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HIGHLIGHTED HANDOUT

BT614T

Industrial Biotechnology (Theory)

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BT614T

Industrial Biotechnology (Theory)

White Biotechnology: Theory — Study Handout

This handout covers BT614T Industrial Biotechnology (Theory) — 'white biotechnology': using microbes and enzymes to manufacture chemicals, fuels, food ingredients and materials at industrial scale. Units follow the production logic: the biology (strains, enzymes), the engineering (fermenters, kinetics), the processes (major product families), and the economics and safety around them.

Learn each product family as: organism/enzyme → substrate → process type → product → application. That four-column pattern answers most descriptive exam questions. 

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Unit 1: Introduction to Industrial Biotechnology

Lectures 1–4

Learning Objectives

- Define industrial (white) biotechnology and its scope
- Distinguish white, red, green and blue biotechnology
- Explain why bioprocesses can beat chemical synthesis
- List the major product sectors

The Colors of Biotechnology

White — industrial: microbes/enzymes making chemicals, fuels, materials.

Red — medical/pharmaceutical: antibiotics, vaccines, therapeutic proteins.

Green — agricultural: GM crops, biopesticides.

Blue — marine: products from marine organisms.

- This course is the **white** branch: factories where the workers are cells.

Why Biology Beats Chemistry (Sometimes)

- **Specificity:** enzymes catalyze one reaction with no side products — fewer purification steps.
- **Mild conditions:** ambient temperature and pressure vs high heat/pressure — energy savings.
- **Renewable feedstocks:** sugars, starch, agricultural waste instead of petroleum.
- **Chirality:** enzymes make single enantiomers — critical for pharmaceuticals.
- Limits: dilute product streams, slow rates vs chemical catalysis, sterility demands, and organisms' product inhibition.

Major Product Sectors

- Bulk organics: ethanol, organic acids (citric, lactic), amino acids.
- Enzymes: amylases, proteases, cellulases for food, detergent, textile industries.
- Biofuels: bioethanol, biodiesel, biogas.
- Biopolymers and biomaterials: PLA, PHA, xanthan gum.
- Food ingredients: vitamins, flavors, single-cell protein.

The big picture: feedstock (cheap, renewable) → biocatalyst (microbe/enzyme) → fermenter (controlled environment) → downstream processing (recovery) → product. Every unit of this course zooms into one box of this pipeline.

Unit Summary

- White biotechnology = industrial production by microbes/enzymes.

- Advantages: specificity, mild conditions, renewable feedstocks, chirality; limits: dilution, rate, sterility.
- Sectors: bulk chemicals, enzymes, biofuels, biopolymers, food ingredients.

Exam Pointers

- ★ Distinguish the four colors of biotechnology.
- ★ Give four advantages and three limitations of bioprocesses vs chemical synthesis.
- ★ Draw and label the bioprocess pipeline.

Unit 2: Microbial Strains: Isolation to Improvement

Lectures 5–8

Learning Objectives

- Describe isolation and screening of industrial strains
- Explain strain preservation methods
- Compare mutation, recombination and genetic engineering for improvement
- Define primary vs secondary metabolites

Finding Producer Strains

Isolation: samples from soil, water, extreme environments — anywhere microbes compete chemically. **Primary screening:** quick qualitative tests (e.g., clear zones on starch agar for amylase producers). **Secondary screening:** quantitative shake-flask trials measuring actual product yield under near-production conditions.

Preservation

- **Subculture:** simple but risks mutation/contamination over time.
- **Lyophilization (freeze-drying):** long-term stable dormancy.
- **Cryopreservation:** -80°C glycerol stocks or liquid nitrogen — the industry standard working cell bank.
- Maintain **master and working cell banks** so production always starts from the same verified generation.

Strain Improvement

Random mutagenesis — UV/chemical mutants screened for overproduction (classical, still powerful; e.g., penicillin yields rose 1000× this way).

Recombination — protoplast fusion, conjugation shuffling beneficial traits.

Genetic/metabolic engineering — targeted: amplify pathway genes, knock out competing branches, install heterologous pathways (e.g., *E. coli* making human insulin or artemisinin precursors).

Primary vs Secondary Metabolites

- **Primary:** essential for growth — amino acids, ethanol, organic acids; produced during exponential (trophophase) growth.
- **Secondary:** not essential — antibiotics, pigments, toxins; produced in stationary phase (idiophase) after growth slows. Fermentation strategy differs: feed for growth first, then trigger production.

