Microbiology

Unit 4 · Microbial Nutrition, Growth and Genetics

How microbes feed, how fast they multiply and how we count them, how they make energy with and without oxygen, and how bacteria and viruses change and share their genes. After this unit you can design a culture medium, keep a pure culture alive, work out generation time and CFU per mL, trace glucose to ATP, and explain how a resistance gene spreads between bacteria.

Taught at 8 of 10 | Usually Sem 3 | 8 chapters

About this unit

This unit follows a microbe through its working life. It starts with what a cell needs to eat and how we feed it in the lab (culture media), then how we isolate one kind of microbe and keep it alive for years (pure cultures and preservation). Next come growth: the growth curve and the arithmetic of doubling, and the methods used to count cells. Then the cell's energy business: aerobic and anaerobic pathways, light-driven ATP synthesis and the secondary metabolites, such as antibiotics, that cells make once growth slows. The unit ends with genetics: how bacteria swap DNA by transformation, transduction and conjugation, and how mutation and recombination change bacterial and viral genomes. Each chapter uses the one before it: you cannot count cells you cannot grow, and you cannot map genes by conjugation without counting recombinant colonies.

Chapters

1. Nutritional requirements and culture media
2. Pure culture methods and preservation of cultures ADVANCED
3. Bacterial growth curve and growth kinetics
4. Measuring growth: counting and enumeration methods
5. Microbial metabolism: aerobic and anaerobic pathways
6. Photophosphorylation and secondary metabolism in microbes ADVANCED
7. Bacterial recombination: transformation, transduction, conjugation
8. Genetics of viruses and bacteria ADVANCED

How to use this book

- Read the lead paragraph and the figure of each chapter first; together they carry the idea.

- Cover the solution of each worked example and do it yourself with a calculator, then compare line by line.
- Answer every Check yourself question in your head or on paper before you open it.

4.1 Nutritional requirements and culture media

Every microbe must take in the elements its cell is built from, a source of energy, and a source of electrons. Microbiologists sort organisms by where these come from, and they design culture media, the food we give microbes in the lab, to match. A good medium lets the wanted organism grow; a clever medium also stops unwanted ones or makes the wanted one easy to spot.

You need: the cell structure of bacteria from Unit 2, *Bacterial morphology and cell structure*.

What a cell needs

Most of a bacterial cell's dry weight comes from six elements: carbon, oxygen, nitrogen, hydrogen, phosphorus and sulfur (remember **CHONPS**). These are the **macronutrients**, needed in large amounts. Carbon builds every organic molecule. Nitrogen goes into amino acids and nucleotides. Phosphorus goes into nucleic acids, phospholipids and ATP. Sulfur goes into the amino acids cysteine and methionine.

Potassium, magnesium, calcium and iron are also needed in fair amounts: Mg^{2+} stabilises ribosomes and membranes, and iron sits in cytochromes. **Micronutrients** (trace elements) such as zinc, copper, manganese, cobalt, molybdenum and nickel are needed in tiny amounts, often as cofactors of enzymes; tap water or impurities in other ingredients usually supply them.

Growth factors are organic compounds a cell needs but cannot make: some amino acids, purines and pyrimidines, and vitamins such as biotin or B12. An organism that needs a growth factor is called **fastidious** if it needs many of them. A mutant that has lost the ability to make one is an **auxotroph** (see 4.8).

Nutritional types

Three questions name an organism's nutritional type. Where does energy come from: light (**photo-**) or chemicals (**chemo-**)? Where do electrons come from: inorganic compounds (**litho-**) or organic compounds (**organo-**)? Where does carbon come from: CO_2 (**auto-**) or organic molecules (**hetero-**)? Join the answers with "-troph".

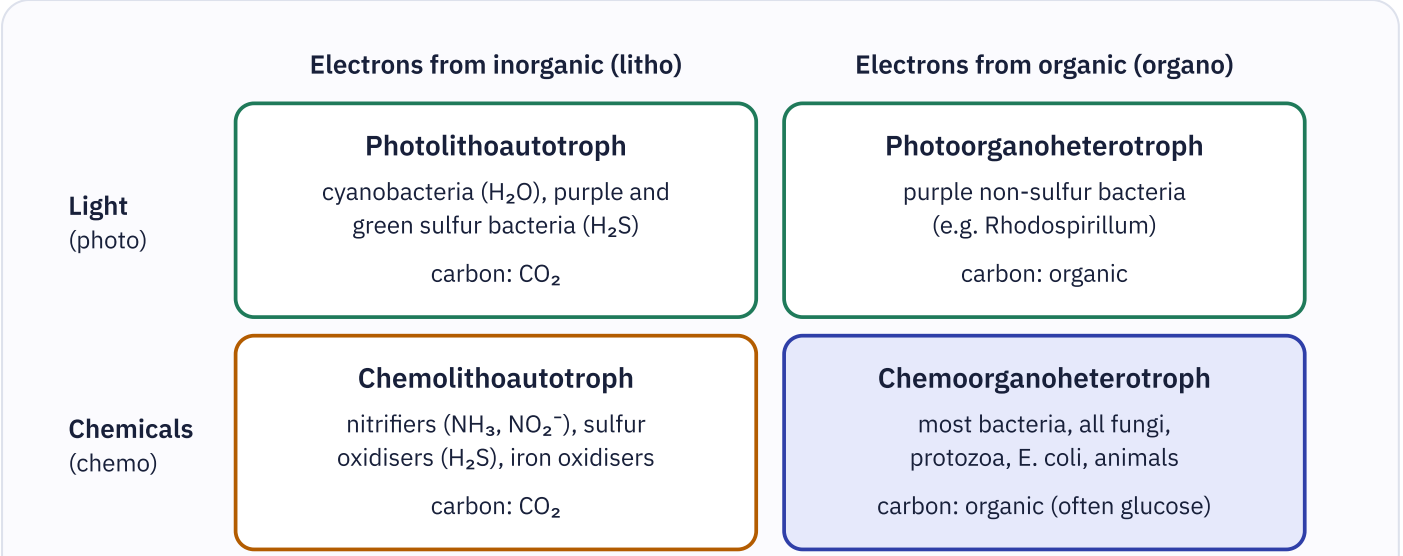


Figure 4.1.1 The four common nutritional types. The bottom-right box holds nearly every pathogen and every industrial heterotroph, which is why most lab media are built on sugar and peptone. Notice that the "auto" organisms are also the "litho" ones in the usual cases.

