BP403T · UNIT III

BP403T Pharmaceutical Biotechnology

Unit III — Microbial Biotransformation & Fermentation Technology

B.Pharm · Semester IV · Pharmaceutical Biotechnology

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Chapter 5 — Microbial Biotransformation

5.1 Learning Objectives

1. Define biotransformation and biocatalysis.
2. Distinguish biotransformation from fermentation.
3. Describe types of biotransformation reactions with examples.
4. Explain advantages and limitations of microbial biotransformation.
5. List pharmaceutical applications of biotransformation.

5.2 Definitions

- **Biotransformation:** Using a microbe's (or its enzyme's) chemistry to convert one compound into another, more useful compound — typically a single, specific chemical step (e.g., adding one oxygen atom to a drug molecule).
- **Biocatalysis:** The broader term — using biological catalysts (enzymes or whole cells) to perform chemical reactions.
- **Substrate:** The starting compound fed to the microbe. **Product:** The converted compound collected.
- **Fermentation (contrast):** The microbe grows AND makes the product from simple nutrients (multi-step, growth-linked). Biotransformation usually uses resting (non-growing) cells for one precise step.

5.3 Terminology

- **Regiospecificity:** The enzyme acts at one exact position on the molecule (like a surgeon operating at one precise spot).
- **Stereospecificity:** The enzyme makes only one mirror-image form (one enantiomer) — crucial because drug enantiomers can differ in effect and safety.
- **Resting cells:** Cells that are alive but not dividing, used just for their enzymes.
- **Cofactor regeneration:** Many enzymes need helper molecules (e.g., NADH); whole cells regenerate these naturally — a big advantage of whole-cell biotransformation over isolated enzymes.

5.4 Explanation

The core idea: Chemists can find it very hard to modify just one corner of a complex molecule without disturbing the rest. Microbial enzymes do it effortlessly and cleanly, in water, at room temperature. So industry feeds a cheap starting compound to a microbe and harvests a valuable, precisely-modified product.

Classic pharmaceutical examples:

1. **Steroid hydroxylation:** Adding an –OH group at exactly carbon-11 of a steroid to make cortisone-type drugs — microbes (*Rhizopus*, *Curvularia* species) do in one step what took chemists dozens of steps. This single biotransformation made cortisone affordable.
2. **Penicillin side-chain modification:** Enzymes (penicillin acylases) snip and swap side chains to create semi-synthetic penicillins like ampicillin and amoxicillin.
3. **Chiral drug synthesis:** Making single-enantiomer drugs (e.g., certain statin or antihypertensive intermediates) using stereospecific microbial enzymes.

Types of biotransformation reactions: oxidation (hydroxylation), reduction, hydrolysis (ester/amide splitting), isomerization, and condensation — each performed by specific microbial enzymes.

Advantages: extreme specificity (position + mirror image), mild conditions (room temperature, water — "green chemistry"), fewer steps, less waste. **Limitations:** substrate may poison the microbe, product may inhibit the enzyme, dilute product streams need extraction, and not every reaction has a known microbial enzyme.

Analogy: Chemical synthesis is like renovating a house with a sledgehammer — powerful but messy. Biotransformation is like hiring a specialist surgeon who operates through a keyhole at exactly the right spot.

Key takeaway: Microbial biotransformation = one precise chemical conversion by microbial enzymes — prized for making steroids, semi-synthetic antibiotics and single-enantiomer drugs cleanly and specifically.

5.5 Classifications

- By catalyst: whole-cell (growing | resting) vs isolated enzyme biotransformation.
- By reaction: oxidation | reduction | hydrolysis | isomerization | C–C bond formation.

5.6 Mechanisms

Steroid 11α-hydroxylation (conceptual): Substrate (e.g., progesterone) enters the fungal cell → cytochrome P450 enzyme complex inserts one oxygen atom at carbon-11 using molecular oxygen and NADPH → 11α-hydroxy product exits. The enzyme's active-site shape allows only the C-11 position to reach the reactive oxygen.

