BIOTECHNOLOGY

UNIT 1 NOTES

BIOTECHNOLOGY

- INTRODUCTION
- ENZYME IMMBOLIZATION
- BIOSENSORS
- PROTEIN ENGINEERING
- USES OF MICROBES
- PRODUCTION OF ENZYMES
- GENETIC ENGINEERING

IMPERFECT PHARMACY

Biotechnology

- Biotechnology is the branch of science that uses living organisms, cells or biological systems to develop useful products such as :
- Medicines
- Vaccines
- Hormones
- Enzymes

Historical Background

- 6000 Bc : Fermentation used to make bread, wine & curd (ancient biotechnology).
- 1676 : Antonie Van Leeuwenhoek discovered microorganisms using a microscope.
- 1796 : Edward Jenner developed the first vaccine (smallpox vaccine).
- 1865 : Gregor Mendel explained the laws of inheritance.
- 1869 : Fredrich Miescher discovered DNA.
- 1928 : Alexander Flaming discovered Penicillin (first antibiotic).
- 1953 : Watson and Crick discovered the double helix structure of DNA.

- 1973: Recombinant DNA technology developed by Cohen & Boyer.
- 1982: First recombinant human insulin (Humulin) produced using biotechnology.
- 1990: First gene therapy trial conducted.
- 2003: Human Genome project completed.
- 2020: mRNA vaccines (like COVID-19 vaccines) developed using advanced biotechnology.

SCOPE OF BIOTECHNOLOGY

Biotechnology has major shape in following areas:

1. Red / Healthcare Biotechnology
2. Green / Agricultural Biotechnology
3. White / Industrial Biotechnology
4. Blue / Marine Biotechnology

1. RED BIOTECHNOLOGY

- Red biotechnology deals with medical & healthcare applications.
- It is used for the production of vaccines, antibiotics, insulins, hormones & monoclonal antibodies.

② GREEN BIOTECHNOLOGY

- Green biotechnology is related to agriculture & plant science.
- It is used to develop high-yield, pest-resistant & disease-resistant crops.

③ WHITE BIOTECHNOLOGY

- White biotechnology is used in industrial & environmental processes.
- It involves the use of micro-organisms & enzymes for production of biofuel, organic acids & biodegradable products.

④ BLUE BIOTECHNOLOGY

- Blue biotechnology focuses on marine & aquatic organisms.
- It helps in developing useful pharmaceutical & industrial products from sea resources.

APPLICATIONS OF BIOTECHNOLOGY

- It is used in production of vaccines & antibiotics.
- It helps in manufacturing insulin, hormones & other biopharmaceuticals.
- It helps in gene therapy for treating genetic disorders.
- It is used to develop genetically modified (GM) crop with better yield.
- Improves food quality & nutritional value.
- It is used in plant tissue culture for rapid plant production.
- It helps in production of enzymes for pharmaceutical & industrial use.
- Used in development of monoclonal antibodies for cancer treatment.
- Helps in research & development of new drugs & vaccines.

ENZYMES

- Enzymes are biological catalysts that increase the speed of chemical reactions in living cells without getting used up.
- They're made up of proteins.
- They're Reusable (not consumed in reaction).

ENZYME IMMOBILIZATION

- Enzyme immobilization is the process of fixing or attaching enzymes on a solid support so that they can't move freely but still remain active.
- Immobilization means trapping or fixing enzymes on a support so they can be reused.

IMPORTANCE OF ENZYME IMMOBILIZATION

- Enzyme can be reused many times.
- Easy separation from product.
- Better stability.
- Continuous production possible.
- Cost -effective in industries.

ADVANTAGES

- Reusable (economical)
- Easy product purification
- Increased stability
- Controlled reaction
- Suitable for industrial use.

Disadvantages

- Initial cost is high.
- Possibility of some loss of enzyme activity.
- Complex preparation method
- Some enzymes becomes unstable.

