Unit-1

Pharmaceutical Biotechnology

B.Pharma 6th Sem Notes

Unit: 1

Brief introduction to Biotechnology with reference to Pharmaceutical Sciences.

- Enzyme Biotechnology- Methods of enzyme immobilization and applications.
- Biosensors- Working and applications of biosensors in Pharmaceutical Industries.
- Brief introduction to Protein Engineering. Use of microbes in industry.
- Production of Enzymes- General consideration – Amylase, Catalase, Peroxidase, Lipase, Protease, Penicillinase. Basic principles of genetic engineering.

Introduction to Biotechnology

Biotechnology:

Biotechnology is the use of living organisms, cells, or biological systems to develop products and processes that benefit human life. The word comes from three Greek words: Bios (life) + Technos (skill/art) + Logos (study).

Simple Definition

Biotechnology = Biology + Technology

It means using nature's own tools (like cells, enzymes, and genes) to make useful things such as medicines, vaccines, and food products.

Biotechnology in Pharmaceutical Sciences

Pharmaceutical biotechnology uses biological systems to discover, develop, and produce medicines. It plays a key role in:

- Drug Discovery – Finding new drug targets using genomics and proteomics
- Drug Production – Making insulin, vaccines, antibiotics using microorganisms
- Gene Therapy – Replacing defective genes to treat diseases
- Diagnostic Tools – Developing biosensors and immunoassays for detecting diseases
- Personalized Medicine – Creating drugs tailored for individual patients

Applications in Pharma Industry

Area	Examples
Antibiotic Production	Penicillin from Penicillium fungi
Recombinant Proteins	Insulin, Erythropoietin, Growth Hormone
Vaccines	Hepatitis B, COVID-19 mRNA vaccines
Monoclonal Antibodies	Herceptin, Adalimumab (Humira)
Enzyme Therapy	Pancreatin, Streptokinase
Gene Therapy	CRISPR-based treatments

Enzyme Biotechnology

Enzyme biotechnology is the branch of biotechnology that uses enzymes (biological catalysts) as tools to carry out specific chemical reactions in industrial, medical, and research applications.

Enzymes are proteins that speed up chemical reactions without being used up. They are highly specific (work on one substrate only), work at mild conditions, and are biodegradable.

Enzyme Immobilization

Immobilization means fixing or attaching enzymes to a solid support (carrier) so they can be reused many times. Think of it like gluing an enzyme to a surface so it stays in place but still works.

Why Immobilize Enzymes?

- Enzymes can be reused many times (cost-effective)
- Easy to separate enzyme from the product
- More stable at high temperatures and harsh conditions
- Continuous processing becomes possible
- Reduced contamination of the product

Methods of Enzyme Immobilization

METHODS OF ENZYME IMMOBILIZATION

FREE ENZYME (Soluble)

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ADSORPTION	COVALENT BINDING	ENTRAPMENT	CROSS-LINKING

Method 1: Adsorption (Physical Attachment)

The enzyme is simply stuck to the surface of a carrier material using weak forces like hydrogen bonds, van der Waals forces, or ionic interactions.

- Examples of carriers: Activated charcoal, Silica, Ion exchange resins, Cellulose
- Advantages: Simple, cheap, enzyme activity is mostly preserved
- Disadvantages: Enzyme can easily detach (leaks off), not suitable for harsh conditions
- Pharma use: Enzyme-based biosensors, chromatography

Method 2: Covalent Binding

The enzyme is chemically bonded (covalently linked) to the carrier surface. The bond is very strong.

- Carriers used: CNBr-activated Sepharose, Glutaraldehyde-treated glass beads
- Binding is through amino, carboxyl, or hydroxyl groups of the enzyme
- Advantages: Very stable, no enzyme leakage
- Disadvantages: Can reduce enzyme activity, expensive
- Pharma use: Glucose biosensors, affinity chromatography, drug manufacturing

Method 3: Entrapment (Gel Entrapment)

The enzyme is trapped inside a gel or polymer network. It cannot escape but small molecules (substrates and products) can pass in and out freely.

- Materials used: Alginate beads, Polyacrylamide gel, Agar, Collagen
- Advantages: Large amounts of enzyme can be immobilized, enzyme is protected
- Disadvantages: Substrate may have difficulty reaching the enzyme (mass transfer limitation)
- Pharma use: Immobilized cell systems, bioreactors for drug synthesis

Method 4: Cross-Linking

Enzyme molecules are chemically linked to each other using bifunctional reagents (agents that react with two molecules at once). No carrier is needed.