5.7 Diagrams (describe for drawing)

- Draw a steroid skeleton with an arrow pointing at C-11 labelled "microbe adds –OH here only".
- Draw whole-cell vs isolated-enzyme: cell as a sac containing enzyme + cofactor recycling loop vs free enzyme needing added cofactor.

5.8 Flowcharts

Biotransformation process: Select microbe/enzyme → Prepare substrate feed → Bioreactor conversion (monitor) → Separate cells → Extract & purify product → QC.

5.9 Infographic Concept Box

🔬 **ONE-PRECISE-STEP CHEMISTRY:** Biotransformation = microbe performs a single, exact chemical edit (steroid C-11 hydroxylation made cortisone affordable; enzyme swapping builds ampicillin). Green, specific, mild — but watch substrate toxicity and dilute streams.

5.10 Tables

Feature	Fermentation	Biotransformation
Cells	Growing, making product from nutrients	Often resting, converting a fed substrate
Steps	Many (whole pathway)	Usually one specific reaction
Product	Built from scratch	Modified from a precursor

Reaction	Example product
Hydroxylation	Corticosteroid intermediates
Deacylation/reacylation	Ampicillin, amoxicillin
Stereospecific reduction	Chiral drug intermediates

5.11 Examples

- *Rhizopus* mould converting progesterone to 11α-hydroxyprogesterone for corticosteroid manufacture.
- Penicillin acylase producing 6-APA, the core for semi-synthetic penicillins.

5.12 Analogies

- Regiospecificity: a key that fits only one lock among a hundred similar locks.

5.13 Pharmacy Relevance

- Many steroid and antibiotic medicines depend on biotransformation steps; pharmacists should know why these processes make drugs affordable and why single-enantiomer purity matters for safety.

5.14 India Context

- Indian bulk-drug manufacturers use fermentation and biotransformation routes for steroids, statins and semi-synthetic antibiotics, supporting affordable medicine production.

5.15 Key Points

1. Biotransformation = microbial conversion of a substrate into a product, usually one specific step.
2. Whole cells regenerate cofactors; isolated enzymes need them supplied.
3. Prized for regiospecificity and stereospecificity under mild, green conditions.
4. Key uses: steroid hydroxylation (cortisone), semi-synthetic penicillins, chiral intermediates.
5. Limitations: toxicity, inhibition, dilute streams.

5.16 Common Confusions

- **Biotransformation vs fermentation:** Fermentation = microbe grows and builds product from simple nutrients; biotransformation = (often resting) cells convert a fed precursor in one step.
- **Biotransformation vs metabolism:** Metabolism is the cell's own chemistry for living; biotransformation is chemistry we exploit for manufacturing.

5.17 Common Mistakes

- Calling every microbial process "fermentation" — biotransformation is the precise term for single-step conversions.
- Forgetting cofactor regeneration as the reason whole cells are often preferred.

5.18 Mnemonics

- Advantages: **"S-M-G"** — Specificity, Mild conditions, Green ("Super Microbe Genius").

5.19 Exam Summary

Microbial biotransformation uses microbial enzymes or whole cells to perform single, highly specific chemical conversions (oxidation, reduction, hydrolysis) under mild conditions. It is essential for steroid manufacture (C-11 hydroxylation), semi-synthetic penicillins and chiral drug intermediates. Whole cells offer cofactor regeneration; limitations include substrate toxicity and dilute products.

5.20 One-Minute Revision

⚡ Biotransformation = one exact chemical edit by microbes. Steroid C-11 hydroxylation, penicillin side-chain swaps, chiral synthesis. Specific + mild + green. Whole cells recycle cofactors.

5.21 Exam Questions

Short answer:

1. Define biotransformation and biocatalysis.
2. Distinguish biotransformation from fermentation.
3. Why are whole cells preferred over isolated enzymes in some conversions?
4. Give two pharmaceutical applications.

