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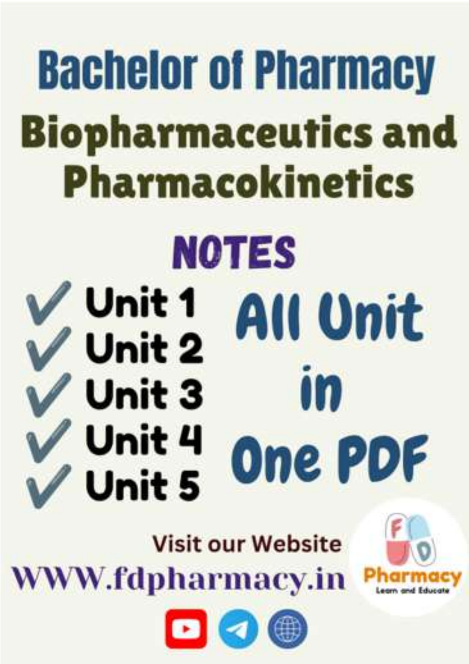
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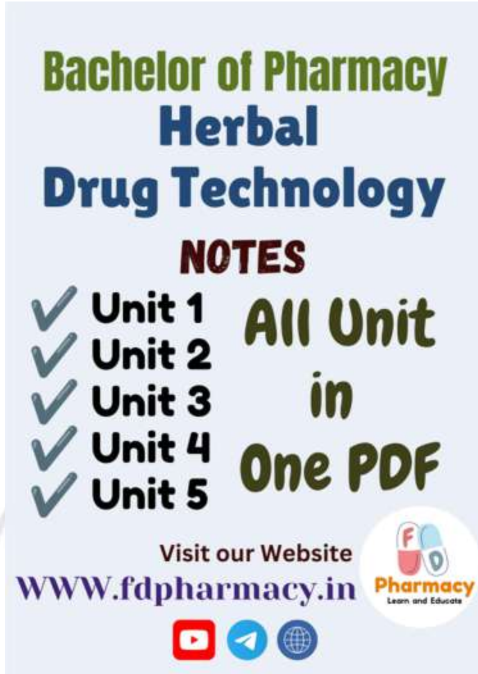
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PHARMACEUTICAL BIOTECHNOLOGY

UNIT 5

TOPIC :

- c) Study of the production of- penicillins, citric acid, Vitamin B12, Glutamic acid, Griseofulvin,

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Production of Penicillin by Fermentation

- Penicillin is the first antibiotic discovered from fungi. It was discovered by Alexander Fleming in 1928 from the mold *Penicillium notatum*.
- Penicillin is a β-lactam antibiotic widely used for the treatment of bacterial infections caused by Gram-positive bacteria.
- Commercial production of penicillin is carried out by submerged aerobic fermentation using high-yield strains of *Penicillium chrysogenum*.

Definition

- Penicillin is a secondary metabolite produced by species of *Penicillium* during fermentation and is used as an antibacterial agent.

Microorganism Used

Main Production Organism

- *Penicillium chrysogenum* (high-yield strain)

Earlier Organism

- *Penicillium notatum*

Types of Penicillin Produced

1. Penicillin G (Benzyl Penicillin)
2. Penicillin V (Phenoxymethyl Penicillin)
3. Ampicillin
4. Amoxicillin

Raw Materials Required

Carbon Sources

- Lactose, Glucose, Molasses

Nitrogen Sources

- Corn steep liquor
- Ammonium sulfate
- Soybean meal

Mineral Sources

- Phosphates, Magnesium salts
- Potassium salts

Precursors

- Phenylacetic acid → Penicillin G
- Phenoxyacetic acid → Penicillin V

Production of Penicillin

Principle

- Penicillin is produced as a secondary metabolite by *Penicillium chrysogenum* under controlled fermentation conditions.

Steps Involved in Production

1. Preparation of Culture Medium

- A nutrient-rich medium containing carbon, nitrogen, minerals, and precursor compounds is prepared.
- The medium should support fungal growth and penicillin production.

2. Sterilization

- Fermenter and medium are sterilized by steam.
- All equipment is maintained under aseptic conditions.
- This prevents contamination.