- Reagent used: Glutaraldehyde (most common)
- Advantages: Very high enzyme loading, stable
- Disadvantages: May reduce activity, brittle structure
- Pharma use: Industrial enzyme preparations, chiral drug synthesis

Applications of Enzyme Biotechnology in Pharma

- Penicillin Acylase – Used to produce semi-synthetic antibiotics (Ampicillin, Amoxicillin)
- L-Asparaginase – Anti-cancer enzyme used in leukemia treatment
- Streptokinase / Urokinase – Clot-dissolving enzymes (thrombolytic drugs)
- Lipase – Used in synthesis of chiral drugs
- Lactase – Immobilized in dairy industry for lactose-free milk
- Glucose Oxidase – Used in blood glucose biosensors for diabetes

Biosensors- Working and applications of biosensors in Pharmaceutical Industries.

Biosensor:

- A biosensor is a device that detects a specific biological or chemical substance and converts the biological signal into a measurable electrical or optical signal.
- Think of a biosensor like a smart detector that uses a biological molecule (like an enzyme or antibody) to recognize a target substance, and then produces a signal that we can measure.

Components of a Biosensor

Component	Role	Example
Bioreceptor	Recognizes the target molecule	Enzyme, Antibody, DNA, Whole cell
Transducer	Converts biological signal to electrical signal	Electrode, Optical fiber, Piezoelectric crystal
Signal Processor	Amplifies and displays the output	Amplifier, Digital display, Computer
Sample	The substance being analyzed	Blood, Urine, Saliva, Serum

Working of a Biosensor (Step by Step)

HOW A BIOSENSOR WORKS

Step 1: Sample containing target molecule is added

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Step 2: Bioreceptor (enzyme/antibody) RECOGNIZES & BINDS to target

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Step 3: Biological reaction produces a change (pH, electron, light)

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Step 4: TRANSDUCER converts biological change into electrical/optical signal

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Step 5: Signal is AMPLIFIED and DISPLAYED as readable output

Types of Biosensors

Based on Bioreceptor:

- Enzymatic Biosensors – Use enzymes as the recognition element (e.g., Glucose biosensor)
- Immunosensors – Use antibody-antigen interactions (e.g., Pregnancy test, COVID test)
- DNA Biosensors – Use DNA hybridization for detecting pathogens or genetic mutations
- Microbial Biosensors – Use whole microorganisms (e.g., BOD measurement)

Based on Transducer:

- Electrochemical Biosensors – Measure electrical changes (most common type)
- Optical Biosensors – Measure light changes (e.g., SPR sensors)
- Piezoelectric Biosensors – Measure mass changes using quartz crystals
- Thermal Biosensors – Measure heat produced during a reaction

Example: Glucose Biosensor (Most Important)

The glucose biosensor (like the diabetic glucometer) works as follows:

- Bioreceptor: Glucose Oxidase enzyme
- Reaction: Glucose + O2 → Gluconic acid + H2O2 (catalyzed by glucose oxidase)
- Transducer: Electrode detects H2O2 produced
- Output: Current proportional to glucose concentration is displayed
- Use: Daily blood sugar monitoring in diabetes patients

Applications of Biosensors in Pharmaceutical Industry

Application	Type of Biosensor	Target Detected
Blood glucose monitoring	Enzymatic electrochemical	Glucose
Drug testing / Abuse detection	Immunosensor	Narcotics, steroids
Therapeutic drug monitoring	Optical biosensor	Drug levels in blood
Pathogen detection	DNA biosensor	Bacteria, viruses
Pregnancy test	Immunosensor	hCG hormone
HIV/AIDS diagnosis	Immunosensor	HIV antibodies
Cholesterol monitoring	Enzymatic	Cholesterol
Cancer biomarker detection	Immunosensor / DNA	PSA, CEA, AFP

Brief introduction to Protein Engineering. Use of microbes in industry.

Protein Engineering:

- Protein engineering is the process of designing and creating new proteins with desired properties, or modifying existing proteins to improve their function.
- Proteins are made of amino acids. By changing the sequence of amino acids (by modifying the gene), we can change the shape, function, stability, and activity of a protein.

Goals of Protein Engineering

- Make enzymes more stable at high temperatures
- Improve drug activity and reduce side effects
- Create new vaccines and antibodies
- Improve enzyme specificity for industrial reactions
- Develop new diagnostic proteins

Methods of Protein Engineering

A) Rational Design (Site-Directed Mutagenesis)

The scientist already knows which amino acid to change. They design a mutation at a specific position in the gene to change the protein.

