Enzyme Biotechnology

Enzyme Biotechnology :-

→ Enzymes are Biocatalysts, helps to carry out the different Biological Reactions.

→ Enzymes are Soluble, amorphous, Proteneous Bioactive Organic compounds produced by living cell.

→ Enzymes are composed of one polypeptide or more Polypeptide chains.

→ Enzymes composed → Protein Part — called Apo Enzymes
→ Non-Protein Part — called Co-Enzyme.

→ Protein Part of enzyme is attached to non Protein Part By covalent or non-covalent bond.

→ Enzymes are classified as
- Exoenzymes (Extracellular)
- Endoenzymes (Intracellular)

☆ Endoenzymes :- these secreted within the cell such as Invertase Enzyme, Uric oxidase, Asparginase etc.

→ These isolated By Breaking the cell By means of a homogenizer

☆ Exo Enzymes :- these secreted outside the cell such as Cellulose, Pectin methyl Astrase.

Enzyme Immobilization :- It is the Technique in which Enzymes are Stored.

→ Enzymes can degrade Easily, So they can Stored through this technology.

→ Enzymes are highly unstable and high cost of Isolation, Purification and recovery of active agents from mixture, So overcome this Problem, Enzymes must be immobilized on Surface of some Solid Spot.

→ The commercial (first) Application of Enzyme technology was released in 1969 in Japan with the use of Asparigillus oryzae Aminoacylase for Industrial Production of amino acids.

Advantages :-

→ It can Increase activity and Stability of Enzyme, Immobilized. Enzymes can be Easily Recover from reactions mixtures and re-used.

→ Immobilized Enzyme can be easily controlled. Enzyme Immobilization Process avoid contamination in Products.

Methods of Enzyme Immobilization

- Surface
  - Adsorption
  - Covalent Bonding
  - Complexation
- within the Surface
  - Incapsulation
  - Entrapement.

Major Components of this Technique is Enzyme, matrix and mode of Interaction of Enzyme with matrix or carrier

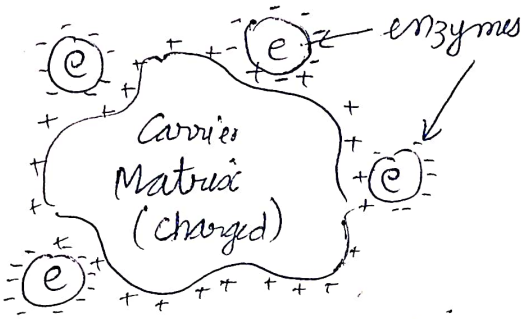
→ The matrix is the carries with which, enzyme can be attached.

→ Matrix must enhance Stability of immobilised Enzymes.

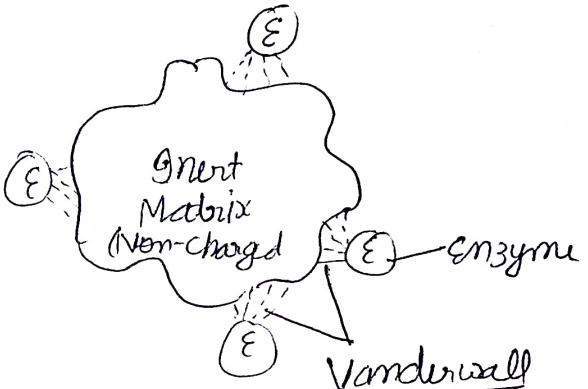
→ An Ideal matrix should be inert, cost effective, Stable, high Rigidity, large surface Area, more Permeability, suitable Shape and highly Resistance to microbial attack.

① Surface Enzyme Immobilization

⇒ **Adsorption**:- Most simple method in which enzyme can adsorb (surface attachment) on charged or non-charged surfaces of carrier matrix.



By Hydrogen Bonding



★ Kinds of matrix used

- Aluminium oxide
- Charcoal
- Starch
- Sepharose
- Cellulose
- Ion exchange resions.

★ Adsorption depends upon PH, ionic Strength, temperature, nature of solvent and concentration of Enzyme and adsorbent.

★ Adsorption occur by → weak Vanderwall forces and Ionic Interactions

↓

Hydrogen Bonding

Disadvantages :-

→ Binding forces between Enzymes and spot are weak.