Exam favorite: 'Why is penicillin harvested in stationary phase?' Because it is a secondary metabolite — the idiophase after nutrient limitation switches on its biosynthesis. Harvesting during log phase would give almost nothing.

Unit Summary

- Isolate → primary screen (qualitative) → secondary screen (quantitative) → preserve as cell banks.
- Improve via mutagenesis, recombination, or targeted metabolic engineering.
- Primary metabolites = growth phase; secondary = stationary (idiophase) — timing the harvest matters.

Exam Pointers

- ★ Outline the strain development pipeline from soil sample to cell bank.
- ★ Compare three preservation methods.
- ★ Distinguish primary and secondary metabolites with two examples each.

Unit 3: Enzymes as Industrial Catalysts

Lectures 9–12

Learning Objectives

- Explain enzyme kinetics: Michaelis–Menten
- Describe factors affecting enzyme activity
- Compare free vs immobilized enzymes
- List major industrial enzymes and uses

Michaelis–Menten Kinetics

$v = V_{\max} \cdot [S] / (K_m + [S])$. $V_{\max}$ = max rate (enzyme saturated); K_m = substrate concentration at half $V_{\max}$ — an inverse affinity measure (low K_m = tight binding).

- Lineweaver–Burk linearization: $1/v = (K_m/V_{\max})(1/[S]) + 1/V_{\max}$ — slope and intercepts give K_m , $V_{\max}$ from data.
- **Inhibition:** competitive (raises apparent K_m ; overcome by more substrate), non-competitive (lowers $V_{\max}$), uncompetitive — know which parameter each changes.

Activity Factors

- **Temperature:** rate rises to an optimum, then denaturation crashes it.
- **pH:** each enzyme has a sharp optimum (ionizable active-site residues).
- Cofactors/coenzymes (metal ions, NAD⁺) and inhibitors in the feedstock.

Immobilization

Fixing enzymes on carriers (adsorption, covalent binding, entrapment, cross-linking) lets them be **reused across batches**, used in **continuous packed-bed reactors**, and often **stabilizes** them. Trade-off: mass-transfer limitation can lower apparent activity. Classic: immobilized glucose isomerase converting glucose → fructose for high-fructose corn syrup.

Industrial Enzyme Roster

Amylases — starch liquefaction/saccharification (brewing, baking).

Proteases — detergents, leather, food tenderizing.

Cellulases/xylanases — paper, textiles, biofuel pretreatment.

Lipases — dairy flavors, biodiesel transesterification.

Glucose isomerase — HFCS. Penicillin acylase — semi-synthetic antibiotics.

Worked: An enzyme has $V_{\max} = 100 \mu\text{mol/min}$, $K_m = 2 \text{ mM}$. At $[S] = 2 \text{ mM}$, $v = 50$ (half-max by definition). At $[S] = 18 \text{ mM}$, $v = 100 \times 18 / 20 = 90$ — near saturation. A competitive inhibitor at fixed dose doubles apparent K_m to 4 mM : at $[S] = 4 \text{ mM}$, $v = 50$ again — more substrate overcomes it.

Unit Summary

- Michaelis–Menten: $v = V_{\max}[S]/(K_m + [S])$; K_m = inverse affinity; inhibitors alter K_m and/or $V_{\max}$ characteristically.
- Activity depends on temperature, pH, cofactors.
- Immobilization enables reuse and continuous reactors; know the major enzymes by industry.

Exam Pointers

- ★ Compute v at given $[S]$ from K_m , $V_{\max}$; interpret a Lineweaver–Burk plot.
- ★ Distinguish competitive and non-competitive inhibition effects.
- ★ Give four immobilization methods and two advantages.

Unit 4: Fermentation Technology and Fermenter Design

Lectures 13–17

Learning Objectives

- Describe the parts and functions of a stirred-tank fermenter
- Compare batch, fed-batch and continuous culture
- Explain sterilization and aseptic operation
- Describe aeration, agitation and foam control

The Stirred-Tank Fermenter

- **Vessel:** stainless steel, pressure-rated, with ports for sensors and feeds.
- **Agitator/impeller:** mixing, oxygen dispersion, heat transfer — Rushton turbines for gas dispersion.
- **Sparger:** introduces sterile air as fine bubbles (oxygen transfer is usually the limiting factor).
- **Baffles:** prevent vortexing, improve mixing.
- **Sensors/control:** pH, dissolved oxygen, temperature, foam probes — linked to automated control loops.
- **Sampling and harvest valves** designed to maintain sterility.