3. Inoculation

- Pure culture of *Penicillium chrysogenum* is introduced into the fermenter.
- The inoculum is prepared separately in seed tanks.
- Proper inoculation ensures maximum antibiotic production.

4. Fermentation

Penicillin is produced by submerged aerobic fermentation.

Conditions Maintained

Parameter	Value
Temperature	24–27°C
pH	6.0–6.5
Aeration	Continuous
Agitation	Continuous
Duration	5–7 days

Process

- Fungal spores germinate and form mycelia.
- Active growth occurs initially.
- During the stationary phase, penicillin production begins.
- Adequate oxygen supply is necessary for high yield.

5. Recovery of Penicillin

After fermentation:

- Mycelium is removed by filtration.
- Filtrate contains dissolved penicillin.
- Penicillin is extracted using organic solvents.

6. Extraction

- The broth is acidified to pH 2–2.5.
- Penicillin is extracted into solvents such as amyl acetate or butyl acetate.
- The antibiotic is then transferred back into an aqueous solution.

7. Purification

- Impurities are removed.
- Crystallization is carried out.
- Pure penicillin salts are obtained.

Examples:

- Sodium Penicillin
- Potassium Penicillin

8. Packaging

The purified product is formulated as:

- Injection
- Capsules
- Tablets
- Oral suspensions

Flow Chart of Penicillin Production

Preparation of Medium
↓
Sterilization
↓
Inoculation with *Penicillium chrysogenum*
↓
Submerged Aerobic Fermentation
↓
Filtration
↓
Solvent Extraction
↓
Purification
↓
Crystallization
↓
Penicillin Product

Applications of Penicillin

Medical Uses

- Streptococcal infections
- Pneumonia
- Tonsillitis
- Syphilis
- Skin infections
- Respiratory tract infections

Pharmaceutical Uses

- Antibiotic formulations
- Injectable preparations
- Oral dosage forms

Production of Citric Acid by Fermentation

- Citric acid is a weak organic acid naturally present in citrus fruits such as lemon and orange. It is one of the most important industrial organic acids and is widely used in pharmaceutical, food, and cosmetic industries.
- Commercially, citric acid is produced mainly by fermentation using Aspergillus niger.

Definition

- Citric acid is a tricarboxylic acid produced by microbial fermentation and used as an acidifying, flavoring, buffering, and chelating agent.

Chemical Formula

$C_6H_8O_7$

Microorganisms Used

Main Organism

- *Aspergillus niger* (most widely used)

Other Organisms

- *Candida lipolytica*
- *Yarrowia lipolytica*
- *Aspergillus wentii*

Raw Materials Required

Carbon Sources

- Molasses
- Sucrose
- Glucose
- Starch hydrolysates

Nitrogen Sources

- Ammonium salts
- Urea

Minerals

- Phosphate salts
- Magnesium salts

Principle

- Under controlled conditions, *Aspergillus niger* converts sugars into citric acid. Limitation of certain nutrients and maintenance of acidic pH favor high citric acid production.

Production Process

1. Preparation of Culture Medium

- A suitable nutrient medium containing sugar, nitrogen, and minerals is prepared.

Requirements

- High sugar concentration
- Low nitrogen content
- Low metal ion concentration

These conditions promote citric acid accumulation.

2. Sterilization

- Fermenter and medium are sterilized by steam.
- Sterile conditions prevent contamination.
- All pipelines and equipment are sterilized.

3. Inoculation

- Pure spores of *Aspergillus niger* are introduced into the fermenter.
- Seed culture is prepared separately.
- Proper inoculum size is maintained.

4. Fermentation

Citric acid is produced by submerged aerobic fermentation.

Conditions Maintained

Parameter	Value
Temperature	28–30°C
pH	2.0–3.5
Aeration	Continuous
Agitation	Continuous
Duration	5–10 Days

Process

- Fungus grows actively in the medium.
- Sugars are metabolized.
- Citric acid accumulates in the fermentation broth.
- Proper oxygen supply is essential for maximum yield.

5. Recovery of Citric Acid

After fermentation:

- Mycelium is removed by filtration.
- Clear filtrate contains citric acid.
- Further purification is carried out.