Enzymes	Carrier Matrix
α- Amylase	Calcium Phosphate
Catalase	Charcoal
Invertase	Charcoal
Aminoglycosides	Agarose gel
Glucose oxidase	Cellophane

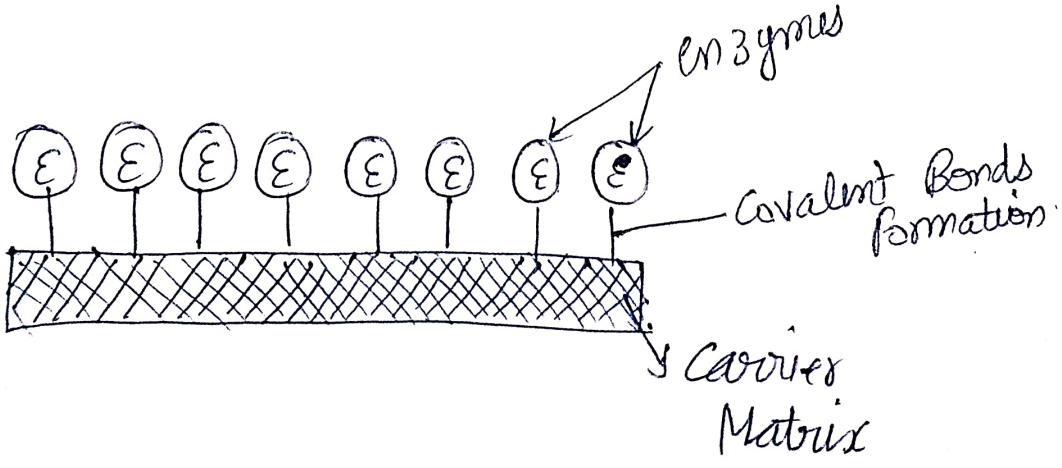
⇒ Covalent Bonding :- In this method, covalent Bond is formed between the chemical groups of Enzyme and chemical groups on surface of carrier.

Advantages

→ Stronger than Adsorption Bonding.

→ Can't be reversed by change in PH or Temperature.

→ Easy and convenient method.



★ Disadvantages

→ May form Permanent Bonds with carrier matrix

→ Difficult to Detach from carrier

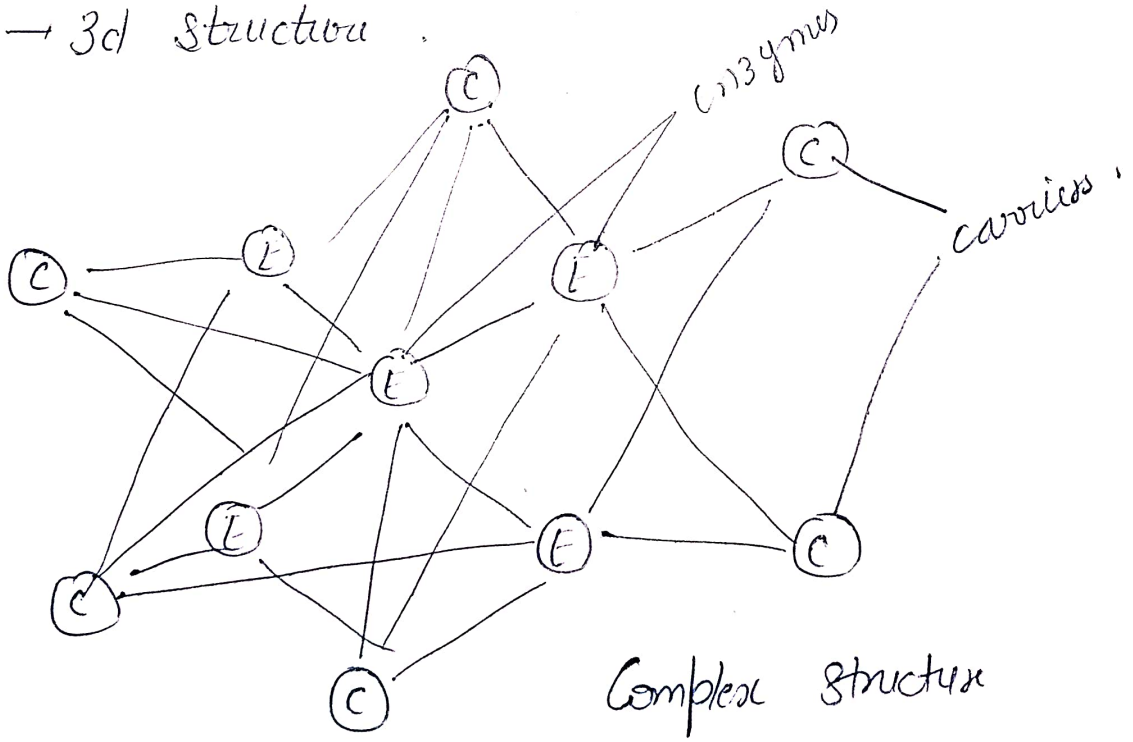
★ Specific groups of Enzymes can form Covalent Bonding (example $-NH_2$, $-COOH$,  , thiol, Imidazole etc)

⇒ Cross-linking Method / Complexation :-

Many number of Carriers and Enzymes can linked and make Solid complex structure.

→ Enzymes may consist multifunctional groups that React with different carriers.

→ 3d Structure.