Methods Of Enzyme Immobilization

They're mainly 5 methods of Enzyme immobilization as follows:

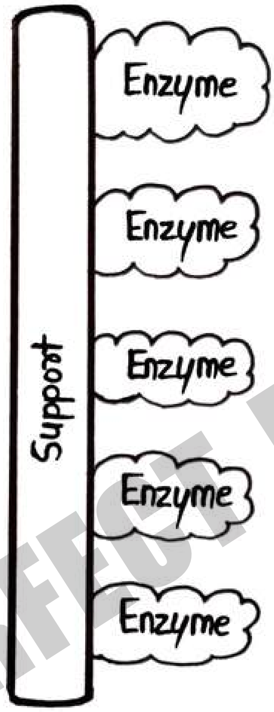
1. Adsorption Method
2. Covalent Bonding
3. Cross linking
4. Entrapment
5. Encapsulation

① Adsorption Method

- This is the simplest method.
- The enzyme is attached to the exterior surface of a support (like charcol) through weak bonds like van der Waals forces or hydrogen bonding.
- Pros: Simple & cheap, doesn't change the enzyme's shape.
- Cons: Because the bonds are weak, the enzyme can "leak" or wash away easily.

Support Materials

- Charcoal
- Glass beads
- Clay
- Cellulose



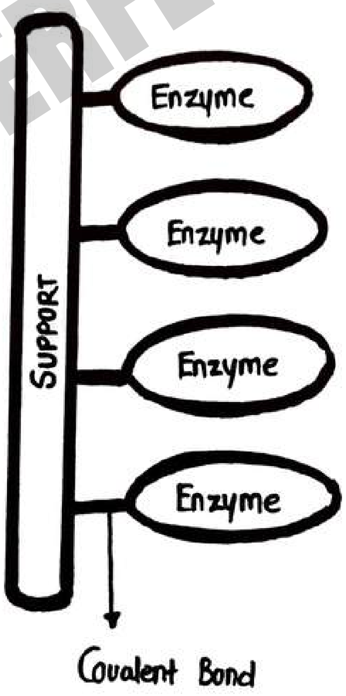
ADSORPTION
(Enzyme Immobilization)

② COVALENT BONDING

- This involves creating a strong chemical bond b/w the enzyme & a support material (like cellulose).
- It is widely used in industries.
- Pros: Very stable, minimal leakage of the enzyme.
- Cons: The chemical reaction can sometimes change the enzyme's shape, making it less effective.

Support Materials

- Agarose
- Cellulose
- Silica Gel

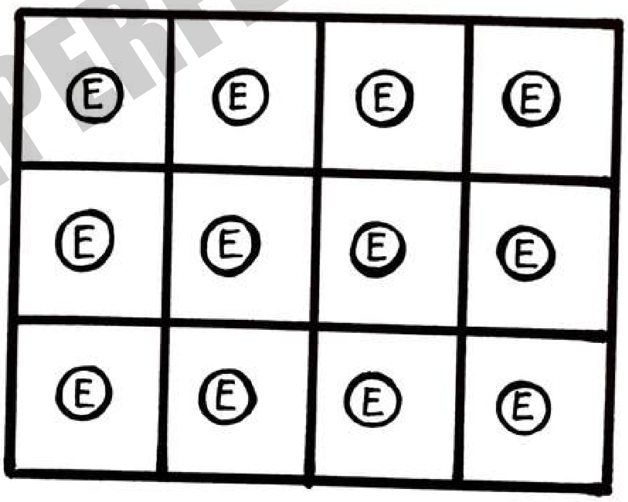


③ ENTRAPMENT

- In this instead of binding, enzyme are trapped inside a gel or matrix.
- This is an excellent method for multienzyme system.
- Pros: The enzyme doesn't physically bond to anything, so its structure remains same.
- Cons: Large substrate molecules might struggle to reach the enzyme inside the matrix.