In practice the names are shortened: **photoautotroph**, **chemoautotroph** (or chemolithotroph), **photoheterotroph** and **chemoheterotroph**. Chemoheterotrophs take energy, electrons and carbon from the same organic molecule, for example glucose.

Culture media

A **culture medium** is a liquid (broth) or solid preparation that supplies the nutrients above at a suitable pH, usually around 7 for bacteria and 5 to 6 for fungi. Solid media are made by adding **agar**, a seaweed polysaccharide, at about 1.5% (15 g/L). Agar melts near 100 °C, sets around 40 °C, and almost no bacterium can digest it, so it stays solid at 37 °C incubation.

Media are classified in three ways:

- **By chemical composition.** A *defined* (synthetic) medium has every ingredient known in exact amount, such as glucose plus mineral salts for E. coli. A *complex* medium contains extracts whose exact make-up is unknown: peptone (digested protein), beef extract or yeast extract. Nutrient broth is complex.
- **By physical state.** Liquid (broth), semi-solid (about 0.5% agar, used to test motility) and solid (about 1.5% agar).
- **By purpose.** *Selective* media suppress unwanted organisms: mannitol salt agar's 7.5% NaCl allows staphylococci. *Differential* media make different organisms look different: on MacConkey agar, lactose fermenters form pink colonies and non-fermenters stay colourless. MacConkey is also selective, because bile salts and crystal violet suppress Gram-positive bacteria. *Enriched* media add blood or serum for fastidious organisms (blood agar, chocolate agar). *Enrichment* broths favour a rare organism in a mixed sample until it outnumbers the rest, such as selenite F

broth for Salmonella from stool or food. *Reducing* media, such as thioglycollate broth, remove oxygen for anaerobes. *Transport* media keep a sample alive without letting it grow.

Blood agar is a good exam case because it is both enriched and differential: β-haemolysis clears the blood fully around a colony, α-haemolysis gives a green zone, and γ means no change.

WORKED EXAMPLE

Question. A lab uses this nutrient agar recipe per litre: peptone 5 g, beef extract 3 g, NaCl 5 g, agar 15 g. (a) How much of each do you weigh for 400 mL? (b) Is the medium defined or complex, and why? (c) What would you add, and at what level, to make it select for staphylococci? (4 marks)

1. Scale factor = 400 mL ÷ 1000 mL = 0.4.
2. Peptone 5 × 0.4 = 2.0 g; beef extract 3 × 0.4 = 1.2 g; NaCl 5 × 0.4 = 2.0 g; agar 15 × 0.4 = 6.0 g. Make up to 400 mL with water, adjust pH to about 7, autoclave at 121 °C for 15 min.
3. Complex: peptone and beef extract are mixtures whose exact chemical make-up is not known.
4. To select for staphylococci, raise NaCl to 7.5% (75 g/L). For 400 mL that is 0.4 × 75 = 30 g NaCl in total, replacing the 2.0 g. Adding mannitol with phenol red (as in mannitol salt agar) also makes it differential: S. aureus ferments mannitol and turns the medium yellow.

EXAM TRAPS

- Selective versus differential: selective decides *who grows*; differential decides *how they look*. MacConkey and mannitol salt agar are both.
- Enriched versus enrichment: enriched is a rich solid medium (blood agar); enrichment is a broth that tilts a mixed population towards one organism.
- Litho and auto are separate questions: one asks about electrons, the other about carbon.
- Agar is a solidifying agent, not a nutrient; few bacteria can break it down.

CHECK YOURSELF

– REMEMBER **Name the six macronutrient elements.** 2 marks

Carbon, hydrogen, oxygen, nitrogen, phosphorus and sulfur (CHONPS).

– REMEMBER **Define a growth factor and give two examples.** 2 marks

An organic compound the cell needs but cannot make, so it must be supplied: amino acids, purines, pyrimidines, vitamins such as biotin.

– UNDERSTAND **Why is MacConkey agar both selective and differential?** 3 marks

Bile salts and crystal violet stop Gram-positive bacteria (selective). Lactose with neutral red makes lactose fermenters pink and non-fermenters colourless (differential).

– UNDERSTAND **Classify Nitrosomonas, which oxidises ammonia and fixes CO_2.** 2 marks

Energy from a chemical, electrons from an inorganic compound (NH_3), carbon from CO_2: chemolithoautotroph.

– APPLY **How much agar and glucose for 250 mL of a medium with 1.5% agar and 0.5% glucose?** 2 marks

1.5% w/v = 1.5 g per 100 mL, so 3.75 g agar; 0.5% = 0.5 g per 100 mL, so 1.25 g glucose.

– APPLY **Suggest a medium to grow a strict anaerobe in broth.** 2 marks

Thioglycollate broth: sodium thioglycollate removes dissolved oxygen, and growth at the bottom of the tube shows an anaerobe.

– ANALYZE **A student's E. coli grows in nutrient broth but not in glucose-salts medium. What does this suggest?** 3 marks

The strain needs a growth factor that the complex medium supplies and the defined one lacks; it is probably an auxotroph. Testing defined medium with single added amino acids would find which one.

BEYOND THE SYLLABUS

Industry designs chemically defined media for recombinant protein production, because a defined medium gives the same batch every time and is easier to justify to regulators. Chromogenic media, where each species gives a different colony colour, are now common in clinical and food labs. Most environmental microbes still refuse to grow on any medium; culturomics and "iChip" style diffusion chambers try to change that. Search "chromogenic agar" and "the great plate count anomaly". This is not for the exam.

4.2 Pure culture methods and preservation of cultures

A **pure culture** (axenic culture) contains only one kind of microorganism, all descended from a single cell. Every identification test, every growth curve and every industrial fermentation starts from one, because a mixed culture gives mixed answers. The trick is to spread cells so thin that each lands alone and grows into its own colony, then keep that strain alive and unchanged for years. Others teach it in the lab course.