Complex Structure

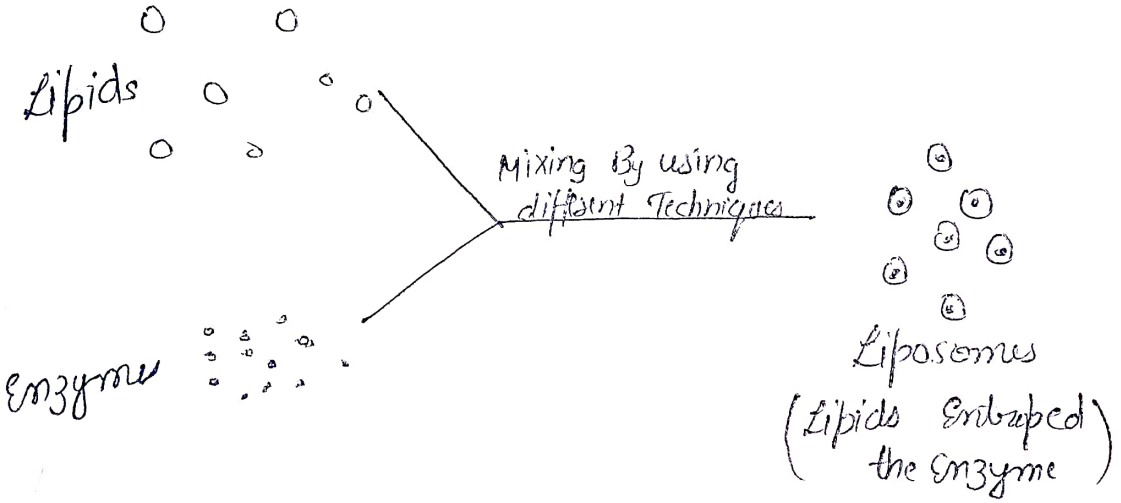
Example of Enzyme - Diazobenzidine, Glutaraldehyde, Toluene etc

advantages - → Easy method
→ less Enzyme leakage Chances
→ Cost Effective

Disadvantages → Enzyme Structural modification chances
→ Conformational changes.

② Within the surface :-

⇒ Micro Encapsulation :- The Enzyme can entraped inside a lipid substance / moleacle. through Chemical Reactions.
→ Smaller in size.



Carriers - Lipid, Lipoprotein etc.

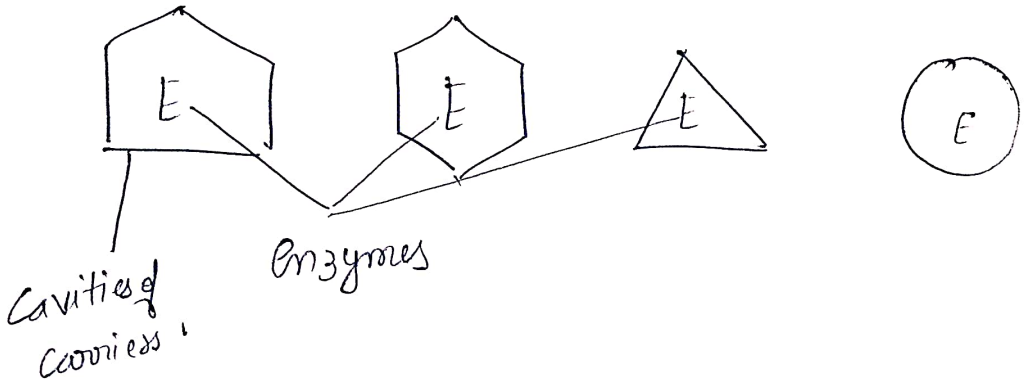
advantages → Better for Lipid / Lipophilic Enzymes.
→ Better Easy and Popular method.

disadvantages - → low Stability
→ Costly

⇒ Entrapment :- Enzymes are Entrapped or enclosed inside a carrier (like cavities)

→ Carriers may have many Shaped cavities shapes.

example - Cellulose, Rubber, glass, Silicon, Starch etc.



Advantages - → Safest method to Conserve Enzymes with more mechanical strength

→ No Special Bonding Required.

→ No chance of denaturation of Enzyme due to Enclosed Structure.

Disadvantages -

→ Enzymes in gas or liquid form may leaked.

→ loading capacity of carrier is low.

BIOSENSORS

These are Artificials devices which measures concentration of Analyte. The Biological materials in Biosensors interact with analyte and Interactions Produces Physical changes (The detectable Part)

* Analyte – Drug in Body

example - Glucometer (Biosensor) can help / used to measure Concentration of Glucose / Blood Sugar (Analytes).

Father of Biosensors :- Leland & Clark.