Common Matrices

- Calcium alginate
- Polyacrylamide gel
- Gelatin

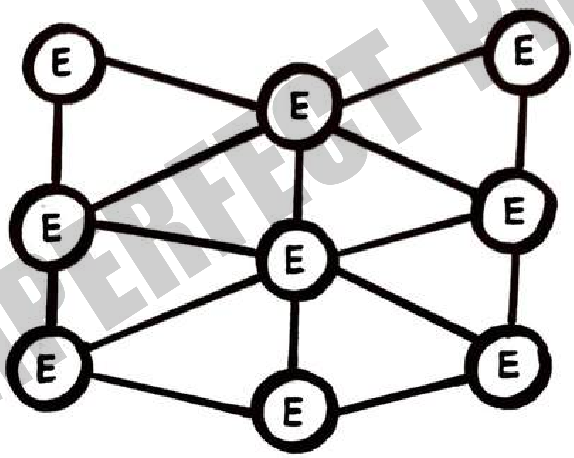


④ Cross - Linking

- This method doesn't use any support material.
- The enzymes are chemically joined to each other using a cross-linking agent (like glutaraldehyde) to form a large, insoluble complex.
- Pros: Very high enzyme concentration.
- Cons: The chemicals used can be harsh & may denature (break) the enzyme.

Comment Agent

- Glutaraldehyde is the most commonly used agent for Cross linking of enzymes.

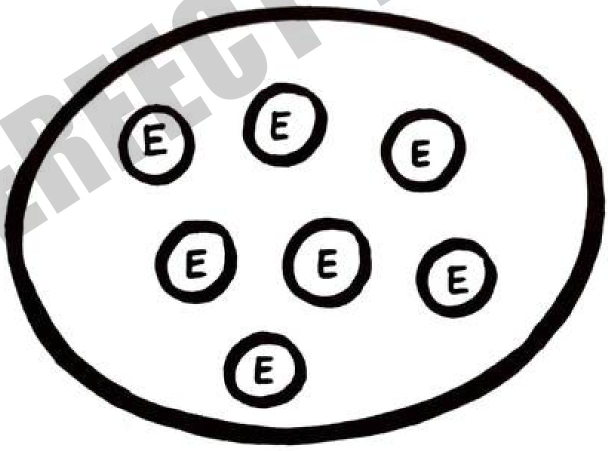


⑤ Encapsulation

- The enzymes are enclosed within a semi-permeable membrane (like a tiny capsule).
- The substrate can go in, & the product can come out, but the enzyme is too big to escape.
- Pros: Good protection from the external environment.
- Cons: The membrane can be a barrier that slows down the reaction rate.

Materials Used

- Nylon membrane
- Polymer membranes



APPLICATIONS

- Used in pharmaceutical industry for drug & antibiotic production.
- Used in food industry (cheese making, lactose-free milk).
- Used in biosensors for diagnostic tests (e.g., glucose testing).
- Used in wastewater treatment to remove pollutants.
- Used in continuous industrial production due to reusability & high stability.

Biosensors

- Biosensors is an analytical device that uses a biological component (like enzyme, antibody or cell) to detect a chemical substance & convert it into a measurable signal, usually electrical.
- Example: Glucose meter, Thermometer etc.

Components Of Biosensors

A Biosensor has following major components:

- Receptor
- Transducer
- Amplifier
- Processor
- Display unit

① Receptor

- The receptor is the biological sensing element of a biosensor that specifically recognizes & binds with the target substance (analyte).
- It may be an enzyme, antibody, DNA, micro-organisms, or cell that produces a biological reaction when the analyte is present.

② Transducer

- The transducer is the part of the biosensor that converts the biological reaction b/w receptor & analyte into a measurable unit signal (usually electrical).
- In simple words, it converts chemical / biological changes into an electrical signal.

