Study of the Production of Penicillin

Introduction

- **Penicillin** is the **first antibiotic** discovered by **Alexander Fleming in 1928** from the fungus *Penicillium notatum* (later *P. chrysogenum* was used for industry due to higher yield).
- It is a **β-lactam antibiotic**, active mainly against **Gram-positive bacteria**.
- It works by **inhibiting cell wall synthesis** (peptidoglycan cross-linking).
- It has been produced commercially since **World War II** and remains one of the most studied antibiotics in **industrial fermentation**.

Microorganism Used

- Early: *Penicillium notatum*.
- Now: **Penicillium chrysogenum** – genetically improved strains for **high yield**.
- Improvement methods: **mutation, selection, recombinant DNA technology**.

Raw Materials

- **Carbon source**: lactose, glucose, sucrose, molasses.
- **Nitrogen source**: corn steep liquor, peptone, yeast extract, ammonium salts.
- **Precursors**: side-chain precursors such as **phenylacetic acid or phenoxyacetic acid** for production of specific penicillins (Penicillin G, Penicillin V).
- **Minerals**: magnesium, potassium, phosphates, sulphates.

Fermentation Process for Penicillin Production

Penicillin production is carried out using **submerged aerobic fed-batch fermentation**.

Stages

1. **Inoculum Preparation**
    - Pure culture of *P. chrysogenum* grown on agar slants.
    - Spores transferred to seed flasks → seed tanks.
    - Stepwise increase in volume until production fermenter is inoculated.
2. **Fermentation**
    - **Type:** Submerged aerobic fed-batch.
    - **Duration:** 120–200 hours (5–7 days).
    - **Conditions:**

        - Temperature: 25–27°C.
        - pH: 6.0–6.5 (maintained by buffers and automatic control).
        - Aeration: continuous sparging of sterile air.
        - Agitation: impellers for mixing and oxygen distribution.
    - **Fed-batch:** Carbon (lactose/glucose) added slowly to prevent catabolite repression.
3. **Penicillin Biosynthesis**
    - Biosynthetic pathway involves three amino acids:
        - **L-cysteine + L-valine + L-α-aminoadipic acid** → tripeptide precursor (ACV).
        - Cyclization → **Isopenicillin N**.
        - Side-chain addition (precursors) → **Penicillin G (benzylpenicillin)** or **Penicillin V (phenoxymethylpenicillin)**.

Harvesting and Recovery

1. **Broth Filtration** – to remove fungal mycelium.
2. **Extraction** – penicillin extracted into organic solvent (butyl acetate/amyl acetate) at acidic pH.
3. **Back extraction** – into aqueous solution at neutral pH.
4. **Purification** – crystallization of penicillin salts (e.g., sodium or potassium penicillin G).
5. **Formulation** – converted into injectable, tablet, or capsule forms.

Downstream Processing (Summary Steps)

1. Cell removal (filtration).
2. Solvent extraction.
3. Back extraction.
4. Crystallization.
5. Drying and packaging.

Production of Penicillin

(You can draw this flowchart in exam)

P. chrysogenum strain

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Inoculum preparation

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Seed culture → Fermenter (fed-batch, aerobic)

| (pH 6-6.5, 25-27°C, aeration, agitation)

Penicillin produced in broth

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Broth filtration → Extraction (organic solvent)

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Back extraction → Purification → Crystallization

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Penicillin G / V (final product)

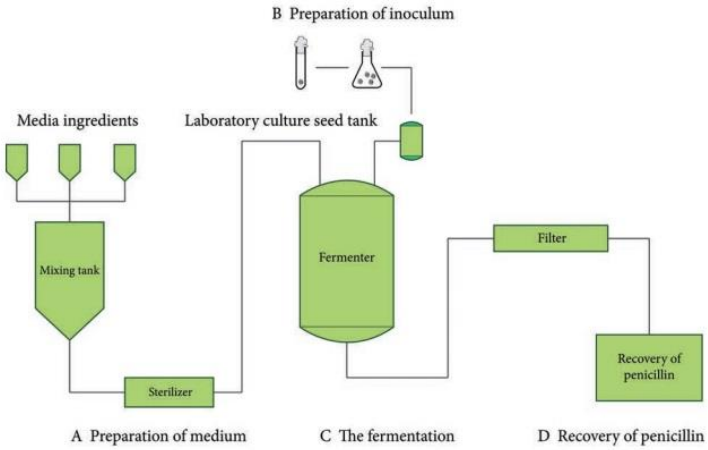

Figure 6.9: Production of Penicillin

Types of Penicillins Produced

- **Natural Penicillins:** Penicillin G, Penicillin V.
- **Semi-synthetic Penicillins:** Ampicillin, Amoxicillin, Methicillin (produced by modifying natural penicillin nucleus).

Advantages of Fed-Batch Process

- Prevents repression by glucose.
- Enhances yield.
- Allows addition of precursors at correct stage for specific penicillins.

Applications of Penicillin

- First-line antibiotic against Gram-positive bacteria (*Streptococcus, Staphylococcus*).

- Used in treatment of pneumonia, syphilis, meningitis, and skin infections.
- Basis for development of semi-synthetic derivatives with broader spectrum and resistance to β-lactamase.

Conclusion

- Penicillin production is a classic example of **industrial microbiology and biotechnology**.
- Modern improvements using **strain engineering and recombinant DNA technology** have significantly increased yields.
- The process remains a **model system for secondary metabolite production** in submerged fermentation.