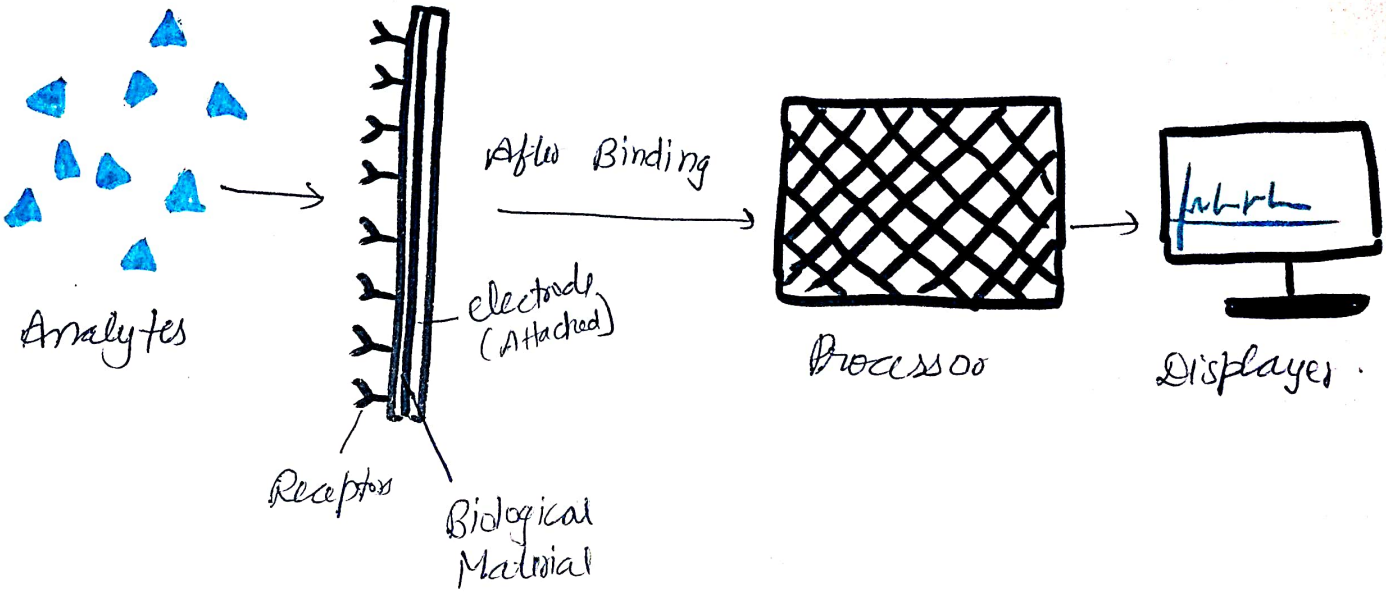
Components of Biosensors :

① Analyte - These may be any drug, chemical, dye Solution etc, main component than can be checked in concentration.

② Biological molecule :- The Part of Body used in Testing. (Blood, urine etc).

③ Transducer :- The component or Part of Biosensor, that generate electric Signal / Impulse and detect changes (Any Thermal, optical or magnetic change etc)

Working :-



When analyte comes to Bind on Biological membrane material attached to electrode surface

↓

Activation of Electrode surface

↓

Production of Electric Signals to Processor

↓

Amplification and detection of Signal

↓

display on Screen.

Advantages :-

→ highly specific (exact disease detected)

→ Durable
→ Easy to use
→ Small sample required

example - Device in ICU
- Pregnancy Kit

★ Applications :

→ Food Analysis
→ Medical diagnosis
→ Study of Biomolecules.
→ Various Analysis of Viruses and Bacterias.
→ Pharmaceuticals and drug Analysis.
→ Drug Adulteration Analysis
→ Industrial effluent Control.
→ Pollution control and monitoring.

Types of Biosensors :

On Basis of Chemical, Electrical and Physical Reactions between Analyte and Biological molecules.

① Calorimetric Biosensors
② Pententiometric Biosensor (used in Bioassay of urea)
③ Amperometric Biosensor (detect movement of electrons from one atom to another)

④ Conductometric Biosensor

⑤ Acoustic wave Biosensor (In Cocain Bioassay)

⑥ optical Biosensor (Change in Light)

PROTEIN ENGINEERING

The modification Techniques Applied to Proteins of Body to Various substances according to the drug Type.

★ Modification of Protein :- (rDNA Technology)

→ our Body consists of Genes and 23 Pair of chromosomes or Net 46 Chromosomes (Total)

- 44 Chromosomes are Autosomes
- 2 Chromosomes are Sex chromosomes.

→ Chromosomes consist of genes and genes made up of Codons (Codons - AUG, UCG, AAA)

→ 3 Codons set make 1 Set and these codons are Genes and cause different functions in Body.

→ Protein Engineering :- It is the modification in the Set of codons to modify & develop new character. (AUG → AGU)

↓

Through rDNA Technology.

Or

Protein Engineering is a Technique in which we modify the Protein Structure by using rDNA Technology and Chemical Treatment for desirable function of Protein

★ rDNA Technology or Recombinant DNA Technology is advance Process and In which we cut gene of human and Bacteria, then fit that Plasmic (Bacteria) in human DNA with Enzyme Restricted Endonuclease (cutting) and DNA Ligase (For Joining)

↓

Make Multiple copies

Objectives :-

→ Improve kinetic Properties

↓

Use (Increase) Pharmacokinetic Properties through Protein Engineering.