Modes of Operation

Batch — everything in at start; run; harvest. Simple, flexible, downtime between runs.

Fed-batch — feed substrate during the run (avoids substrate inhibition, extends production) — the workhorse for antibiotics and recombinant proteins.

Continuous (chemostat) — fresh medium in, culture out at the same rate; steady state at dilution rate $D = \mu$. Highest productivity, but contamination or strain degeneration ruins long runs.

Sterility and Process Control

- **Medium sterilization:** batch (121°C, 15–20 min) or continuous HTST; filter-sterilize heat-labile feeds.
- **Aseptic design:** steam-sterilizable seals, sterile air filtration (0.2 μm), positive pressure.
- **Oxygen transfer:** k_La is the key parameter — raised by agitation, aeration rate and bubble size; viscous broths (fungal) are the hard case.
- **Foam:** mechanical breakers or antifoam agents (which can hurt oxygen transfer — a real trade-off).

Chemostat math: at steady state $\mu = D$. Biomass $X = Y(S_{\text{in}} - S)$. Washout occurs if $D > \mu_{\text{max}}$ — the culture is flushed out. Exam questions love asking for the washout condition.

Unit Summary

- Stirred tank: vessel, impeller, sparger, baffles, sensors — built for mixing, oxygen transfer and sterility.
- Batch (simple), fed-batch (avoids inhibition — industry standard), continuous/chemostat (steady state, washout risk).
- Sterilize medium and air; manage k_La and foam.

Exam Pointers

- ★ Label a fermenter diagram and state each part's function.
- ★ Compare the three culture modes (4 points).
- ★ In a chemostat, what happens when D exceeds μ_{max} ?

Unit 5: Microbial Growth Kinetics

Lectures 18–20

Learning Objectives

- Describe the growth curve phases
- Apply the Monod equation
- Define yield coefficients and productivity
- Relate kinetics to process design

The Growth Curve

Lag — adaptation, no net growth. Log/exponential — $\mu = \mu_{\max}$, balanced growth. Stationary — nutrients exhausted, growth = death (secondary metabolites here). Death — decline.

Monod Kinetics

$\mu = \mu_{\max} \cdot S / (K_s + S)$ — the Michaelis–Menten of growth; K_s = substrate at half $\mu_{\max}$.

Yield: $Y_{X/S}$ = biomass formed / substrate consumed. Product yield $Y_{P/S}$ similarly. —

Productivity = product formed / (volume × time) — the economic bottom line.

- Doubling time $t_d = \ln 2 / \mu$. Biomass $X = X_0 e^{(\mu t)}$ in batch exponential phase.

Kinetics → Design

- High K_s organisms need higher residual substrate — affects chemostat operating point.
- Product inhibition (ethanol, acids) caps batch titers — fed-batch or in-situ product removal helps.
- Oxygen-limited growth: μ capped by kLa — scale-up must preserve oxygen transfer, not just geometry.

Worked: $\mu_{\max} = 0.5 \text{ h}^{-1}$, $K_s = 0.2 \text{ g/L}$, $S = 1.8 \text{ g/L} \rightarrow \mu = 0.5 \times 1.8 / 2.0 = 0.45 \text{ h}^{-1}$; $t_d = 0.693 / 0.45 \approx 1.54 \text{ h}$. With $Y_{X/S} = 0.5$ and 20 g/L substrate consumed, biomass = 10 g/L .

Unit Summary

- Lag → log → stationary → death; secondary metabolites belong to stationary phase.
- Monod $\mu = \mu_{\max} \cdot S / (K_s + S)$; yields link substrate to biomass/product; productivity is the economic metric.
- Kinetics dictates feeding strategy, chemostat setpoints and scale-up criteria.

Exam Pointers

- ★ Compute μ , doubling time and biomass from Monod data.
- ★ Sketch and label the growth curve; mark where antibiotics are produced.
- ★ Why is oxygen transfer the usual scale-up bottleneck?

Unit 6: Downstream Processing

Lectures 21–24

Learning Objectives

- Outline the downstream sequence
- Compare cell separation methods
- Explain cell disruption for intracellular products
- Describe purification: precipitation, chromatography, drying

The Recovery Sequence

Downstream processing (DSP) is often **60–80% of production cost**: 1) **cell separation** (if product is extracellular), 2) **cell disruption** (if intracellular), 3) **concentration/purification**, 4) **polishing and formulation**. Heuristic: **remove the most abundant impurity with the cheapest step first** (e.g., filtration before chromatography).