Long answer:

5. Describe microbial biotransformation with types, advantages, limitations and pharmaceutical applications.

MCQs:

6. Steroid 11-hydroxylation is an example of: (a) fermentation (b) biotransformation (c) PCR (d) cloning — Answer: (b)
7. 6-APA is the core for: (a) cephalosporins (b) semi-synthetic penicillins (c) statins (d) steroids — Answer: (b)

Viva:

8. Why is stereospecificity important in drug manufacture? (Enantiomers can differ in action/safety; single-enantiomer drugs are safer.)

Application-based:

9. A company wants to add –OH at one specific carbon of a steroid cheaply. Chemical or microbial route — justify. (Microbial: regiospecific, one step, mild, green.)

5.22 References

- Vogel, H. C. and Todaro, C. M. Fermentation and Biochemical Engineering Handbook. William Andrew.
- Doran, P. M. Bioprocess Engineering Principles. Academic Press.
- Glick, B. R., Pasternak, J. J. and Patten, C. L. Molecular Biotechnology: Principles and Applications of Recombinant DNA. ASM Press.

Chapter 6 — Fermentation Technology, Industrial Products and Blood Products

6.1 Learning Objectives

1. Define fermentation and describe its principles.
2. Describe a fermenter and its key parts.
3. Explain process control parameters in fermentation.
4. Outline industrial production of alcohol, penicillin, citric acid and vitamin B12.
5. Describe collection, processing and storage of blood products.

6.2 Definitions

- **Fermentation:** Growth of microorganisms under controlled conditions to make a desired product — the cells both multiply and manufacture (unlike single-step biotransformation).
- **Fermenter (bioreactor):** The tank (vessel) where fermentation happens, with controls for air, stirring, temperature, pH and sterility.
- **Upstream processing:** Everything before fermentation — strain selection, medium preparation, sterilization, inoculum build-up.
- **Downstream processing:** Everything after — separating cells, purifying and finishing the product (often the costliest part).
- **Blood products:** Therapeutic preparations from human blood — whole blood, packed red cells, platelets, plasma, and fractions like albumin, clotting factors and immunoglobulins.

6.3 Terminology

- **Batch / fed-batch / continuous:** Batch = fill, grow, harvest, clean; fed-batch = nutrients added during the run (avoids overload); continuous = fresh medium in, product out, nonstop.
- **Inoculum:** The starter culture added to the fermenter (like curd starter added to milk).
- **Sterilization-in-place (SIP):** Steaming the whole fermenter sterile without opening it.
- **Primary vs secondary metabolites:** Primary = made during active growth (e.g., alcohol, citric acid); secondary = made after growth slows (e.g., penicillin antibiotics).
- **Aseptic:** Free from contaminating microbes — the golden rule of fermentation.

6.4 Explanation

Fermentation principles: Give the right microbe the right food, air (or no air), temperature and pH, keep everything sterile, and it will convert cheap raw materials into valuable products. The fermenter is a carefully controlled artificial home.

Parts of a fermenter (know these): vessel (stainless steel tank), agitator/impeller (stirring), sparger (air bubbles in), baffles (improve mixing), jackets/coils (heating–cooling), pH/temperature/dissolved-oxygen sensors, sampling port, and foam control.

Process control — the five dials: temperature, pH, dissolved oxygen (aeration + agitation), foam, and nutrient feeding. Lose control of any one and the batch can fail or get contaminated.