→ Elimination of allosteric inhibition

↓

Eliminate By Protein Engineering

(Allosteric Inhibition - change in shape of Receptor so that, Enzyme / Protein not Bind to it)

↓

Use Receptor Structural changes.

→ Increase Substrate and Reaction Spefically.

↓

By technique we change the drug Size / Shape acc. to Receptor.

→ Increase Thermostability

↓

Stabilise Temperature.

→ Addition in optimal PH
→ Increase and decrease in optimal Temperature
→ Increase Rate of Reaction.
→ Increase Shelf life.
→ Increase Quality of Product.

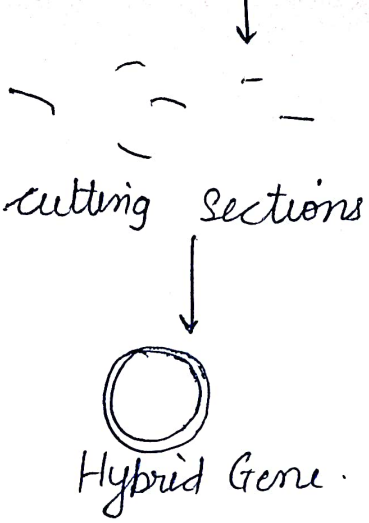
Methods of Protein Engineering
- Site directed Mutagenesis
- Protein Engineering By Gene Families
- Protein Engineering By chemical Modification.

① Site directed Mutagenesis:- Mutation in any site of gene By Protein Engineering.

→ RDNA Technology used.
→ In Technology, Site of gene
- Substituted
- Deleted or
- change in gene → To get desired gene

→ gene are of mainly 26 Types.
→ Also new/Hybrid gene can be formed by cutting different gene Segment to get desirable Properties.

Different genes



(2) Protein Engineering through Chemical Modification

* Enzyme used - glutaraldehyde.
* Alteration in Structures, Bonds, shapes of Protein through Breaking and Formation.

(3) By Gene Families :-

In this, the gene consist of Codons can be altered.

↓

Cutting through Restriction Endonuclease.

↓

Joining through DNA ligase

↓

Formation of a new codon Pair as Required according to characteristics

↓

Also make multiple copies from it.

Codons → Cutting (AUG) → A / U / G → GUA (New Codon)

Applications :

* Herbal Applications :- development of hybrid seed, crop, Plant & medicine.
  → Treatment of Cancer, cardiac disease.

* Environmental Application :- detoxify the food Products (Example - By Enzyme oxidation)

* Food Industry :-
  → Wheat glutin used to make different Products
  → By Protease, amylase Lipase.

Use Of Microbes In Industry

→ Microbes are microscopic organisms exist in single form or in colonies.

→ used to form Probiotic

→ Widely used in Industry for of dairy, Beverages etc. Production under suitable growth condition of media.

→ Production of desired Enzyme obtained through genetic engineering.

→ The Process of recovery, Isolation and purification is easy by microbe than Animal & Plant.

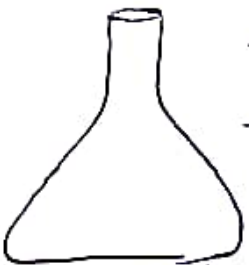
→ Most Enzyme used Industrially By microbes.

example - Aspergillus Nigor (Fungus) ⟶ Bulk Enzyme (Citric Acid)

General Consideration :-

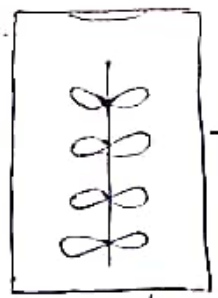
(Inoculation - Microorganism Population in Suitable medium)

★ Enzyme Production By Microbes :-



Inoculation development

Transfer In ⟶



Fermentor

⟶ Cells disruption (Breaks) of microbes

↓

Filteration

↓

Removal of Nucleic acid from cell Burst

↓

Salt - Treatment

↓

Salt Treatment

↓

Store in cool storage for some time (with salt)

↓

Filteration

↓

Final Purification (Chromatography) → Pass By Breeze Drying → Enzyme Powder.

★ Selection of Microbes :-

→ Microbes having some characteristics like :-

- → that's metabolite is minimal.
- → that can Produce maximum amount of desired Enzyme in small time.
- → Prepared inoculum in liquid medium.

★ Formation of Medium :-

- → Must have nutrients Essential for microbe growth.
- → To produce Sufficient Quantity of Enzyme.
- → Easily available & low cost.

⇒ Substrate used in medium :-

- → Yeast Extract, Molasses, wheat, Pulses, agar.
- → The Medium PH Should optimal for microbe growth and Enzyme Production.

★ Production Process (continous Process)

- → Batch Process (single step)
- → Continuous Process.