Getting a pure culture

All plating methods rest on one idea: one well-separated colony is assumed to come from one cell (strictly, one **colony-forming unit**, since a chain or clump can also give one colony).

- **Streak plate.** A sterile loop drags the sample across the agar in three or four sections, flaming or changing the loop between them, so each section gets fewer cells than the last.
- **Spread plate.** A small volume (usually 0.1 mL) of a diluted sample is spread over the surface with a sterile glass or plastic spreader. Colonies grow on the surface.
- **Pour plate.** Diluted sample is mixed with melted agar cooled to about 45 °C and poured. Colonies grow both on and inside the agar; heat-sensitive or strictly aerobic organisms may suffer.
- **Single-cell methods.** A micromanipulator under the microscope picks one cell. Flow sorting and limiting dilution in microwell plates do the same at scale.

Enrichment broths (see 4.1) come first when the organism is rare in the sample.

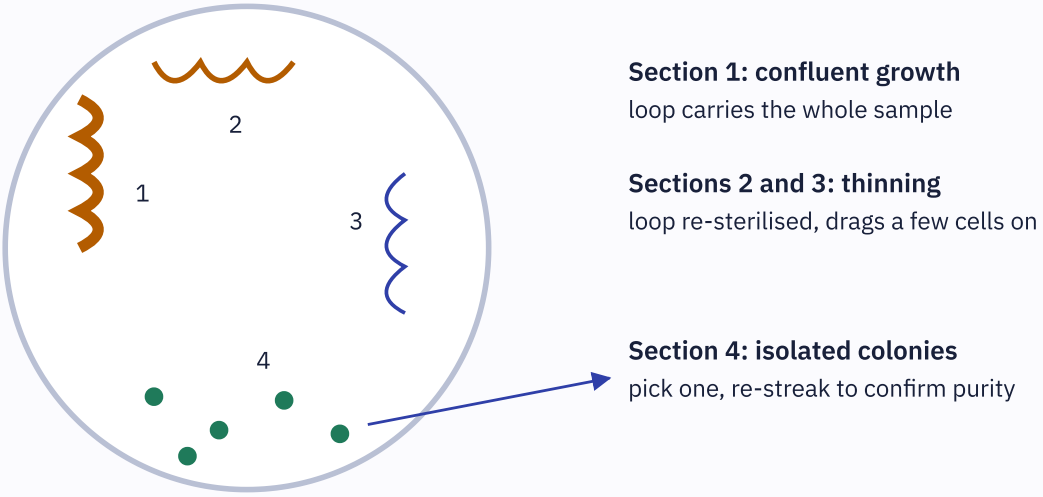


Figure 4.2.1 A four-quadrant streak plate after incubation. Cell number falls with each section, so only the last sections show single colonies. One colony is picked and streaked again before it is called pure.

Keeping it alive: preservation

The aim is to stop growth (so no mutations pile up and no contaminant enters) while keeping cells viable. Colder and drier means longer storage.

1. **Periodic subculture.** Transfer to fresh slants every few weeks and store at about 4 °C. Cheap, but each transfer risks contamination and selects mutants.
2. **Mineral oil overlay.** Sterile paraffin oil over a slant cuts oxygen and drying; works for months to a few years for many fungi and bacteria.
3. **Freezing in glycerol.** Cells in broth with about 15–20% glycerol are frozen at −80 °C. Glycerol is a cryoprotectant: it limits ice crystal damage. This is the everyday lab method.
4. **Liquid nitrogen.** Storage at −196 °C (or in the vapour phase above it) stops all metabolism; viability is kept for decades.
5. **Lyophilisation (freeze-drying).** Cells in a protectant such as skim milk are frozen, then water is removed under vacuum by sublimation. Sealed ampoules store at room temperature and are easy to post, which is why culture collections use them.

National collections hold reference strains: in India, MTCC (Microbial Type Culture Collection) at CSIR-IMTECH Chandigarh and NCIM at CSIR-NCL Pune; abroad, ATCC (USA) and DSMZ (Germany).

WORKED EXAMPLE

Question. A soil suspension has about 10^8 bacteria per mL. You want 30 to 300 colonies on a spread plate of 0.1 mL. Which tenfold dilution should you plate? (3 marks)

1. Cells plated = concentration × dilution × 0.1 mL.
2. At 10^{-5}: $10^8 \times 10^{-5} \times 0.1 = 100$ colonies. At 10^{-6}: 10 colonies. At 10^{-4}: 1000 colonies.
3. Plate 10^{-5} (expect about 100) and, as a safety margin, 10^{-4} and 10^{-6} too, because the starting count is only an estimate.

EXAM TRAPS

- A colony comes from one CFU, which may be more than one cell; that is why you re-streak.
- Pour plates grow colonies inside the agar too; spread plates only on the surface.
- Lyophilisation removes water by sublimation from the frozen state, not by heating.
- Glycerol stocks belong at −80 °C; at −20 °C viability falls much faster.

CHECK YOURSELF

- REMEMBER **Define a pure culture.** 1 mark

  A culture with only one kind of microorganism, descended from a single cell or CFU.

- REMEMBER **Name two Indian culture collections.** 2 marks

  MTCC (CSIR-IMTECH, Chandigarh) and NCIM (CSIR-NCL, Pune).

- UNDERSTAND **Why does a streak plate give single colonies only in the last sections?** 2 marks

  Each section is started by dragging a few cells from the previous one with a re-sterilised loop, so the number of cells falls step by step until cells land apart.

- APPLY **Choose a method to send 50 strains by post to another lab.** 2 marks

  Lyophilised ampoules: dry, stable at room temperature, no cold chain needed.

- ANALYZE **Why is repeated subculture a poor way to keep an industrial strain for years?** 3 marks

  Each transfer means growth, so mutations accumulate and faster-growing low-yield variants can take over; each transfer also risks contamination. Freezing or freeze-drying stops growth.

BEYOND THE SYLLABUS

Biotech companies keep a "master cell bank" and "working cell banks" of each production strain, frozen in many identical vials, so every batch starts from the same cells. Microfluidic droplets now isolate thousands of single cells at once. Search "cell banking ICH Q5D" and "droplet microfluidics single cell isolation". This is not for the exam.