③ AMPLIFIER

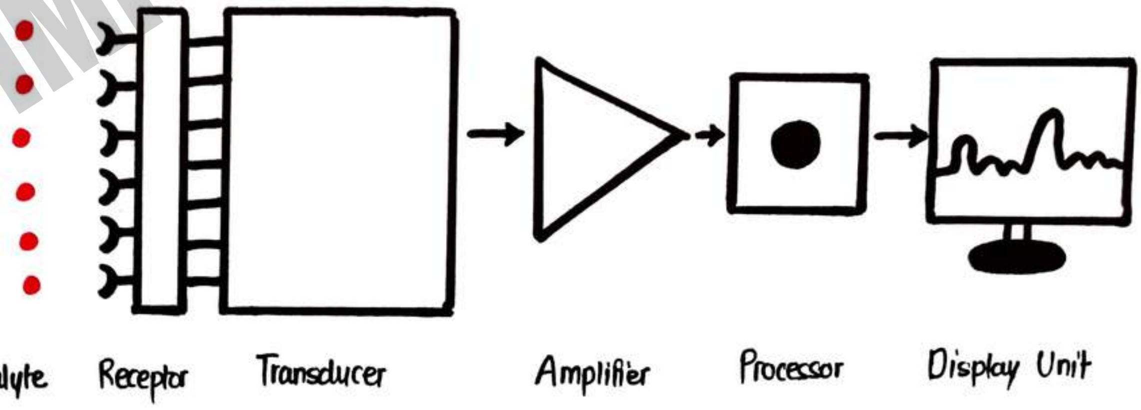
- The amplifier increases the strength of the weak signal produced by the transducer.
- This makes the signal strong enough for accurate detection & measurement.

④ PROCESSOR

- The processor analyzes & processes the amplified signal.
- It converts the signal into meaningful data & calculates the concentration of the analyte.

⑤ DISPLAY UNIT

- The display unit shows the final result in a readable form such as numbers, graph, or digital reading.
- Example: The glucose level shown on a glucometer screen.



PRINCIPLE OF BIOSENSORS

- The principle of a biosensor is based on the specific interaction b/w a biological element (receptor) & the target substance (analyte).
- When the analyte reacts with the bioreceptor, a biochemical change occurs, which is converted into an electrical signal by the transducer.
- This signal is then amplified, processed & displayed as a measurable result.

TYPES OF BIOSENSORS

1. Calorimetric Biosensors
2. Potentiometric Biosensors
3. Acoustic Wave Biosensors
4. Amperometric Biosensors
5. Optical Biosensors

1. CALORIMETRIC BIOSENSORS

- These biosensors measure the heat produced during a biochemical reaction.
- When the analyte reacts with the bioreceptor, heat is released or absorbed.
- The change in temperature is measured & used to detect the substance.
- Example: Enzyme Thermistor

② Potentiometric Biosensors

- These biosensors measure the change in voltage (potential) during a reaction.
- They detect ion concentration changes in the solution.
- They're simple, stable & commonly used.
- Example: pH sensors.

③ Acoustic Wave Biosensors

- These biosensors work on the principle of sound waves or vibrations.
- When the analyte binds to the sensor surface, the mass changes.
- This causes a change in wave frequency, which is measured.
- Example: Quartz Crystal Microbalances

④ Amperometric Biosensors

- These biosensors measures the electric current produced during a biochemical reaction.
- The current is directly proportional to the concentration of analyte.
- They're highly sensitive & widely used in medical devices.
- Example: Glucometer

⑤ Optical Biosensors

- These biosensors detects changes in light.
- When the analyte interacts with the bioreceptor, light properties change.
- These changes are measured to detect the substance.
- Example: Fibre-optic Biosensors.

APPLICATION OF BIOSENSORS

Biosensors are widely used in following major cases:

- Medical field: Used for blood glucose monitoring (glucometer), disease diagnosis, pregnancy tests & cholesterol detection.

- Pharmaceutical field: Used in drug analysis, quality control & monitoring of fermentation & drug production process.

- Environmental Monitoring: Used to detect pollutants, pesticides, heavy metals & to check water quality.

- Food industry: Used for detecting food contamination, toxins & ensuring food safety & quality.

- Agriculture: Used to detect pesticides, soil nutrients & plant diseases.