→ optimal growth Condition →
- → Nutrient
- → PH
- → Temperature
- → Oxygen

→ Enzyme should removed when produced from Metabolites (Residue)

★ Recovery and Purification of Enzyme :- Intracellular enzyme Recovery is more Tough / harder than the Extracellular

→ In Intracellular.

→ cell lysis required.

Amylase :- This Enzyme Converts Starch into Sugar.

Starch —Amylase→ Sugar (Chemical digestion)

→ Amylase Present in Saliva

→ Amylase is First Enzyme to be discovered and Isolated.

→ α- Amylase obtained from rice, animal, Plant, microbes.

Production of Amylase → Submerged Fermentation
→ Solid State Fermentation

① Sub-Merged Fermentation :- The culture media rapidly utelised (Eat) By microbes

↓

Need to Replace Constantly the Substrate (Culture Media)

→ Medium can be utelised (used) must be Sterilised and Product Purified Easily.

→ Temperature, PH etc. must be at optimum level.

→ Microbes used that can required liquid substrate. (exan- Broth and Molasses)

② Solid State Fermentation :- (SSF)

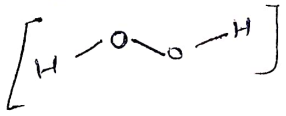
- Microbes require less moisture for growth.
→ Substrate / culture Media not utilized (used) rapidly.
→ SSF require simple Equipment.

Importance of Amylase :-

→ Production of Sweetener in food Industry.
→ good heat stability.
→ used for converting glucose into Fructose also

Catalase :- Convert Hydrogen peroxide into water and oxygen.

H_2O_2 (Hydrogen Peroxide) $\xrightarrow{\text{Decomposition}}$ $H_2O + O_2$



→ Enzyme Prevent accumulation of H_2O_2 & organ, tissue damaged by Peroxide
→ Catalase Present in the liver.
→ used in Preservation of food in Industry.
→ used H_2O_2 in water to treat waste water Treatment.

Peroxidase :- Enzyme used to decompose water or H_2O_2

★ Peroxidase → Heme Peroxidase
→ Non-heme Peroxidse (No heme Present)

Heme Peroxidase :- → PCO XS
→ PCATS

* PCOXS - Peroxidase cyclo oxygenase superfamily
* PCATS - The Peroxidase catalase superfamily.

Other Examples - Glutathione Peroxidase (GPX)
Manganese Peroxidase (MnP)
Thyroid Peroxidase (TPO)

→ Peroxidase produced by number of microorganisms and Plant.

→ It catalyse Variety of Reactions in presence of Peroxides such as H_2O_2.

Exam Rxn :

$$H_2O_2 + 2AH_2 \xrightarrow{\text{Peroxidase}} 2H_2O + 2AH$$

Importance :

→ Play Important Role in Innate Immunity (By Birth)

→ In Appoptosis (Program Cell Death) and Cell Signalling Role

Applications :

→ Peroxidase used in uric acid detection kits.

→ Peroxidase comined with cholesterol oxidase
↓
used for detection kits of Cholesterol.

Lipase : The Enzyme use to Break Dietary Fat in Body into Fatty acids and glycerol.

$$\text{Dietary Fat} \xrightarrow{\text{Lipase}} \text{Fatty Acids} + \text{Glycerol}$$

→ Lipase is produced by Stomach Cells used in digestion of Fat like Butter etc

→ Lipase found in Pancreas also (Pancreatin Lipase) and act on Small Intestine.

Importance :-

→ Maintain glyceride level in Body By Breakdown
→ used to obtain PUFA from animal & Plant Lipid.
→ Added in Detergent to remove fat or Lipid Stain without Fabric damage

Protease :- Enzyme that Break Protein into Amino-Acids.

→ Hydrolyse the Peptide Bond of Amino Acids.

Protein $\xrightarrow{\text{Protease}}$ Amino Acids

→ Protein Breaks By Protease.
→ Protease Produced by → Bacteria (Pseudomonas Clostidium etc.)
→ Fungi (Aspergillus niger, Aspergillus Flavus etc)

→ Protease → Exopeptidas
→ Endopeptidas

- Exopeptidas - Breaks Protein at Terminal ends.
- Endopeptidas - Breaks Protein in chains. (Targeted)

Importance :-

→ Required to digestion of Protein
→ used in therapy like oncology, Inflammation and Immune Regulation.