4.3 Bacterial growth curve and growth kinetics

In microbiology, "growth" means an increase in the number of cells, not the size of one cell. A bacterium divides by **binary fission**: one cell becomes two, two become four, so the population doubles every **generation time**. When a few cells are put into fresh broth in a closed flask (a **batch culture**), the count follows a typical curve of four phases. Kinetics turns that curve into numbers you can calculate and design with: generation time, specific growth rate, and the Monod equation that links growth to food.

The four phases

1. **Lag phase.** Cells adjust to the new medium: they make enzymes, ribosomes and ATP, and repair damage. Cell number barely changes, though cells are very active. Lag is long if the inoculum is old, cold or from a different medium, and short or absent if exponentially growing cells go into the same medium.
2. **Log (exponential) phase.** Cells divide at a constant, maximum rate for that medium and temperature. A plot of log(number) against time is a straight line. Cells are most uniform here, so physiological studies and most antibiotics (which act on growing cells) work best now.
3. **Stationary phase.** Growth rate falls to zero because a nutrient runs out, waste (acids, for example) builds up, or oxygen is limiting. New cells are balanced by dying cells. Many **secondary metabolites** are made here (see 4.6), and some bacteria start to form endospores.
4. **Death (decline) phase.** Viable count falls, usually exponentially but much more slowly than it rose. Some cells survive a long time by living on the remains of others.

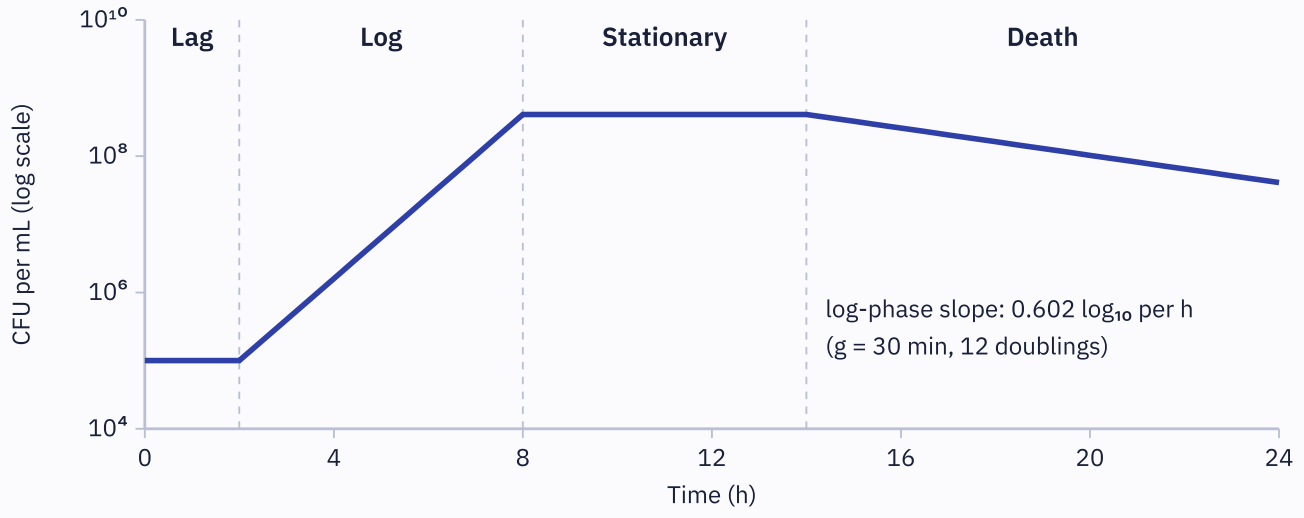


Figure 4.3.1 A batch growth curve computed for 10^5 CFU/mL inoculated with a 2 h lag, a 30 min generation time for 6 h, a stationary phase, then a slow death of 0.1 $\log_{10}$ per hour. On a log axis the log phase is a straight line; its slope gives the growth rate.

The arithmetic of doubling

Start with N_0 cells. After n generations there are

$$N = N_0 \times 2^n$$

$$\log N = \log N_0 + n \log 2$$

$$n = (\log N - \log N_0) / \log 2 = 3.32\ (\log N - \log N_0)$$

Here log is base 10, and 1/log 2 = 1/0.301 ≈ 3.32. If the log phase lasts a time t, then

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generation time   g = t / n
growth rate constant k = n / t   (generations per hour) = 1 / g
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Engineers prefer the **specific growth rate** μ (mu), defined from biomass X: dX/dt = μX, so $X = X_o$ e^(μt). The doubling time t_d and μ are linked by t_d = ln 2 / μ = 0.693 / μ. Do not mix k and μ: μ = k × ln 2 = 0.693 k.

Growth and substrate: Monod kinetics

Growth rate depends on how much of the limiting nutrient (substrate) is present. Jacques Monod described this with an equation of the same shape as Michaelis–Menten enzyme kinetics:

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μ = μmax × S / (Ks + S)
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μmax is the maximum specific growth rate (h^{-1}), S is the limiting substrate concentration (g/L), and Ks is the saturation constant: the value of S at which μ = μmax/2. A small Ks means the organism grows well even at low food levels. Growth yield $Y_{X/S}$ = ΔX / ΔS tells how many grams of cells form per gram of substrate used.

Continuous culture: the chemostat

In a **chemostat**, fresh medium flows in at rate F (L/h) and culture flows out at the same rate, so volume V stays constant. The **dilution rate** D = F / V (h^{-1}). At steady state cells grow exactly as fast as they are washed out, so **μ = D**. The steady-state substrate level is $\bar{S}$ = Ks D / (μmax − D), and biomass is $\bar{X} = Y (S_o - \bar{S})$, where S_o is the substrate in the feed. If D is pushed above about μmax, cells cannot keep up and are **washed out**. A **turbidostat** instead holds turbidity constant by adjusting flow.

Two other patterns appear in exams. **Diauxic growth**: E. coli in glucose plus lactose uses glucose first, pauses (a second lag) while it switches on the lac genes, then grows on lactose, giving a two-step curve. **Synchronous growth**: all cells divide together for a few generations, produced by filtration or temperature shifts, useful for studying the cell cycle.