Industrial products (one paragraph each):

1. **Alcohol (ethanol):** Yeast (*Saccharomyces cerevisiae*) ferments sugars (molasses, grains) without oxygen → ethanol + CO_2. Used in tinctures, syrups, disinfectants and as a solvent. Distillation purifies it.
2. **Penicillins:** The mould *Penicillium chrysogenum* (improved strains) makes penicillin in aerated fed-batch fermenters; phenylacetic acid is fed as side-chain precursor. Penicillin is a secondary metabolite — produced after growth phase. Downstream: filtration, solvent extraction, crystallization.
3. **Citric acid:** The mould *Aspergillus niger* converts molasses/sucrose to citric acid under specific low-pH, high-sugar conditions. Used as an acidulant, antioxidant synergist and effervescent-tablet ingredient.
4. **Vitamin B12:** Produced by bacteria (*Propionibacterium*, *Pseudomonas* species) — the only vitamin made by fermentation at scale. Used in anaemia treatment and supplements.

Blood products — collection, processing, storage:

- **Collection:** Donated blood collected into bags with anticoagulant (e.g., CPDA), screened for infections (HIV, hepatitis B/C, syphilis, malaria as per national norms).
- **Processing:** Centrifugation separates whole blood into components — packed red blood cells, platelet concentrates, fresh frozen plasma; plasma can be fractionated into albumin, immunoglobulins and clotting factors.
- **Storage:** Red cells at 2–6 °C, platelets at 20–24 °C with agitation, plasma frozen at –30 °C or below. Every unit is labelled, tracked and expires on schedule — "vein to vein" traceability.

Analogy: A fermenter is like a pressure cooker with dials — same vessel, but you control heat, stirring and air precisely, and the "food" inside is microbes cooking up medicine.

Key takeaway: Fermentation = controlled microbial manufacturing with upstream, fermentation and downstream stages. Its products include alcohol, penicillin, citric acid and B12. Blood products follow a parallel discipline: screened collection, component separation, and strict cold storage.

6.5 Classifications

- Fermentation modes: batch | fed-batch | continuous; submerged (liquid) | solid-state.
- Metabolites: primary (growth-associated) | secondary (post-growth).
- Blood components: cellular (RBC, platelets, WBC-poor) | plasma | plasma fractions (albumin, immunoglobulins, clotting factors).

6.6 Mechanisms

Penicillin biosynthesis (conceptual): Amino acids (cysteine + valine + α-aminoadipic acid) join into a tripeptide → cyclization forms the β-lactam + thiazolidine rings (isopenicillin N) → side-chain exchange with fed phenylacetic acid → penicillin G.

6.7 Diagrams (describe for drawing)

- Draw a fermenter: label vessel, impeller, sparger, baffles, jacket, sensors, inlet/outlet.
- Draw blood bag → centrifuge → three layers (RBC bottom, buffy coat, plasma top) → component bags.

6.8 Flowcharts

Fermentation pipeline: Strain → Inoculum → Medium prep & sterilization → Fermentation (controlled) → Harvest → Downstream (separate → purify → finish) → QC & pack. **Blood:** Donation & screening → Centrifugation → Components → Labelled storage → Transfusion.

6.9 Infographic Concept Box

🏭 **MICROBE FACTORIES:** Fermenter = steel home with 5 dials (temperature, pH, oxygen, foam, feed). Products: alcohol (yeast), penicillin (mould, secondary metabolite), citric acid (*A. niger*), B12 (bacteria). Blood: collect → screen → spin → separate → store cold (RBC 2–6 °C, platelets ~22 °C, plasma frozen).

6.10 Tables

Product	Organism	Type
Ethanol	*Saccharomyces cerevisiae*	Primary metabolite
Penicillin	*Penicillium chrysogenum*	Secondary metabolite
Citric acid	*Aspergillus niger*	Primary metabolite
Vitamin B12	*Propionibacterium* spp.	Primary metabolite

Blood component	Storage
Packed red cells	2–6 °C
Platelets	20–24 °C with agitation
Fresh frozen plasma	–30 °C or below

6.11 Examples

- Molasses from a sugar mill fermented to ethanol for tinctures and sanitizers.
- A blood bank in Lucknow separating one donation into red cells, platelets and plasma for three different patients.