- Step 1: Study the protein structure using X-ray crystallography or NMR
- Step 2: Identify which amino acid to change
- Step 3: Use molecular biology techniques to change the gene (mutagenesis)
- Step 4: Express the mutant protein and test its properties
- Example: Engineering tPA (tissue Plasminogen Activator) for better clot dissolution

B) Directed Evolution (Random Mutagenesis + Selection)

This mimics Darwin's natural selection in the lab. Random mutations are introduced, and the best protein is selected and evolved further.

- Step 1: Introduce random mutations in the gene (using chemicals or UV)
- Step 2: Express thousands of mutant proteins
- Step 3: Test/screen all proteins for desired activity
- Step 4: Take the best one and repeat the process
- Example: Engineering enzymes for industrial catalysis (Nobel Prize 2018 – Frances Arnold)

Applications of Protein Engineering:

- Insulin analogues – Modified insulin (Glargine, Lispro) with better action profiles
- Monoclonal antibodies – Engineered antibodies for cancer treatment (Trastuzumab, Bevacizumab)
- Improved enzymes – Thermostable DNA polymerase (Taq polymerase) for PCR
- Protein vaccines – Engineered viral proteins for safer vaccines
- Antibody-Drug Conjugates (ADC) – Antibody linked to a drug for targeted cancer therapy

Use of Microbes in Industry

Introduction

Microorganisms (bacteria, fungi, yeast, algae) are tiny living machines that can produce a huge variety of useful substances. Industrial microbiology involves using microbes on a large scale to produce products of commercial value.

Microbes Used in Industry

Microorganism	Type	Industrial Use
Penicillium notatum	Fungus	Production of Penicillin antibiotic
Aspergillus niger	Fungus	Citric acid, gluconic acid, enzymes (amylase)
Saccharomyces cerevisiae	Yeast	Alcohol fermentation, bread making, insulin production
Lactobacillus spp.	Bacteria	Lactic acid, yogurt, cheese production
Bacillus subtilis	Bacteria	Protease, amylase enzyme production
Streptomyces spp.	Bacteria	Streptomycin, tetracycline antibiotics
Clostridium spp.	Bacteria	Acetone-butanol fermentation (ABE process)
Rhizobium spp.	Bacteria	Nitrogen fixation in agriculture
E. coli (recombinant)	Bacteria	Recombinant insulin, human growth hormone

Industrial Applications of Microbes

A) Pharmaceutical Industry

- Antibiotic Production – Penicillin (Penicillium), Streptomycin (Streptomyces), Erythromycin
- Hormone Production – Recombinant human insulin using E. coli
- Vaccine Production – Hepatitis B surface antigen using Saccharomyces cerevisiae
- Enzyme Production – Amylase, Protease, Lipase from Aspergillus and Bacillus
- Vitamin Production – Vitamin B12 from Pseudomonas, Riboflavin from Ashbya gossypii

B) Food Industry

- Fermentation – Beer, wine, bread production using Saccharomyces cerevisiae
- Dairy Products – Cheese, yogurt using Lactobacillus and Streptococcus
- Organic Acids – Citric acid from Aspergillus niger for soft drinks

C) Environmental Industry

- Bioremediation – Pseudomonas breaks down oil spills and pesticides
- Sewage Treatment – Microbes decompose organic waste
- Biogas Production – Methanobacterium produces methane from organic waste

D) Agricultural Industry

- Biofertilizers – Rhizobium fixes nitrogen from the air
- Biopesticides – Bacillus thuringiensis (Bt) produces toxins that kill insects

Production of Enzymes- General consideration – Amylase, Catalase, Peroxidase, Lipase, Protease, Penicillinase. Basic principles of genetic engineering.

Production of Enzymes – Industrial Considerations

General Considerations for Enzyme Production

Industrial enzyme production involves growing microorganisms in large fermenters and then extracting and purifying the enzymes. The process must be efficient, cost-effective, and produce high-quality enzymes.