WORKED EXAMPLE 1: GENERATION TIME

Question. A culture in log phase rises from 2×10^3 to 6.4×10^4 cells/mL in 2.5 h. Find the number of generations, the generation time and μ. (5 marks)

1. $\log N_0 = \log (2 \times 10^3) = 3.301$; $\log N = \log (6.4 \times 10^4) = 4.806$.
2. $n = 3.32 \times (4.806 - 3.301) = 3.32 \times 1.505 = 5.0$ generations. (Check: $2^5 = 32$ and $2 \times 10^3 \times 32 = 6.4 \times 10^4$.)
3. $g = t / n = 150 \text{ min} / 5 = 30 \text{ min}$.
4. $\mu = \ln 2 / t_d = 0.693 / 0.5 \text{ h} = 1.39 \text{ h}^{-1}$.

WORKED EXAMPLE 2: CHEMOSTAT

Question. An organism has μmax = 0.8 h^{-1}, Ks = 0.2 g/L and Y = 0.5 g cells per g glucose. A 2 L chemostat is fed 10 g/L glucose at 0.8 L/h. Find D, the steady-state glucose and biomass, and the biomass productivity. (6 marks)

1. $D = F / V = 0.8 / 2 = 0.4 \text{ h}^{-1}$. Since D < μmax, there is no washout, and $\mu = 0.4 \text{ h}^{-1}$.
2. $\bar{S} = Ks\ D / (\mu max - D) = 0.2 \times 0.4 / (0.8 - 0.4) = 0.08 / 0.4 = 0.2$ g/L.
3. $\bar{X} = Y (S_0 - \bar{S}) = 0.5 \times (10 - 0.2) = 4.9$ g/L.
4. Productivity $= D \times \bar{X} = 0.4 \times 4.9 = 1.96$ g cells per L per h.

EXAM TRAPS

- Use $\log_{10}$ with the 3.32 factor, or ln with 1/ln 2 = 1.443; mixing them gives answers off by 2.3 times.
- Generation time is only calculated from points inside the log phase.
- k (generations per h) and μ (per h, natural log) are different numbers: $\mu = 0.693\ k$.
- Lag phase is not inactivity; cells are busy making enzymes.
- Favourite question: "Draw and explain the bacterial growth curve" (8 marks). Label axes, use a log y-axis, and give a reason for each phase.

CHECK YOURSELF

- REMEMBER **Name the phases of a batch growth curve in order.** 2 marks

  Lag, log (exponential), stationary, death (decline).

- REMEMBER **Write the Monod equation and define Ks.** 2 marks

  $\mu = \mu_{max} S/(K_s + S)$; Ks is the substrate concentration at which μ is half of μmax.

- UNDERSTAND **Why does a culture enter stationary phase?** 3 marks

  A nutrient becomes limiting, toxic waste builds up, or oxygen and space run short; division slows until births equal deaths.

- UNDERSTAND **Explain diauxic growth.** 3 marks

  With two sugars, the cell uses the preferred one (glucose) first; catabolite repression keeps lactose genes off. When glucose runs out there is a lag while lac enzymes are made, then a second log phase on lactose.

- APPLY **How many cells after 4 h from 100 cells if g = 20 min?** 2 marks

  $n = 240/20 = 12$; $N = 100 \times 2^{12} = 100 \times 4096 = 4.1 \times 10^5$ cells.

- APPLY **$\mu = 0.35\ h^{-1}$. Find the doubling time.** 2 marks

  $t_d = 0.693/0.35 = 1.98$ h, about 2 h.

- ANALYZE **In worked example 2, what happens if the feed rate is raised to 1.8 L/h?** 3 marks

  $D = 0.9\ h^{-1}$, which exceeds $\mu_{max} = 0.8\ h^{-1}$. Cells cannot grow fast enough to replace those washed out, so the culture washes out.

BEYOND THE SYLLABUS

Industrial fermentations mostly run as **fed-batch**: substrate is fed slowly to hold μ below the level where E. coli makes acetate or yeast makes ethanol, which raises final cell density. Models with substrate inhibition (Haldane, also called Andrews) and maintenance energy (Pirt) extend Monod. Single-cell "mother machine" microfluidics now track growth of individual cells for hundreds of generations. Search "fed-batch exponential feeding" and "Pirt maintenance coefficient". This is not for the exam.

4.4 Measuring growth: counting and enumeration methods

To draw a growth curve, test a drug or pass a food sample, you must put a number on a population too small to see. Methods fall into two families: **direct** methods count cells (under a microscope, as colonies, or electronically), and **indirect** methods measure something that rises with cell number (turbidity, dry weight, ATP). Some count only living cells, others count dead ones too, and that difference is the most common exam question.

Direct counts

- **Direct microscopic count.** A counting chamber (Petroff–Hausser for bacteria, the Neubauer haemocytometer for yeast and larger cells) holds a known, tiny volume over a ruled grid. Cells per mL = average count per square ÷ volume of one square (in mL) × dilution factor. Quick, but it counts dead cells too, and small cells are hard to see unless stained.
- **Electronic counting.** A Coulter counter detects each cell as a change in electrical resistance when it passes through a small opening. A flow cytometer counts cells by light scatter and fluorescence, and with live/dead stains it can separate viable from dead cells.

Viable counts

The **standard plate count** counts only cells able to multiply, as **colony-forming units** (CFU). The sample is diluted tenfold step by step (1 mL into 9 mL), then plated by spread plate (0.1 mL) or pour plate (often 1 mL). Only plates with 30 to 300 colonies are used: fewer gives large sampling error, more gives crowded colonies that merge.