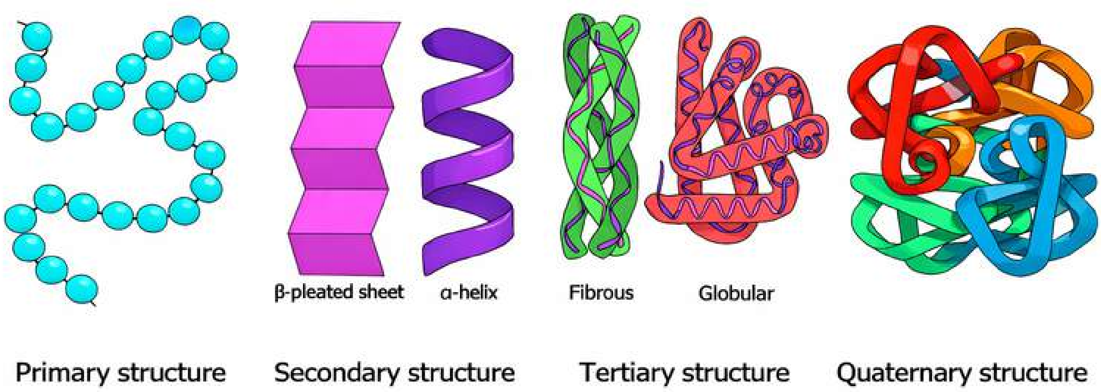
Protien Engineering

- Protein engineering is a branch of biotechnology in which scientists modify proteins to improve their properties or create new functions.
- It is done using Recombinant DNA technology or Chemical treatment to get our desirable functions.

Protiens

- Protiens are made of amino acids, & their function depends on their structure (arrangement of amino acids).
- On the basis of arrangement of amino acids proteins can be devided into 4 classes/levels

① Primary structure
② Secondary structure
③ Tertiary structure
④ Quaternary structure



- Now, if we change the sequence of amino acids, the protein's shape & function can also change.
- Hence, protein engineering means intentionally changing the structure of a protein to make it better or more useful.

NEED OF PROTIEN ENGINEERING

Natural proteins are not always perfect for industrial or medical use. They may:

- Work only at specific temperature
- Be unstable at high heat
- Be sensitive to pH changes
- Works slowly
- Act on only 1 specific substrate

Now to overcome all these problems, scientist modify protein:

- To increase enzyme activity & efficiency
- To make proteins more stable to heat, pH & chemicals.
- To improve specificity of enzymes.
- To produce better therapeutic proteins like insulin, vaccines etc.
- To reduce side effects.
- To create new functions.

APPROACHES FOR PROTIEN ENGINEERING

Two major approaches for protein engineering includes:

1. Rational Design Approach
2. Directed Evolution

① RATIONAL DESIGN

- In this approach, scientists first study the 3D structure & function of the protein.
- Specific amino acids in the protein are changed using genetic engineering.
- Changes are made in a planned way to improve activity, stability or specificity.
- It requires proper knowledge of protein structure & mechanism.

② DIRECTED EVOLUTION

- In this method Random mutations are introduced into the gene that codes for protein.
- Many variants of protein are produced & screened.
- The best performing protein (with desired property) is selected.
- It doesn't need detailed structural knowledge.
- It is time consuming method & need large number of experiments.

TECHNIQUES OF PROTIEN ENGINEERING

- Protein engineering techniques are the methods used to modify proteins to improve thier function, stability, activity or specificity.
- Main techniques includes:

1. Site Directed Mutagenesis
2. PE by Gene Families
3. PE through Chemical Modifications

1. SITE - DIRECTED MUTAGENESIS

- Site - directed Mutagenesis is a molecular biology technique used to introduce a specific & intentional change in DNA sequence.

Principle

- IF we change the DNA sequence → amino acid changes → protein structure & function change.

Basic Steps

- Select the target gene.
- Design primers containing the desired mutation.
- Perform PCR to copy the mutated gene.
- Removes original DNA.
- Insert mutated DNA into bacteria
- Mutated protein is produced.

② PE By Gene Families

- Protein Engineering by gene families means using similiar genes that belong to the same family to create improved or new proteins.

Mechanism

- Scientists compare similiar genes from different organisms.
- Useful parts of different genes are combined.
- Hybrid or modified proteins are produced.
- Example: Designing a heat stable enzymes by comparing enzymes from different thermophillic & normal bacteria.