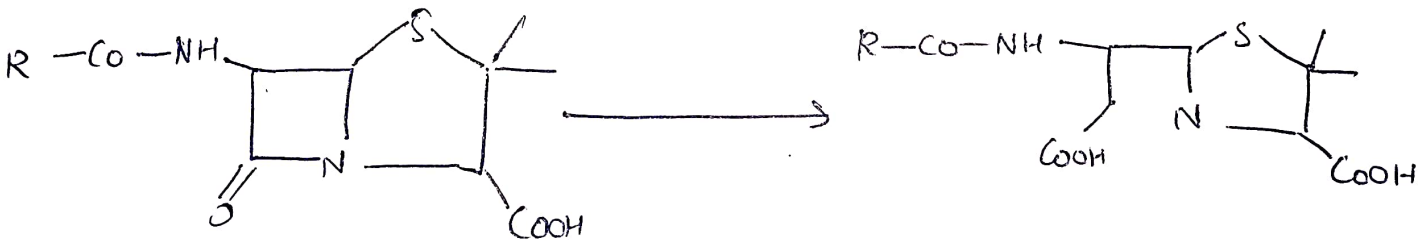
→ Required for Blood Coagulation
→ Help in Cell division, growth, Apoptosis and other living functions.
→ used in wound Healing.

Examples of Protease

- Thrombin
- Rennin
- Trypsin
- Plasmic
- Pepsin etc.

Penicillinase :- Enzyme that Breaks Penicillin (Antibiotic) into Penicillinoic acid.

→ Penicillin Acts as an Antibiotic, Kill or Inhibit the growth of Bacteria.
→ Penicillinase Breaks β-lactam (Beta-lactam) Ring of Penicillin.



Importance

→ Antibacterial Action.
→ Respirator Infection Treatment.
→ Infection in Skin
→ Bone Infection etc.

Basic Principles Of Genetic Engineering

The process of formation of Desired DNA through a host cell.

Organism → Contain Desired gene with Vector DNA help → r-DNA (Desired gene + Vector DNA) → Transfoored to host cell → Desired Product

Process :-

① Isolation of DNA :- Lysis (Breakdown) of Cell (with Enzyme)

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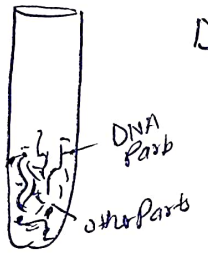
Precipitation of DNA obtained after treating with chilled Ethanol

↓

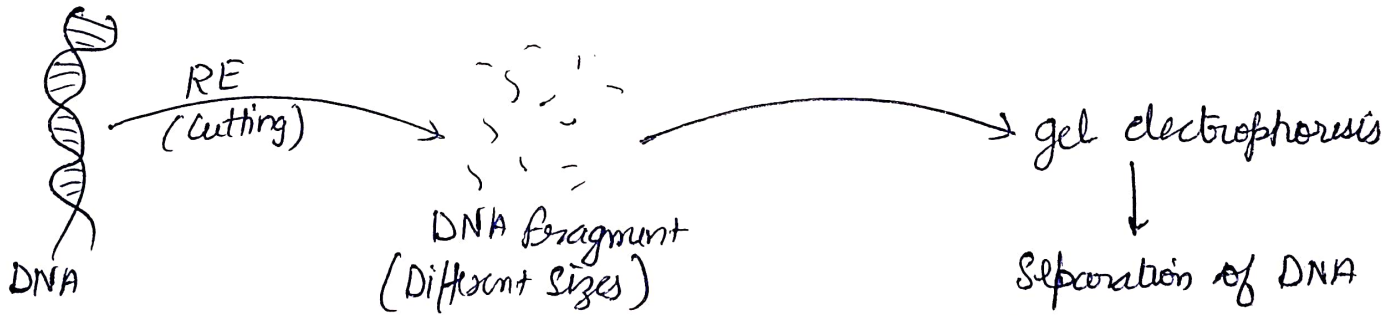
DNA Precipitation + other Remaining Parts

↓



Removal of DNA from Test Tube (Spooling)

② Cutting of DNA :- Restriction Endonuclease Enzyme used to cut the DNA Fragments



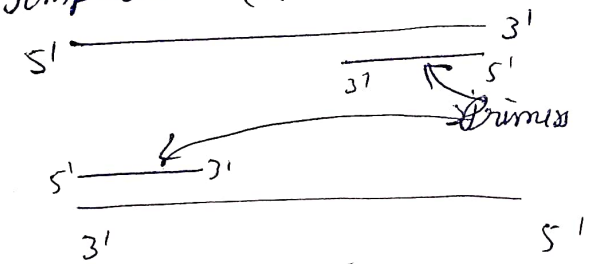
③ Amplification of DNA :- Process of Increasing No. of DNA.
(By PCR — Polymerase chain Reactions)