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CFU per mL = colonies counted ÷ (volume plated in mL × dilution)
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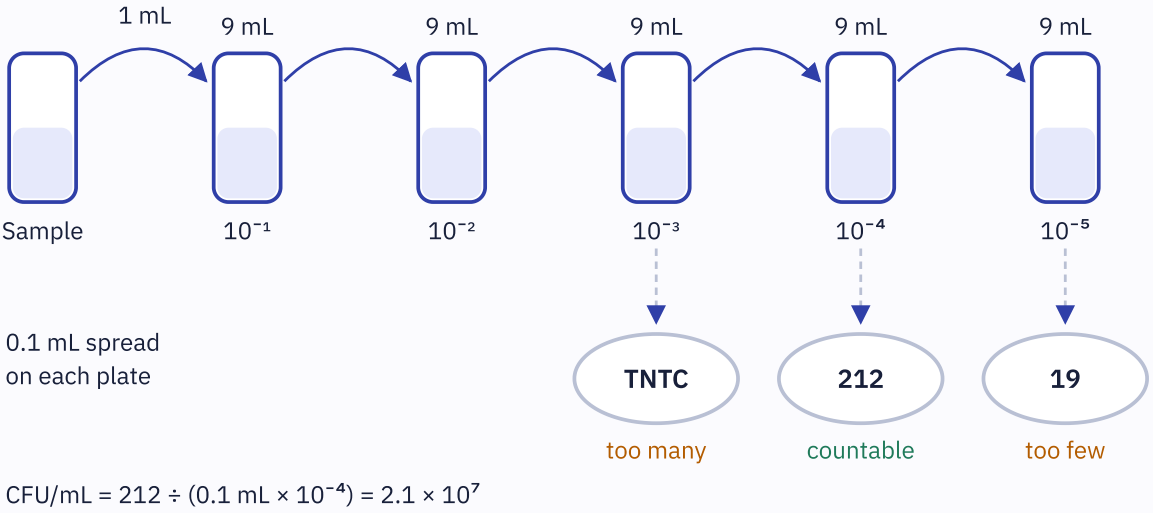


Figure 4.4.1 A tenfold serial dilution with 0.1 mL spread from the last three tubes. Only the 10^{-4} plate falls in the 30 to 300 range, so only it is used; the 10^{-5} plate agrees roughly ($19 \div 10^{-6} = 1.9 \times 10^{7}$) but is too few to trust. TNTC means too numerous to count.

Membrane filtration is used when cells are few, as in drinking water. A large volume (often 100 mL) is passed through a membrane with pores small enough (commonly 0.45 µm) to trap bacteria, and the membrane is laid on a selective medium. Each colony is one CFU in the filtered volume, so results are reported per 100 mL.

The **most probable number** (MPN) method estimates numbers statistically. Several tubes of broth at each of three dilutions are inoculated; the pattern of tubes showing growth (or gas, for coliforms) is read against a published MPN table. It suits samples, such as water, milk or food, where cells are too few or too clumped for plates, and it works with selective broths.

Indirect methods

- **Turbidity.** Cells scatter light, so a spectrophotometer reading of optical density (OD, often at 600 nm) rises with cell number. It is fast and does not destroy the sample, but needs a calibration curve against plate counts for each organism, and becomes non-linear at high density (dilute the sample). McFarland standards are reference suspensions for the same idea in clinical labs.
- **Dry weight.** Cells are centrifuged or filtered, washed, dried and weighed. Used for fungi and in fermenters (g dry cell weight per L).
- **Cell activity.** ATP by firefly luciferase light (rapid hygiene tests on food surfaces), protein or DNA content, oxygen uptake or CO_2 output.

Rapid methods such as ATP bioluminescence, impedance measurement, immunological tests (ELISA) and PCR give answers in hours instead of days.

WORKED EXAMPLE 1: PLATE COUNT

Question. 1 g of paneer is blended in 9 mL of saline (10^{-1}), then diluted tenfold to 10^{-5}. 0.1 mL from the 10^{-4} tube gives 87 colonies on plate count agar. Find CFU per g. (4 marks)

1. The plate is in range (30 to 300), so it can be used.
2. Total dilution of that tube = 10^{-4} (the first 1 g in 9 mL counts as the 10^{-1} step).
3. CFU per g = $87 \div (0.1 \times 10^{-4}) = 87 \times 10^{5} = 8.7 \times 10^{6}$ CFU/g.

WORKED EXAMPLE 2: HAEMOCYTOMETER

Question. A brewery yeast sample is diluted 1:10 and loaded on a Neubauer haemocytometer (depth 0.1 mm). Five of the 0.2 mm × 0.2 mm squares hold 24, 27, 22, 25 and 22 cells. Find yeast cells per mL of the original sample. (4 marks)

1. Mean per square = $(24 + 27 + 22 + 25 + 22) \div 5 = 120 \div 5 = 24$.
2. Volume of one square = $0.2 \times 0.2 \times 0.1 = 0.004$ mm³. Since 1 mL = 1000 mm³, this is 4×10^{-6} mL.
3. Cells per mL in the diluted sample = $24 \div (4 \times 10^{-6}) = 6 \times 10^{6}$.
4. Undo the 1:10 dilution: $6 \times 10^{6} \times 10 = 6 \times 10^{7}$ cells/mL (living and dead).

EXAM TRAPS

- Plate counts give CFU (living cells able to multiply); chamber counts and turbidity include dead cells.
- Divide by the dilution (10^{-4}), which is the same as multiplying by the dilution factor (10^{4}). Do not do both.
- Remember the volume plated: 0.1 mL on a spread plate adds another factor of 10.
- OD is linear only over a limited range; a very turbid culture must be diluted before reading.

CHECK YOURSELF

– REMEMBER **What range of colonies makes a plate countable?** 1 mark

30 to 300 colonies.

– REMEMBER **Name two direct and two indirect methods.** 2 marks

Direct: microscopic chamber count, plate count (also Coulter counter, flow cytometry). Indirect: turbidity, dry weight (also ATP, oxygen uptake).

– UNDERSTAND **Why is membrane filtration used for drinking water?** 2 marks

Safe water has very few bacteria; filtering a large volume such as 100 mL collects enough cells to count.

– UNDERSTAND **Why must turbidity be calibrated against plate counts?** 2 marks

OD depends on cell size and shape, so the OD-to-cell-number relation differs between organisms and growth phases.

– APPLY **1 mL of the 10^{-6} dilution in a pour plate gives 145 colonies. CFU/mL?** 2 marks

$145 \div (1 \times 10^{-6}) = 1.45 \times 10^{8}$ CFU/mL.

