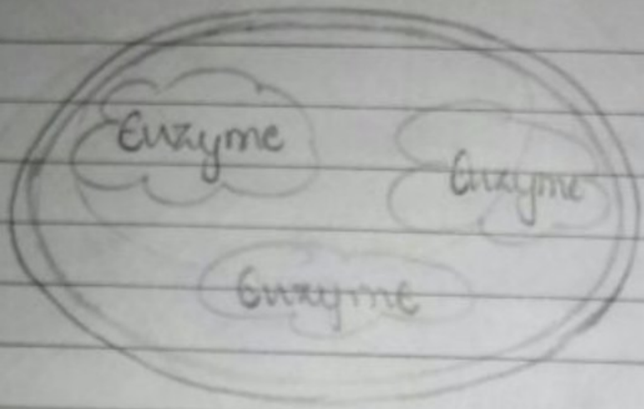
Advantages -
- very little desorption.
- Higher stability
- Cheap & simple.

Disadvantage -
- The enzyme may get denaturised by the poly-functional reagent.
- They may cause changes in the active site.

(5) Encapsulation -

This type of immobilisation is done by enclosing the enzymes in a membrane capsule. The Capsule will be made up of semi-permeable membrane like nitrocellulose or nylon.

In this method, the effectiveness depends upon the stability of enzyme inside capsule.



Advantage -

- Cheap & simple method.
- Large quantity of enzyme can be immobilised by encapsulation.

Disadvantage -

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

→ Write a detail note on Genetic engineering?

Genetic engineering refers to the direct manipulation of DNA to alter & organism characteristics in a particular way.

It is a set of technique that are used to achieve 3 goals:

- To reveal the process of how genes are inherited & expressed.
- To provide effective treatment to various diseases (particularly various disorders).
- To generate economic benefits which includes improved plants & animals for agriculture & efficient prodn of valuable biopharmaceuticals.

History of Genetic Engineering:-

- In 1951, the term Genetic engineering was first point by Jack Williamson in his science fiction novel.
- In 1953, James Watson & Francis Crick proposed the double helix structure of DNA.
- In 1972, Paul Berg created the first recombinant DNA molecule by combining the DNA from the monkey virus SV40 (Simian virus 40) with that of lambda virus (Bacteriophag).
- In 1973, Herbert Boyer & Stanley Cohen created the first transgenic organism by inserting antibiotic resistance genes into the plasmid of an E.coli bacteria.
- In 1980s,

(i) Invention of PCR (Polymerase chain Rx^n)
(ii) Development of genetic fingerprinting.
(iii) Transgenic mice produced carrying human gene.

(iv) Transgenic plants produced resistance to a herbicide.

- In 1990s
(i) GM (Genetically modifies) used to make Chymosin (an enzyme used in making hard cheese).
(ii) First gene therapy trials on human.

- In 1996, the birth of the first cloned animal (sheep).

Application of Genetic Engineering in Medicine :-

(1) Treatment -

(A) Insulin prodn:

→ Human insulin produced through Genetic Engineering since 1982 -:
Human insulin gene inserted into the bacterium E-coli to produce synthetic human insulin for the treatment insulant dependent diabetes.
In past, insulin was obtain from a cow or pig pancreas that has many problems.

(B) Producing growth hormone to treat growth retardation (Dwarfism).

(C) Producing fertilization injection (containing FSH hormone) for treating infertility.

(d) Making human albumin, antihemophilic factor & many other drugs.

(2) other genetic a f

(ii) Vac Today to that virus This develo that Gene for Gerlo dis vacci

(iii) Ge for

He Su

(iv)

(e) other biopharmaceuticals, under development through genetic engineering include anticancer drug & a possible vaccines for AIDS, Malaria, etc.

(ii) Vaccination -
Today the microorganism (such as yeast) is used to produce virus, antigen, used as a vaccine, that stimulate human immune system against virus.
This procedure has been done successfully for development of a vaccine against hepatitis B virus that is now widely used.
Genetically engineer vaccine hold great promise for the future.
Genetic engineer vaccine may be useful to prevent diseases that have resistant to traditional vaccination including HIV, tuberculosis

(iii) Genes therapy -
Gene therapy is also been tested as a treatment for cystic fibrosis, skin cancer, breast cancer, brain cancer & AIDS.
However, most of these treatment are only partially successful.

(iv) Tissue Engineering -
Acc. to their source, stem cells are divided into adult which are multipotent & embryonic stem cells.
Stem cells may be useful for the repair of damaged tissues or may be used to grow new organs.

Stem cells in the pulp of primary teeth, characterised as multipotent cell have the potential to be used in both dental & medical application.
Treatment of peridontal disease, Spinal code injurying, diabetes, Stroke, heart attack, burn, rhumatoid arteries regenerate many types of tissues in the body.

Techniques of Genetic Engineering:-

In genetic engineering, recombination can also refers to artificial & deliberable recombination of pieces of DNA offen from different organism & creating recombinant DNA. A prime example of such a use of genetic recombination is gene targeting which can be used to add, delete or otherwise change & organism genes. This technique is important for biomedical researcher as an allow them to study the effect of specific genes. Genetic recombinated is catalysed by many different enzymes is called Recombinases.

There are following method for genetic recombination
(i) Transformation
(ii) Conjugation
(iii) Transduction.

* In Transformation, abacterium takes up a piece of DNA floating in its environment.
* In transduction, DNA is accidentally moved from one bacterium to another by a virus.
* In conjugation, DNA is transferred b/w bacteria through a tube b/w cells.