Steps for DNA Amplification :-

- Denaturation
- Annealing
- Extension

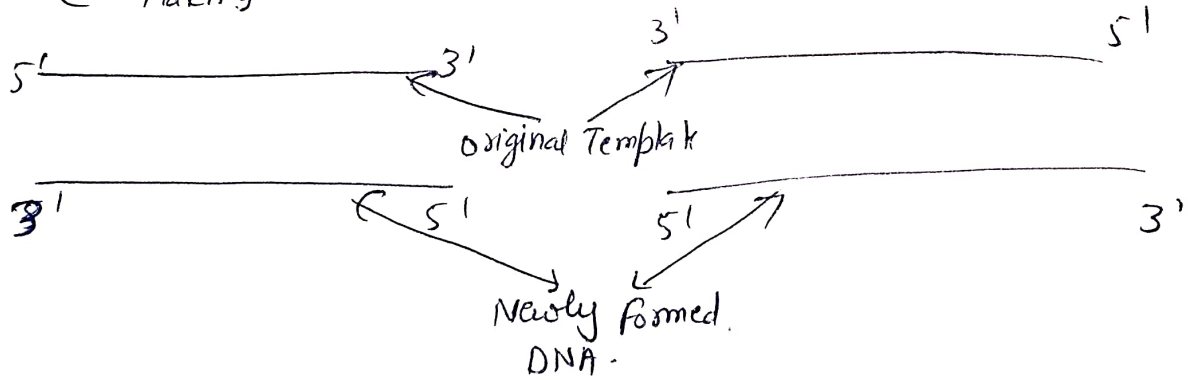
① Denaturation - Break DNA (Double Stranded) into single Templates

② Annealing :- Add the Primers (small DNA set) to Parent/original Templates. (at 54°C)

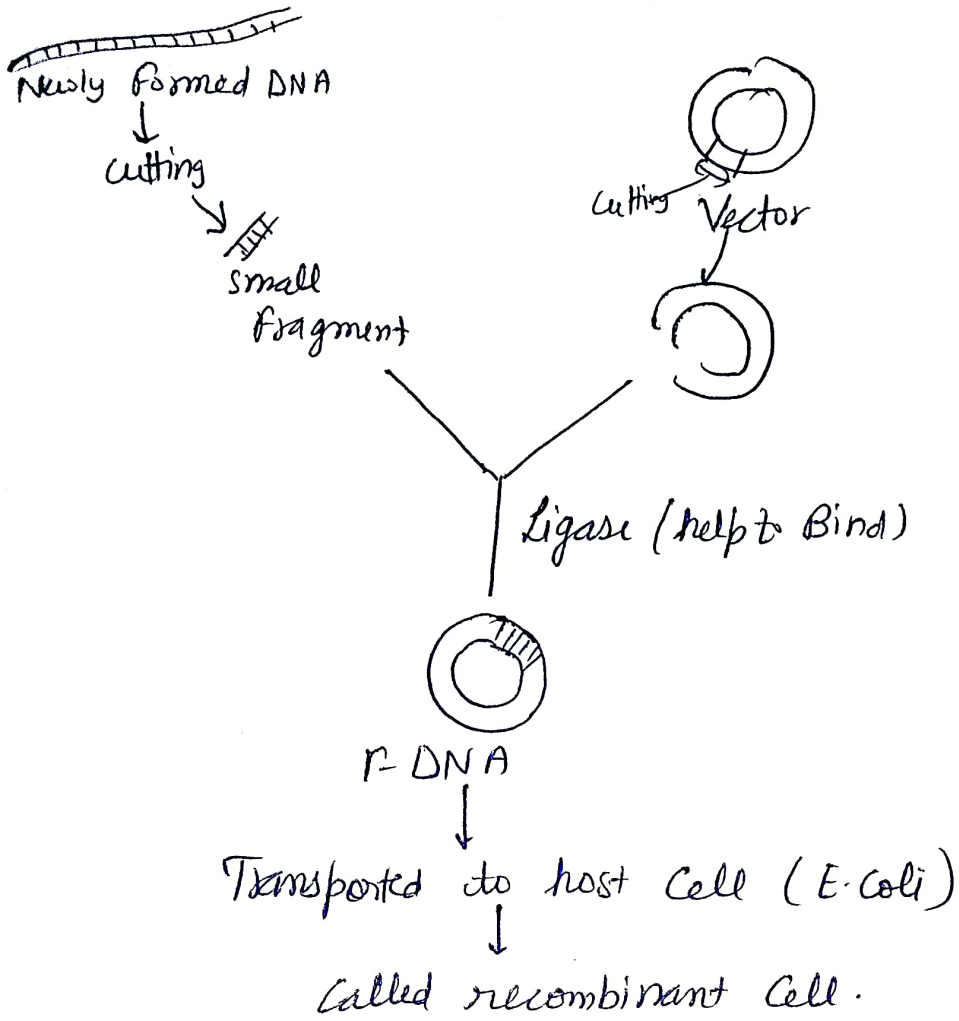


③ Extension :- Elongation of Primers equal to length of original DNA strands. (at 72°C)

(2 DNA To 4 DNA Making)



④ Formation of r-DNA :- Formed DNA Segment (small Part) can inserted in Vector (other organism DNA) to form recombinant DNA (r-DNA)


Newly formed DNA
cutting
small fragment
cutting
Vector
Ligase (help to Bind)
r-DNA
Transported to host Cell (E.Coli)
Called recombinant Cell.