6. Precipitation

- Calcium hydroxide [$Ca(OH)_2$] is added.
- Calcium citrate precipitate is formed.

Reaction

Citric Acid + Calcium Hydroxide
↓
Calcium Citrate

7. Acidification

- Calcium citrate is treated with sulfuric acid.
- Citric acid is released.
- Calcium sulfate (gypsum) is formed as a by-product.

8. Purification

- Filtration removes impurities.
- Activated charcoal may be used for decolorization.
- Concentration and crystallization are performed.

9. Crystallization and Drying

- Pure citric acid crystals are obtained.
- Crystals are dried and packed for industrial use.

Flow Chart of Citric Acid Production

Preparation of Medium
↓
Sterilization
↓
Inoculation with *Aspergillus niger*
↓
Aerobic Fermentation
↓
Filtration
↓
Calcium Citrate Formation
↓
Treatment with Sulfuric Acid
↓
Purification
↓
Crystallization
↓
Citric Acid

Applications of Citric Acid

Pharmaceutical Uses

- Flavoring agent
- Buffering agent
- Effervescent tablets
- Anticoagulant in blood storage
- pH adjustment

Food Industry

- Soft drinks
- Fruit juices
- Candies
- Preservatives

Cosmetic Industry

- Skin care products
- Shampoos

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Vitamin B12 (Cobalamin) – Production by Fermentation

- Vitamin B12, also known as Cobalamin, is a water-soluble vitamin essential for normal growth, red blood cell formation, DNA synthesis, and proper functioning of the nervous system.
- Vitamin B12 is unique among vitamins because it contains cobalt in its chemical structure. It is mainly synthesized by microorganisms and is commercially produced through microbial fermentation.

Sources of Vitamin B12

Natural Sources

- Liver
- Meat
- Fish
- Poultry
- Eggs
- Milk and dairy products

Industrial Source

- Microbial fermentation

Functions of Vitamin B12

1. Helps in the formation of red blood cells.
2. Essential for DNA synthesis.
3. Maintains healthy nerve cells.
4. Prevents megaloblastic anemia.
5. Supports normal metabolism.
6. Required for growth and development.
7. Helps in energy production.

Production of Vitamin B12

Principle

- Vitamin B12 is produced commercially by fermentation using selected microorganisms. The microorganisms synthesize Vitamin B12 during their growth under controlled conditions.

Microorganisms Used

Bacteria

- **Propionibacterium shermanii**
- **Pseudomonas denitrificans**
- Bacillus species
- Streptomyces species

Among these, **Pseudomonas denitrificans** is widely used for large-scale industrial production due to its high yield.

Fermentation Process

1. Preparation of Culture Medium

A suitable nutrient medium is prepared containing:

Carbon Sources

- Glucose
- Molasses
- Sucrose

Nitrogen Sources

- Corn steep liquor
- Soybean meal
- Ammonium salts

Minerals

- Phosphates
- Magnesium salts
- Iron salts

Cobalt Source

- Cobalt chloride
- Cobalt sulfate

Importance: Cobalt is an essential component of Vitamin B12 and must be present in the medium.

2. Sterilization

- Fermenter, pipelines, and culture medium are sterilized using steam.
- Sterilization prevents contamination by unwanted microorganisms.
- Aseptic conditions are maintained throughout the process.

3. Inoculation

- Pure culture of the selected microorganism is introduced into the sterilized fermenter.
- Inoculum is prepared in seed tanks before transfer.
- Proper inoculation ensures maximum Vitamin B12 production.

4. Fermentation

Conditions Maintained

Parameter	Range
Temperature	28–32°C
pH	6.5–7.5
Aeration	Continuous
Agitation	Continuous
Duration	3–7 days

Process

- Microorganisms grow and multiply in the medium.
- Vitamin B12 is synthesized as a metabolic product.
- Aeration and agitation ensure adequate oxygen supply and nutrient distribution.

5. Recovery of Vitamin B12

After fermentation:

1. Fermentation broth is collected.
2. Cells are separated by filtration or centrifugation.
3. Vitamin B12 is extracted from microbial cells.
4. Heat treatment may be used to release intracellular Vitamin B12.