6.12 Analogies

- Fed-batch feeding: feeding a baby small spoonfuls instead of the whole meal at once — prevents choking (substrate inhibition).

6.13 Pharmacy Relevance

- Pharmacists use fermentation products daily (alcohol in preparations, penicillin antibiotics, citric acid in effervescents, B12 injections) and manage blood-bank-adjacent products like albumin and immunoglobulins with strict storage rules.

6.14 India Context

- India has large fermentation capacity (antibiotics, vitamins, alcohol) and a vast voluntary blood-donation network with component-separation facilities; national guidelines govern donor screening and storage.

6.15 Key Points

1. Fermentation = controlled microbial growth for product manufacture; stages: upstream → fermentation → downstream.
2. Control the five dials: temperature, pH, oxygen, foam, feeding; sterility is non-negotiable.
3. Alcohol, citric acid, B12 = primary metabolites; penicillin = secondary metabolite (fed-batch, aerated).
4. Blood: screened collection → centrifugation into components → temperature-specific storage with traceability.

6.16 Common Confusions

- **Primary vs secondary metabolites:** Primary made during growth (alcohol); secondary made after growth slows (penicillin). Different timing, different control strategy.
- **Upstream vs downstream:** Upstream = before/inoculation; downstream = after/harvest purification.

6.17 Common Mistakes

- Saying penicillin is made without oxygen — *Penicillium* needs aeration; it is the yeast alcohol process that is anaerobic.
- Storing platelets in the fridge — platelets need ~22 °C with agitation; cold damages them.

6.18 Mnemonics

- Fermenter parts: **"V-A-S-B-J"** — Vessel, Agitator, Sparger, Baffles, Jacket ("Very Active Stirring Brings Joy").
- Blood storage: **"6-22-Frozen"** — RBC 2–6 °C, platelets ~22 °C, plasma frozen.

6.19 Exam Summary

Fermentation is controlled microbial manufacturing in fermenters (vessel, agitator, sparger, baffles, jacket, sensors) with tight control of temperature, pH, oxygen, foam and feeding, in batch, fed-batch or continuous modes. Industrial products include ethanol (yeast), penicillin (secondary metabolite of *P. chrysogenum*), citric acid (*A. niger*) and vitamin B12 (bacteria). Blood products are collected with anticoagulant, infection-screened, separated by centrifugation into components, and stored at component-specific temperatures with full traceability.

6.20 One-Minute Revision

⚡ Fermenter 5 dials: temp, pH, O_2, foam, feed. Alcohol/citric acid/B12 = primary; penicillin = secondary (needs air, fed-batch). Blood: screen → spin → separate → 6 °C / 22 °C / frozen.

6.21 Exam Questions

Short answer:

1. Define upstream and downstream processing.
2. List five parts of a fermenter.
3. Why is penicillin produced in fed-batch mode?
4. Name the storage temperatures of three blood components.

Long answer:

5. Describe the construction of a fermenter and the process control parameters.
6. Outline the industrial production of penicillin OR citric acid.
7. Describe collection, processing and storage of blood products.

MCQs:

8. Penicillin is a: (a) primary metabolite (b) secondary metabolite (c) enzyme (d) vitamin — Answer: (b)
9. Citric acid is produced by: (a) yeast (b) *A. niger* (c) *E. coli* (d) algae — Answer: (b)
10. Platelets are stored at: (a) 2–6 °C (b) 20–24 °C with agitation (c) –30 °C (d) room temp still — Answer: (b)

Viva:

11. Why must fermentation be aseptic? (Contaminants eat the nutrients, make toxins, ruin the batch.)

Application-based:

12. A blood bank's refrigerator fails overnight. Which components are at risk and what action? (RBCs and thawed plasma at risk; quarantine, check temperature logs, discard per protocol if out of range; platelets were at room temp anyway.)

6.22 References

- Doran, P. M. Bioprocess Engineering Principles. Academic Press.
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