③ PE Through Chemical Modification

- In this method, Protein structure is modified using chemical reagents.
- Specific chemical groups are added to the protein.
- Certain amino acids are chemically altered.

Example

- Adding polyethylene glycol (PEGlyation) to increase stability.

Application Of PE

- Improves enzyme stability & activity.
- Develops better medicines (insulin, antibodies).
- Used in Vaccine & Drug design.
- Produces industrial enzymes (detergants, food)
- Helps in Waste treatment.

Microbes

- Microbes are very tiny living organisms that we can't see with naked eyes.
- We can see them only with a microscope.
- Microbes are found everywhere – in air, water, soil, food & even inside our body.
- Some microbes are harmful & cause (illness) diseases, but many are useful & help humans in different ways.
- Example of microbes includes:
  - Bacteria
  - Fungi
  - Viruses
  - Protozoa
  - Algae

Use Of Microbes In Industry

- Microbes are widely used in different industries because they grow fast & produce useful substances.

① Food Industry

- Curd & Yogurt : Made by bacteria like "Lactobacillus".
- Bread : Made using yeast (fungi).
- Cheese : Prepared using bacteria & fungi.
- Fermented foods : Like idli, dosa batter.

② PHARMACEUTICAL INDUSTRY

ⓐ Antibiotics

- Many antibiotics are made from microbes.
- Penicillin is produced from the fungus penicillium.
- Streptomycin is produced from the bacterium Streptomyces.
- These antibiotics help to kill or stop the growth of harmful bacteria.

ⓑ Vaccines

- Vaccines are prepared using weakened or killed microbes.
- They help our body develop immunity against diseases like polio, hepatitis B etc.
- Microbes are grown in controlled laboratory conditions to prepare safe vaccines.

ⓒ Hormones

- Microbes are genetically modified to produce human hormones.
- Example: Insulin is produced using genetically engineered bacteria like E. coli.
- This insulin is used to treat diabetes.

ⓓ Vitamins

- Some microbes produce vitamins such as:
  - Vitamin B12
  - Riboflavin (Vit B2)
- These vitamins are used in supplements & medicines.

(e) Enzymes

- Microbes produce enzymes used in medicine, such as:
  - Streptokinase - Used to dissolve blood clots.
  - Asparaginase - Used in Cancer treatment.

(3) ALCOHOL & BEVARAGES

- Alcohol & Wine: Produced by Yeast through fermentation.

(4) AGRICULTURE

- Biofertilizers: Like Rhizobium helps in nitrogen fixation.
- Biopesticides: Used to kill harmful insects.

(5) WASTE TREATMENT

- Microbes help in sewage treatment by breaking down waste materials.
- Used in biogas production.

Production Of Enzymes

- Enzymes are biological catalysts (proteins) that speed up chemical reactions.
- In Biotechnology industries, enzymes are mainly produced by micro-organisms like bacteria & fungi because they grow fast & give high yeild.

Steps In Industrial Enzyme Production

- Selection of micro-organisms
- Fermentation process
- Production of enzymes
- Extraction & Purification

① Selection Of Microorganism

- A suitable microbe is selected that produces the required enzyme.
- Example :- Bacillus ⟶ produces amylase.
  - Aspergillus ⟶ produces citric acid & enzymes.

② Fermentation Process

- The selected microbe is grown in large tanks called fermenters under controlled conditions (temperature, pH, oxygen).
- This process is called fermentation.
- There are two types:
  - Submerged fermentation (liquid medium)
  - Solid-state fermentation (solid medium)

③ Production Of Enzymes

- During growth, microbes produce enzymes either:
- Inside the cell (intracellular enzyme)
- Outside the cell (extracellular enzyme)
- Most industrial enzymes are extracellular because they're easier to collect.