Separation and Disruption

- **Centrifugation**: fast, continuous (disc-stack); struggles with small density differences.
- **Filtration**: rotary vacuum or membrane (micro/ultrafiltration); fouling is the enemy.
- **Disruption**: high-pressure homogenization, bead milling (mechanical); enzymatic lysis, sonication — release intracellular product, then remove debris by centrifugation.

Purification and Finishing

- **Precipitation**: ammonium sulfate or solvent — cheap bulk capture.
- **Chromatography**: ion exchange, affinity (highly specific, expensive), size exclusion — the polishing steps for pharma-grade purity.
- **Membrane**: ultrafiltration concentrates; diafiltration exchanges buffers.
- **Drying/formulation**: spray drying, lyophilization; add stabilizers for shelf life.

Example — extracellular enzyme: broth → centrifugation (remove cells) → ultrafiltration (concentrate) → ion exchange (purify) → spray dry. Intracellular recombinant protein: broth → homogenize → centrifuge (remove debris) → affinity chromatography → diafiltration → lyophilize. Note how the first step flips with product location.

Unit Summary

- DSP = 60–80% of cost: separate → disrupt (if needed) → purify → formulate.
- Centrifugation/filtration for bulk; precipitation for capture; chromatography for polish.
- Product location (intra vs extracellular) decides the opening steps.

Exam Pointers

- ★ Outline DSP for an extracellular vs an intracellular product.
- ★ Why is DSP the dominant cost? Give two reasons.

- ★ Compare centrifugation and filtration for cell harvesting.

Unit 7: Major Product Families I: Acids, Amino Acids, Solvents

Lectures 25–28

Learning Objectives

- Describe citric acid fermentation
- Explain amino acid production (glutamate, lysine)
- Describe ethanol and ABE fermentations
- Apply the organism→substrate→process→product pattern

Citric Acid

Organism: *Aspergillus niger*. **Substrate:** molasses/sucrose. **Process:** aerobic submerged fermentation; low pH and manganese deficiency trigger overflow metabolism — citric acid excreted instead of being burned in the TCA cycle. **Product:** food acidulant, ~2 million tons/year — one of the largest fermentation products.

Amino Acids

- **Glutamic acid (MSG):** *Corynebacterium glutamicum*; biotin limitation makes the membrane leaky so glutamate is excreted.
- **Lysine:** *C. glutamicum* auxotrophs/mutants with deregulated aspartate pathway (feedback inhibition removed) — animal feed supplement.
- Trick used: **auxotrophic mutants** and feedback-deregulation to force overproduction of a primary metabolite.

Solvents: Ethanol and ABE

- **Ethanol:** *Saccharomyces cerevisiae* on sugar/starch; anaerobic; product inhibition caps ~12–15%; distilled. Fuel ethanol is a giant white-biotech market.
- **ABE (acetone-butanol-ethanol):** *Clostridium acetobutylicum*; biphasic (acidogenic then solventogenic); historically huge, revived for biobutanol (better fuel than ethanol).

Pattern drill — Citric acid: A. niger → molasses → aerobic batch, Mn-deficient → citric acid → food/beverage. Lysine: C. glutamicum mutant → glucose → aerobic fed-batch → lysine → feed. Ethanol: S. cerevisiae → sugar → anaerobic batch → ethanol → fuel/beverage. Memorize one line each.

Unit Summary

- Citric acid: *A. niger*, overflow metabolism under Mn deficiency.
- Amino acids: pathway-deregulated *C. glutamicum* mutants.
- Ethanol (yeast, anaerobic) and ABE (*Clostridium*, biphasic) — the solvent fermentations.

Exam Pointers

- ★ Why does manganese deficiency boost citric acid production?

- ★ How do auxotrophic mutants overproduce lysine?
- ★ Compare ethanol and ABE fermentations (organism, phases, products).

Unit 8: Major Product Families II: Enzymes, Biopolymers, Biofuels

Lectures 29–32

Learning Objectives

- Describe industrial enzyme production
- Explain biopolymers: PHA, PLA, xanthan
- Outline biofuel generations
- Evaluate sustainability claims

Bulk Enzymes

Produced by submerged fermentation of **Bacillus** (proteases, amylases), **Aspergillus/Trichoderma** (cellulases, amylases) — often recombinant strains secreting the enzyme extracellularly for easy recovery. Sold as concentrates standardized by activity units. Markets: detergents (protease/lipase/cellulase), starch processing, textiles, baking.