6. Purification

- Crude extract is purified using adsorption and chromatography techniques.
- Impurities are removed.
- Concentrated Vitamin B12 solution is obtained.

7. Conversion into Stable Forms

Purified Vitamin B12 is converted into pharmaceutical forms such as:

- Cyanocobalamin
- Hydroxocobalamin
- Methylcobalamin

These forms possess better stability and therapeutic value.

Flow Chart of Vitamin B12 Production

Culture Medium Preparation
↓
Sterilization
↓
Inoculation with *Pseudomonas denitrificans* / *Propionibacterium shermanii*
↓
Fermentation (3–7 Days)
↓
Cell Separation
↓
Extraction of Vitamin B12
↓
Purification
↓
Concentration
↓
Formation of Cyanocobalamin/Methylcobalamin
↓
Packaging

Applications of Vitamin B12

Pharmaceutical Uses

- Treatment of Vitamin B12 deficiency
- Treatment of pernicious anemia
- Management of megaloblastic anemia
- Treatment of neuropathy and nerve disorders
- Nutritional supplements

Medical Uses

- Prevention of anemia
- Maintenance of nervous system health
- Support for growth and development
- Improvement of metabolic functions

Production of Glutamic Acid by Fermentation

- Glutamic acid is a non-essential amino acid that plays an important role in protein synthesis and cellular metabolism. It is widely produced by microbial fermentation and is used in the food, pharmaceutical, and biotechnology industries.
- The sodium salt of glutamic acid, known as Monosodium Glutamate (MSG), is extensively used as a flavor enhancer.

Definition

- Glutamic acid is an amino acid produced by microbial fermentation from carbohydrates and is used in the manufacture of MSG and pharmaceutical products.

Chemical Formula

$C_5H_9NO_4$

Microorganisms Used

Main Organisms

- *Corynebacterium glutamicum*
- *Brevibacterium flavum*
- *Brevibacterium lactofermentum*

Among these, Corynebacterium glutamicum is the most commonly used industrial microorganism.

Raw Materials Required

Carbon Sources

- Molasses
- Glucose
- Sucrose
- Starch hydrolysates

Nitrogen Sources

- Ammonium sulfate
- Ammonium nitrate
- Urea

Mineral Sources

- Phosphates
- Magnesium salts
- Potassium salts

Growth Factors

- Biotin (controlled amount)

Principle

- Under controlled fermentation conditions, microorganisms convert sugars into glutamic acid through metabolic pathways. The produced glutamic acid is secreted into the fermentation broth and later recovered and purified.

Production Process

1. Preparation of Culture Medium

A suitable nutrient medium containing:

- Carbon source
- Nitrogen source
- Minerals
- Growth factors

is prepared to support microbial growth and glutamic acid production.

2. Sterilization

- Fermenter and medium are sterilized using steam.
- Sterile conditions are maintained throughout the process.
- Contamination is prevented.

3. Inoculation

- Pure culture of *Corynebacterium glutamicum* is introduced into the fermenter.
- Seed culture is prepared separately before transfer.
- Proper inoculum size is maintained.

4. Fermentation

Glutamic acid is produced by submerged aerobic fermentation.

Conditions Maintained

Parameter	Value
Temperature	30–33°C
pH	7.0–8.0
Aeration	Continuous
Agitation	Continuous
Duration	2–4 Days

Process

- Microorganisms utilize sugars as an energy source.
- Cell growth occurs rapidly.
- Metabolic activities lead to glutamic acid formation.
- Glutamic acid accumulates in the fermentation broth.

5. Recovery of Glutamic Acid

After fermentation:

- Cells are removed by filtration or centrifugation.
- The filtrate contains dissolved glutamic acid.

6. Isolation

- The broth is acidified to about pH 3.2.
- Glutamic acid crystallizes out of solution.
- Crystals are separated by filtration.

7. Purification

- Crystals are washed to remove impurities.
- Recrystallization is carried out.
- Highly pure glutamic acid is obtained.

8. Drying and Packaging

- Purified crystals are dried.
- Product is packed and stored under suitable conditions.