④ Extraction & Purification

- The enzyme is separated from the culture medium by:
  - Filtration
  - Centrifugation
  - Purification techniques

Uses Of Industrial Enzymes

- Detergent industry → Protease, Lipase
- Food industry → Amylase, Invertase
- Pharmaceutical industry → Streptokinase
- Textile industry → cellulose
- Paper industry → Xylanase

General Considerations

① Amylas
② Catalase
③ Peroxidase
④ Lipase
⑤ Protease
⑥ Penicillinase

① Amylase

- Amylase is an enzyme that converts starch into simple sugars like maltose & glucose.
- It is present in humans, plants & Microbes.
- It is one of the most important industrial enzymes, especially in the food & fermentation industries.
- There are three main types:

a) Alpha - amylase
b) Beta - amylase
c) Glucoamylase

Common Industrial Microbes:

- Bacillus licheniformis
- Bacillus subtilis
- Aspergillus niger
- Aspergillus oryzae

Industrial Production

- It is produced by submerged fermentation in large fermenters.
- After fermentation, the enzyme is filtered & purified.
- They're usually produced as an extracellular enzyme, so it is easier to collect.

Uses

- Bread making
- Brewing industry
- Starch processing
- Textile & paper industry

② CATALASE

- Catalase breaks down hydrogen peroxide (H_2O_2) into water & Oxygen.
- Hydrogen peroxide is toxic to cells, so catalase protects cells from damage.

Industrial Production

- Microorganisms used: Aspergillus niger, Micrococcus luteus, Bacillus species.
- It is produced by fermentation process.
- After growth, cells are separated & enzyme is purified.
- Sometimes it may produced intracellularly, so cell distruption may be needed.

Uses

- Textile industry
- Food packaging
- Contact lens cleaning solutions
- Laboratory applications.

③ PEROXIDASE

- Peroxidase is an enzyme that catalyzes oxidation reactions using hydrogen peroxide.
- It is widely used in diagnostics & environmental treatment.

Industrial Production

- It is obtained from plants like horseradish or produced by microbes.
- Microbial production uses fermentation methods.
- After fermentation, enzyme is purified by filtration & chromatography.

Uses

- Diagnostic kits (blood glucose tests)
- Wastewater treatment (removal of phenolic compounds)
- Food industry

④ LIPASE

- Lipase breaks down fats (lipids) into fatty acids & glycerol.
- It works at the interface of oil & water

Industrial Production

- Microorganisms used: Candida rugosa, Aspergillus niger, Rhizopus oryzae
- Produced by submerged or solid-state fermentation.
- Mostly extracellular, so easy to recover.
- Enzyme is purified & may immobilized for reuse.

Uses

- Detergents (removes oil stains)
- Cheese & dairy products
- Biodiesel production
- Pharmaceutical preparations.

⑤ PROTEASE

- Protease breaks down proteins into peptides & amino acids.
- It is one of the largest produced industrial enzymes worldwide.
- Proteases are of three types:

a) Acid Protease

b) Neutral Protease

c) Alkaline Protease

Industrial Production

- Microorganisms used : Bacillus subtilis, Aspergillus oryzae
- Produced by large-scale fermentation.
- Alkaline protease is widely produced for detergents.
- After fermentation, enzyme is filtered & purified.

Uses

- Detergents
- Leather processing
- Pharmaceutical formulations

⑥ PENICILLINASE

- Penicillinase is an enzyme that breaks down the antibiotic Penicillin by opening its beta-lactam ring.
- It is mainly produced by certain bacteria & is responsible for antibiotic resistance.

Industrial Production

- Microorganisms used : Staphylococcus aureus, Bacillus cereus.
- It's produced by fermentation under controlled conditions.
- It can be intracellular or extracellular.
- It's further Extracted & purified for laboratory and research use.

Uses

- Reseach on antibiotic resistant.
- Testing penicillin sensitivity.
- Quality control in pharmaceutical labs.

Genetic Engineering

- The basic principle of genetic engineering is to change or modify the DNA of an organism by inserting, deleting or altering specific genes to obtain desired characteristics or useful products.
- In simple words it is the technique of cutting, modifying, & joining DNA to produce a new gene or protein.