Biopolymers

PHA (polyhydroxyalkanoates) — bacterial storage granules (e.g., *Alcaligenes/Ralstonia*); truly biodegradable; cost is the barrier.

PLA (polylactic acid) — lactic acid fermented (*Lactobacillus*) then polymerized; compostable packaging.

Xanthan gum — *Xanthomonas campestris* exopolysaccharide; food thickener, oil-drilling mud.

Biofuels by Generation

1G — food crops (corn/sugarcane ethanol, palm biodiesel): food-vs-fuel conflict.

2G — lignocellulosic waste (straw, bagasse): pretreatment + cellulases + fermentation; the white-biotech showcase, still cost-challenged.

3G — algae: high oil yields per hectare; dewatering cost is the hurdle.

Biogas — anaerobic digestion of waste to methane; simplest, most robust.

Sustainability analysis framework: feedstock (waste vs food?), energy balance (EROI), water use, land-use change, and end-of-life (biodegradable?). Apply to any biofuel/biopolymer claim — exams reward this critical lens over cheerleading.

Unit Summary

- Bulk enzymes: *Bacillus/Aspergillus* fermentations; detergents and starch are the big markets.
- PHA (bacterial, biodegradable), PLA (from fermented lactic acid), xanthan (*Xanthomonas*).
- Biofuels 1G→3G + biogas; judge each on feedstock, EROI, water, land and end-of-life.

Exam Pointers

- ★ Name the producer organisms for protease, cellulase and xanthan.
- ★ Compare 1G, 2G and 3G biofuels on feedstock and key challenge.
- ★ Evaluate PLA packaging with the sustainability framework.

Unit 9: Bioprocess Economics, Scale-up and Biosafety

Lectures 33–36

Learning Objectives

- Estimate bioprocess economics: CAPEX, OPEX, productivity
- Explain scale-up criteria and challenges
- Describe biosafety levels and containment
- Discuss regulatory and ethical aspects

Economics

- **CAPEX:** fermenters, DSP equipment — stainless steel dominates; scale economies via the 0.6 power rule ($\text{cost} \propto \text{capacity}^{0.6}$).
- **OPEX:** substrate (often the largest cost — hence waste feedstocks), utilities (aeration, cooling, sterilization energy), labor, DSP consumables.
- **Key metrics:** titer (g/L), yield (g/g), productivity (g/L/h) — improving any of the three transforms economics; DSP cost share must fall as titer rises.

Scale-up

Lab → pilot → production. You cannot keep everything constant: maintain **kLa** (oxygen transfer) or **power per volume** or mixing time — choose the critical one for your process. Common failures: oxygen starvation in viscous broths, heat-removal limits (surface/volume falls with scale), longer sterilization heat-up creating more byproducts.

Biosafety and Regulation

BSL-1 — well-characterized non-pathogens (most industrial strains). BSL-2 — moderate-risk agents. BSL-3/4 — serious pathogens (not typical industry).

- **Containment:** closed fermenters, sterilize all exhaust gas and effluents (kill tanks), validate with containment tests.
- **GMO regulation:** contained-use directives, risk assessment of recombinant strains, labeling rules for products.
- **Ethics:** food-vs-fuel land use, biopiracy (benefit-sharing for strains sourced from biodiversity), dual-use awareness.

Case: A citric-acid plant scales 10 L → 100 m³ and yield collapses. Diagnosis: kLa not maintained — the big tank is oxygen-starved in the viscous Aspergillus broth; also sterilization took hours longer, degrading medium. Fixes: more agitation/aeration power, fed-batch feeding, or a production strain less sensitive to oxygen. Always check oxygen first in aerobic scale-up failures.

Unit Summary

- Economics: substrate dominates OPEX; titer/yield/productivity are the levers; 0.6 rule for CAPEX scaling.
- Scale-up: keep kLa or P/V constant; watch oxygen, heat removal, sterilization time.

- Biosafety: BSL levels, exhaust/effluent sterilization, GMO contained-use rules.

Exam Pointers

- ★ A plant doubles capacity. Estimate the CAPEX increase via the 0.6 rule.
- ★ List three classic scale-up failures and fixes.
- ★ What containment measures apply to a recombinant BSL-1 production strain?

Key Terms Glossary

All key terms from this handout in one place — use for quick revision.

White — industrial: microbes/enzymes making chemicals, fuels, materials.

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Green — agricultural: GM crops, biopesticides.

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