– APPLY **100 mL of well water is filtered and 18 coliform colonies grow. Report the result.** 1 mark

18 coliforms per 100 mL; the water is unfit to drink without treatment.

– ANALYZE **A microscopic count gives 5×10^{8} cells/mL but the plate count gives 1×10^{8} CFU/mL. Give two reasons.** 3 marks

The microscope counts dead cells too, and chains or clumps form one colony from several cells. Some live cells may also fail to grow on that medium.

BEYOND THE SYLLABUS

Quantitative PCR (qPCR) counts gene copies and can detect pathogens in food in hours; digital PCR gives absolute counts without a standard curve. Many soil and sea microbes are "viable but non-culturable": alive, yet they never form colonies, so plate counts can miss most of a community. FSSAI and the BIS standards set the microbiological limits used by Indian food labs. Search "viable but non-culturable" and "FSSAI microbiological standards". This is not for the exam.

4.5 Microbial metabolism: aerobic and anaerobic pathways

Metabolism is the sum of a cell's chemical reactions. **Catabolism** breaks food down to release energy, trapped as ATP, and electrons, carried as NADH. **Anabolism** (biosynthesis) spends that ATP and those electrons to build cell parts. A chemoheterotroph such as E. coli breaks glucose to pyruvate, and then what happens depends on whether oxygen, another electron acceptor, or none at all is available. The three answers are aerobic respiration, anaerobic respiration and fermentation.

You need: glycolysis and the TCA cycle from Biochemistry; redox potential means how strongly a substance pulls electrons.

Glucose to pyruvate: three routes

- **Embden–Meyerhof–Parnas (EMP) pathway**, ordinary glycolysis: glucose → 2 pyruvate, net 2 ATP and 2 NADH. ATP here is made by **substrate-level phosphorylation**: a phosphate is passed directly from a high-energy intermediate to ADP.
- **Entner–Doudoroff (ED) pathway**, used by Pseudomonas and Zymomonas among others: glucose is oxidised to 6-phosphogluconate, then split by KDPG aldolase. It gives 1 ATP, 1 NADH and 1 NADPH per glucose.
- **Pentose phosphate pathway**: runs alongside EMP. Its main job is not ATP but NADPH (for biosynthesis) and sugar building blocks, ribose-5-phosphate for nucleotides and erythrose-4-phosphate for aromatic amino acids.

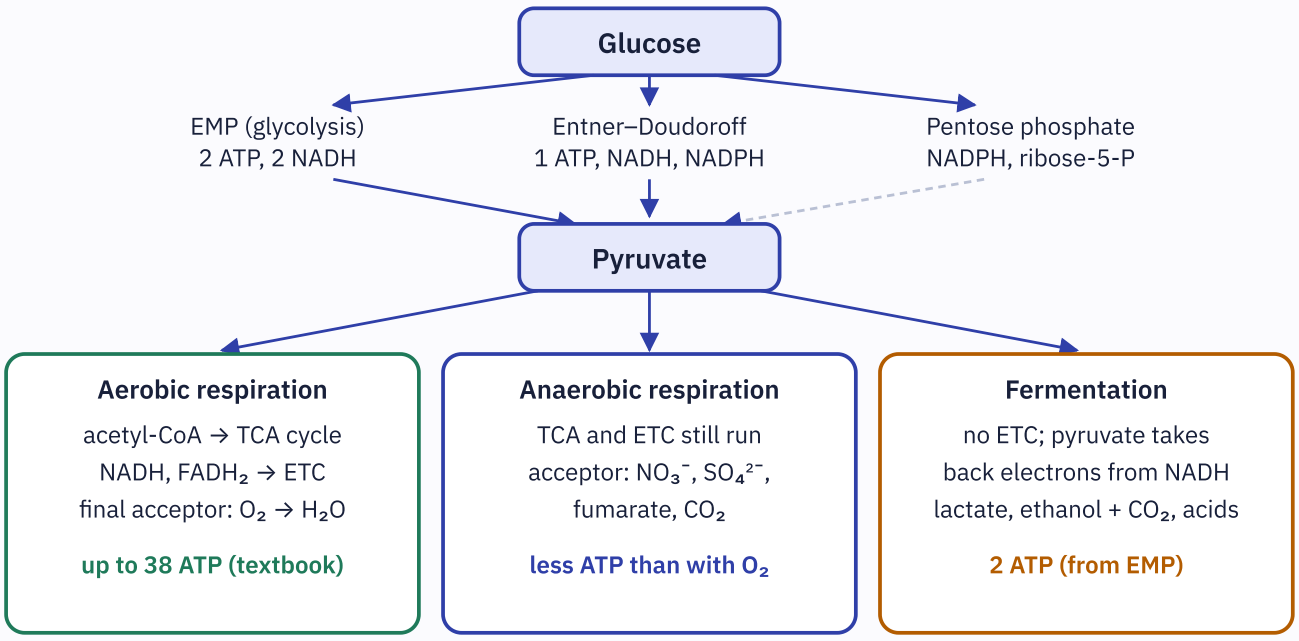


Figure 4.5.1 The main routes of glucose catabolism in bacteria. The three routes meet at pyruvate. What happens next is set by the electron acceptor: oxygen, another inorganic or organic acceptor, or an internal organic molecule (fermentation). ATP yield falls from left to right.

Aerobic respiration

Pyruvate is oxidised to acetyl-CoA (releasing CO_2 and NADH), and acetyl-CoA enters the **TCA cycle**, which oxidises it fully to CO_2 while filling NADH and $FADH_2$. These carriers hand electrons to the **electron transport chain** (ETC). In bacteria the ETC sits in the plasma membrane, since there are no mitochondria. As electrons pass to O_2, protons are pumped out of the cell, and the proton gradient (the **proton motive force**) drives ATP synthase. This is **oxidative phosphorylation**.

The classic textbook count for one glucose in a prokaryote is 38 ATP: 4 by substrate-level phosphorylation, and 34 from 10 NADH (× 3) and 2 $FADH_2$ (× 2). With the modern values of about 2.5 ATP per NADH and 1.5 per $FADH_2$ the total is about 32. Real bacteria often get less because their chains pump fewer protons. Exams usually accept 38 for prokaryotes; say which convention you use.