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Flow Chart of Glutamic Acid Production

Preparation of Culture Medium

↓

Sterilization

↓

Inoculation with *Corynebacterium glutamicum*

↓

Submerged Aerobic Fermentation

↓

Filtration/Centrifugation

↓

Acidification

↓

Crystallization

↓

Purification

↓

Drying

↓

Glutamic Acid

Applications of Glutamic Acid

Pharmaceutical Uses

- Amino acid preparations
- Nutritional supplements
- Parenteral nutrition products

Food Industry

- Production of Monosodium Glutamate (MSG)
- Flavor enhancer in foods

Biotechnology

- Fermentation products
- Culture media supplements

Production of Griseofulvin by Fermentation

- Griseofulvin is an antifungal antibiotic produced by certain species of *Penicillium*. It is used for the treatment of fungal infections of the skin, hair, and nails caused by dermatophytes.
- Griseofulvin acts by inhibiting fungal cell division and preventing the growth of fungi. Commercial production is carried out by microbial fermentation.

Definition

- Griseofulvin is an antifungal secondary metabolite produced by *Penicillium* species through fermentation and used in the treatment of dermatophytic fungal infections.

Chemical Formula

$C_{17}H_{17}ClO_6$

Microorganism Used

Main Producing Organism

- *Penicillium griseofulvum*

Other Species

- *Penicillium patulum*
- *Penicillium nigricans*

Among these, Penicillium griseofulvum is the most commonly used industrial strain.

Principle

- Under suitable fermentation conditions, *Penicillium griseofulvum* produces griseofulvin as a secondary metabolite. The antibiotic accumulates in the fermentation broth and is subsequently extracted and purified.

Raw Materials Required

Carbon Sources

- Glucose
- Sucrose
- Molasses

Nitrogen Sources

- Corn steep liquor
- Peptone

Mineral Sources

- Magnesium salts
- Phosphates
- Potassium salts

Production Process

1. Preparation of Culture Medium

A nutrient-rich medium containing carbon, nitrogen, and mineral sources is prepared.

The medium should support fungal growth and griseofulvin production.

2. Sterilization

- Fermenter and culture medium are sterilized by steam.
- Aseptic conditions are maintained.
- Contamination by unwanted microorganisms is prevented.

3. Inoculation

- Pure spores of *Penicillium griseofulvum* are inoculated into the fermenter.
- Seed culture is prepared separately before inoculation.

4. Fermentation

Griseofulvin is produced by submerged aerobic fermentation.

Conditions Maintained

Parameter	Value
Temperature	25–28°C
pH	5.5–6.5
Aeration	Continuous
Agitation	Continuous
Duration	5–7 Days

Process

- Fungal growth occurs initially.
- During the stationary phase, griseofulvin is synthesized.
- Adequate oxygen supply is essential for maximum production.

5. Recovery of Griseofulvin

After completion of fermentation:

- Fungal mycelium is separated by filtration.
- The antibiotic is present in the biomass and broth.
- Extraction is carried out using suitable organic solvents.

6. Extraction

- Solvents such as acetone or chloroform are used.
- Griseofulvin is dissolved and separated from impurities.
- Crude extract is obtained.

7. Purification

- The crude product is purified by recrystallization.
- Impurities are removed.
- Highly pure griseofulvin crystals are obtained.

8. Drying and Packaging

- Purified crystals are dried.
- Product is packed under suitable conditions.
- Stored in moisture-free containers.

Flow Chart of Griseofulvin Production

Preparation of Culture Medium
↓
Sterilization
↓
Inoculation with *Penicillium griseofulvum*
↓
Submerged Aerobic Fermentation
↓
Filtration
↓
Solvent Extraction
↓
Purification
↓
Crystallization
↓
Drying
↓
Griseofulvin

Mechanism of Action

Griseofulvin:

- Binds to fungal microtubules.
- Inhibits mitosis (cell division).
- Prevents fungal growth and reproduction.
- Acts mainly against dermatophytes.

Applications of Griseofulvin

Medical Uses

Fungal Infections of:

- Skin (Tinea corporis)
- Scalp (Tinea capitis)
- Feet (Athlete's foot)
- Groin (Tinea cruris)
- Nails (Onychomycosis)

Pharmaceutical Uses

- Tablets
- Capsules
- Oral suspensions

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