Components Of Genetic Engineering

- Donor DNA (Desired gene)
- Restriction Endonucleases
- Vector (Plasmid / Phage)
- DNA ligase
- Host cell
- Recombinant DNA

Steps Involved In GE

It involves following steps:

- Isolation of Desired Gene
- Cutting of DNA
- Selection of Vector
- Ligation
- Introduction into Host cell
- Clonning & Expression

① Isolation Of Desired Gene

- First, the gene of interest (useful gene) is identified & isolated from the donor organism.
- Example: Insulin gene from human cells.
- The main purpose is to obtain the specific DNA fragment that codes for a desired protein.

② Cutting Of DNA

- Special enzymes called restriction endonucleases act like molecular scissors.
- They cut DNA at specific sequences.

③ Selection Of Vector

- A vector is a DNA molecule used to carry the desired gene into a host cell.
- The most important vector are Plasmids, Bacteriophages & Cosmid.

④ Ligation

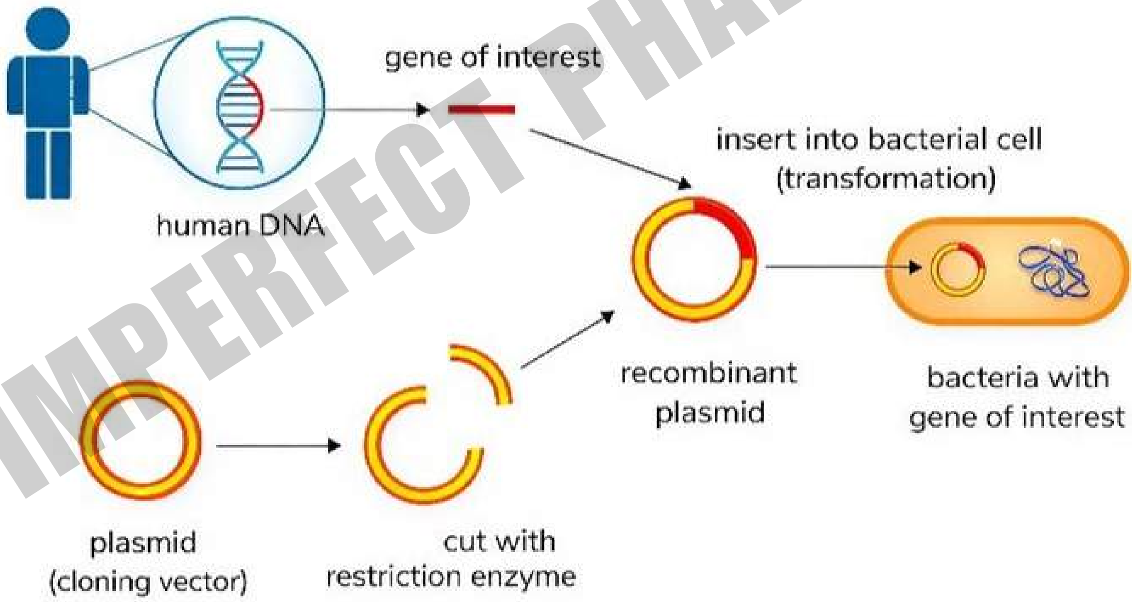
- The desired gene is joined with the vector using an enzyme called DNA ligase.
- This forms Recombinant DNA (rDNA).
- Desired gene + Vector DNA = Recombinant DNA

⑤ Introduction Into Host Cell

- The recombinant DNA is introduced into a suitable host cell such as:
  - Bacteria (E.coli)
  - Yeast
  - Animal cells

⑥ CLONING & EXPRESSION OF GENE

- Inside the host:
  - The host cell multiplies.
  - The inserted gene replicates.
  - The gene produces the desired protein (expression).
- **Example:** Bacteria produce large amounts of insulin.



APPLICATIONS OF GENETIC ENGINEERING

- Insulin production
- Vaccine production
- Gene therapy
- Production of Enzymes
- Agricultural crop improvement.

THANK YOU

FOR CHOOSING IMPERFECT PHARMACY AS YOUR STUDY PARTNER

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