General Steps in Enzyme Production:

Step 1: Selection of Microorganism (Strain Selection)

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Step 2: Optimization of Growth Conditions (Media, pH, Temperature, Oxygen)

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Step 3: Large-Scale Fermentation (Batch, Fed-Batch, or Continuous)

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Step 4: Cell Harvesting (Centrifugation or Filtration)

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Step 5: Enzyme Extraction (Cell Disruption for intracellular enzymes)

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Step 6: Purification (Precipitation, Chromatography, Ultrafiltration)

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Step 7: Formulation and Quality Control (Standardization, Stabilization)

Factors for Enzyme Production:

- Microorganism choice – Must be safe (GRAS status), high-yield producer
- Carbon source – Glucose, starch, cellulose (cheap substrates preferred)
- Nitrogen source – Ammonia, peptone, yeast extract
- pH control – Each enzyme requires specific pH
- Temperature – Optimal range maintained throughout fermentation
- Aeration and Agitation – Dissolved oxygen is critical for aerobic organisms

- Inducer addition – Some enzymes are produced only when substrate is present (inducible enzymes)

Amylase

Amylase is an enzyme that breaks down starch (amylose and amylopectin) into simple sugars like maltose, glucose, and dextrins.

- Type: Endoamylase (alpha-amylase), Exoamylase (beta-amylase, glucoamylase)
- Sources: Aspergillus oryzae, Bacillus amyloliquefaciens, Bacillus subtilis
- Production conditions: pH 5.5-7.0, Temperature 30-37°C, Aerobic conditions
- Substrate (Inducer): Starch is used as inducer and carbon source

Applications of Amylase:

- Pharma: Digestive enzyme supplement (Pancreatin), treating pancreatic insufficiency
- Food industry: Starch liquefaction in brewing, glucose syrup production
- Textile: Desizing of fabrics
- Paper industry: Paper sizing

6.3 Catalase

Catalase is an enzyme that breaks down hydrogen peroxide (H_2O_2) into water and oxygen. It protects cells from oxidative damage.

Reaction: $2H_2O_2 \rightarrow 2H_2O + O_2$

- Sources: Aspergillus niger, Micrococcus lysodeikticus, bovine liver
- Production conditions: pH 7.0, Temperature 30-37°C, Aerobic
- Inducer: H_2O_2 or oxidative stress

Applications of Catalase:

- Pharma: Used in wound care to remove H_2O_2 residue
- Food industry: Cheese production (removing H_2O_2 used as preservative)
- Contact lens cleaning solutions
- Textile: Removing hydrogen peroxide after bleaching of fabrics

6.4 Peroxidase

Peroxidase is an enzyme that catalyzes the oxidation of various substrates using hydrogen peroxide as the oxidant.

Reaction: Substrate + $H_2O_2 \rightarrow$ Oxidized product + H_2O

- Sources: Horseradish (Armoracia rusticana), Coprinus cinereus fungus
- Most used type: Horseradish Peroxidase (HRP)
- Production conditions: pH 6.0-7.0, Temperature 25-30°C

Applications of Peroxidase:

- Pharma (Most Important): Used in ELISA (Enzyme-Linked Immunosorbent Assay) for diagnosis
- Immunohistochemistry – Detecting proteins in tissue sections
- Biosensor component – For detection of H_2O_2 in clinical samples
- Wastewater treatment – Removing phenols and aromatic compounds

Lipase

Lipase is an enzyme that breaks down fats and oils (triglycerides) into fatty acids and glycerol.

Reaction: Triglyceride + H_2O → Fatty acids + Glycerol

- Sources: Candida rugosa, Rhizopus arrhizus, Aspergillus niger, Pseudomonas
- Production conditions: pH 7.0-8.0, Temperature 30-40°C, Aerobic with good aeration
- Inducer: Oils (olive oil, soybean oil) are used as inducers and carbon sources

Applications of Lipase:

- Pharma: Lipase supplement in digestive enzyme capsules (Pancrelipase)
- Pharma: Synthesis of chiral drugs (used in asymmetric synthesis)
- Food industry: Cheese ripening, baked goods
- Detergent industry: Removing fat stains in laundry detergents
- Biodiesel production: Transesterification of oils to biodiesel

6.6 Protease

Protease (also called Proteinase or Peptidase) is an enzyme that breaks down proteins into smaller peptides and amino acids by hydrolyzing peptide bonds.

- Types: Endopeptidases (cut within the protein), Exopeptidases (cut from ends)
- Sources: Bacillus subtilis, Aspergillus oryzae, Streptomyces griseus, Papaya (Papain)
- Production conditions: pH 7.0-9.0 (neutral to alkaline), Temperature 30-55°C
- Inducer: Casein, gelatin, or skimmed milk are used as protein inducers

Applications of Protease:

- Pharma: Trypsin and Chymotrypsin – anti-inflammatory and tissue digestion enzymes
- Pharma: Streptokinase – fibrinolytic enzyme for treating blood clots
- Pharma: Collagenase – used in wound debridement
- Detergent industry: Removing protein stains (blood, food)
- Leather industry: Dehairing of animal hides
- Food industry: Meat tenderization, beer clarification

6.7 Penicillinase (Beta-Lactamase)

Penicillinase (Beta-lactamase) is an enzyme produced by bacteria that breaks down the beta-lactam ring of penicillin, making the antibiotic ineffective. This is the main mechanism of antibiotic resistance.

Reaction: Penicillin + H2O → Penicilloic acid (inactive)

- Sources: Staphylococcus aureus, Bacillus cereus, Bacillus licheniformis (naturally produce it)
- Production: Induced by penicillin in the growth medium

Applications of Penicillinase:

- **Pharmaceutical manufacturing:** Used in the production of semi-synthetic antibiotics (6-APA)
- **Step:** Penicillinase (Penicillin Acylase) removes the side chain from penicillin G to give 6-APA (6-Aminopenicillanic acid), which is then chemically modified to produce Ampicillin, Amoxicillin, Cloxacillin, etc.
- **Antibiotic resistance studies:** Used in research to understand and combat antibiotic resistance
- **Clinical microbiology:** Detection of beta-lactamase producing bacteria

Enzyme	Source Microorganism	Key Application in Pharma
Amylase	Aspergillus oryzae, B. subtilis	Digestive supplement, starch processing
Catalase	Aspergillus niger	Wound care, food preservation
Peroxidase	Horseradish, Coprinus	ELISA diagnostics, biosensors
Lipase	Candida rugosa, Rhizopus	Digestive enzyme, chiral drug synthesis
Protease	B. subtilis, A. oryzae	Anti-inflammatory, fibrinolytic agents
Penicillinase	S. aureus, B. cereus	Semi-synthetic antibiotic production (6-APA)

Basic Principles of Genetic Engineering:

Genetic Engineering:

- Genetic engineering (also called Recombinant DNA Technology or Gene Cloning) is the direct manipulation of an organism's DNA using laboratory techniques to add, remove, or alter genetic material.
- Simple idea: Take a gene from one organism → Put it into another organism → Make that organism produce the desired protein.
- Example: Take the human insulin gene → Put it into E. coli bacteria → E. coli now makes human insulin!

Tools of Genetic Engineering

Tool	What it Does	Example
Restriction Enzymes	Cut DNA at specific sequences (molecular scissors)	EcoRI, HindIII, BamHI
DNA Ligase	Joins cut DNA pieces together (molecular glue)	T4 DNA Ligase
Vectors	Carry foreign DNA into host cell	Plasmids, Bacteriophages, Cosmids
PCR (Polymerase Chain Reaction)	Amplifies (copies) specific DNA sequences	Taq DNA Polymerase
Gel Electrophoresis	Separates DNA fragments by size	Agarose gel
DNA Sequencing	Reads the sequence of DNA bases	Sanger method, Next-gen sequencing
CRISPR-Cas9	Precisely cuts and edits DNA	Gene therapy, knockout studies

Basic Steps in Genetic Engineering

STEPS IN RECOMBINANT DNA TECHNOLOGY

Step 1: ISOLATION of the Desired Gene (e.g., Insulin gene from human cells)

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Step 2: CUT the gene using Restriction Enzymes (creates sticky ends)

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Step 3: SELECT a VECTOR (Plasmid) and cut it with the same restriction enzyme

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Step 4: JOIN the gene into the vector using DNA Ligase → Recombinant DNA

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Step 5: INTRODUCTION into Host Cell (Transformation of E. coli)

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Step 6: SELECTION of cells that have taken up the recombinant DNA

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Step 7: EXPRESSION – Host cell reads the gene and produces the protein

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Step 8: EXTRACTION and PURIFICATION of the desired protein

Restriction Enzymes – The Molecular Scissors

Restriction enzymes recognize specific short DNA sequences (4-8 base pairs) called restriction sites and cut the DNA at these sites.

- EcoRI recognizes: 5'-GAATTC-3' and cuts between G and A
- Cutting produces 'sticky ends' – short single-stranded overhanging sequences
- The same restriction enzyme is used to cut both the gene and the vector
- This ensures that the gene can be inserted into the vector perfectly

Vectors – The Carriers

A vector is a DNA molecule used to carry the foreign gene into the host cell.

Types of Vectors:

- Plasmids – Small circular DNA found naturally in bacteria. Most commonly used. Examples: pBR322, pUC19
- Bacteriophages – Viruses that infect bacteria. Used for cloning larger DNA fragments. Example: Lambda phage
- Cosmids – Hybrid of plasmid and phage. Can carry even larger DNA inserts

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