Anaerobic respiration

Some bacteria run the ETC with a final electron acceptor other than O_2. Because these acceptors pull electrons less strongly, less energy is released and less ATP made. Examples:

- Nitrate (NO_3^-) → nitrite → N_2: **denitrification** by Pseudomonas and Paracoccus; E. coli stops at nitrite.

- Sulfate (SO_4^{2-}) → H_2S: **sulfate reducers** such as Desulfovibrio, which blacken mud and corrode iron pipes.
- CO_2 → CH_4: **methanogens**, which are archaea, in biogas plants and the rumen.
- Fumarate → succinate: E. coli without oxygen.

Organisms are grouped by their oxygen use: **obligate aerobes** need O_2; **facultative anaerobes** (E. coli, yeast) respire with O_2 and ferment or respire anaerobically without it; **obligate anaerobes** (Clostridium, methanogens) are harmed by O_2; **microaerophiles** (Campylobacter) need a little.

Fermentation

In **fermentation** there is no ETC. Its only purpose is to reoxidise NADH to NAD^+ so that glycolysis can keep going; an organic molecule made from pyruvate takes the electrons. ATP comes only from glycolysis: 2 ATP per glucose by EMP. Fermentations are named after their products:

- **Homolactic**: pyruvate → 2 lactate (Lactobacillus, Lactococcus: curd, yogurt).
- **Heterolactic**: lactate + ethanol + CO_2 through the phosphoketolase pathway, 1 ATP (Leuconostoc: idli and dosa batter rise partly from this CO_2).
- **Alcoholic**: pyruvate → acetaldehyde + CO_2 → ethanol (Saccharomyces, Zymomonas: toddy, beer, bread).
- **Mixed acid**: lactate, acetate, succinate, formate, ethanol, H_2 and CO_2 (E. coli; methyl red test positive). **Butanediol** fermentation (Enterobacter; Voges–Proskauer test positive) gives neutral products.
- **Butyric and acetone–butanol**: Clostridium species; the Weizmann process made acetone this way.

Biosynthesis draws its precursors from the same pathways. Pyruvate, acetyl-CoA, oxaloacetate and α-ketoglutarate feed amino acid, lipid and nucleotide synthesis, which makes the TCA cycle **amphibolic** (both catabolic and anabolic). Reactions that refill drained cycle intermediates, such as pyruvate carboxylation to oxaloacetate, are **anaplerotic**. Bacteria growing on acetate use the **glyoxylate cycle** to make sugars from two-carbon units.

WORKED EXAMPLE 1: ENERGY FROM DIFFERENT ACCEPTORS

Question. Standard reduction potentials ($E^{\circ\prime}$) are: $NAD^+/NADH$ −0.32 V, O_2/H_2O +0.82 V, NO_3^-/NO_2^- +0.42 V. Calculate $\Delta G^{\circ\prime}$ for passing two electrons from NADH to O_2 and to nitrate, and explain why aerobic respiration gives more ATP. (F = 96.5 kJ per V per mol.) (5 marks)

1. $\Delta G^{\circ\prime} = -n\,F\,\Delta E^{\circ\prime}$, where n = electrons moved (2) and $\Delta E^{\circ\prime} = E^{\circ\prime}$(acceptor) − $E^{\circ\prime}$(donor).
2. To O_2: $\Delta E^{\circ\prime} = 0.82 - (-0.32) = 1.14$ V; $\Delta G^{\circ\prime} = -2 \times 96.5 \times 1.14 = -220$ kJ/mol.
3. To nitrate: $\Delta E^{\circ\prime} = 0.42 - (-0.32) = 0.74$ V; $\Delta G^{\circ\prime} = -2 \times 96.5 \times 0.74 = -143$ kJ/mol.
4. Oxygen releases about 1.5 times more free energy per NADH, so more protons are pumped and more ATP made. Facultative organisms use O_2 first when it is present.

WORKED EXAMPLE 2: ATP BALANCE

Question. How many glucose molecules must Lactococcus ferment to make as much ATP as E. coli makes from one glucose by aerobic respiration (textbook values)? (3 marks)

1. Aerobic respiration: 38 ATP per glucose. Homolactic fermentation: 2 ATP per glucose.
2. 38 ÷ 2 = 19 glucose molecules.
3. That is why fermenters use sugar fast and pile up products (lactic acid in curd), which industry then harvests.

EXAM TRAPS

- Fermentation is not "anaerobic respiration": fermentation has no ETC and no external acceptor.
- Substrate-level phosphorylation (in glycolysis and the TCA cycle) differs from oxidative phosphorylation (ATP synthase driven by a proton gradient).
- In prokaryotes the ETC is in the plasma membrane; writing "mitochondria" loses marks.
- Methanogens are archaea, not bacteria.

CHECK YOURSELF

– REMEMBER **Give the net yield of the EMP and ED pathways per glucose.** 2 marks

EMP: 2 ATP, 2 NADH. ED: 1 ATP, 1 NADH, 1 NADPH.

– REMEMBER **Name three final electron acceptors used in anaerobic respiration.** 2 marks

Nitrate, sulfate, CO_2 (also fumarate, Fe^{3+}).

– UNDERSTAND **Why must fermenting cells regenerate NAD^+?** 2 marks

Glycolysis needs NAD^+ to accept electrons at the glyceraldehyde-3-phosphate step; without regeneration it stops and so does ATP supply.

– UNDERSTAND **Why is the TCA cycle called amphibolic?** 2 marks

It oxidises acetyl-CoA for energy and also supplies precursors (oxaloacetate, α-ketoglutarate) for biosynthesis.

– APPLY **$\Delta G^{\circ\prime}$ for NADH (−0.32 V) to fumarate/succinate (+0.03 V)?** 2 marks

$\Delta E^{\circ\prime} = 0.35$ V; $\Delta G^{\circ\prime} = -2 \times 96.5 \times 0.35 = -68$ kJ/mol.

– APPLY **A culture is methyl red positive and VP negative. Which fermentation?** 2 marks

Mixed acid fermentation, typical of E. coli.

– ANALYZE **Yeast in a sealed sugar solution makes ethanol, but stirred with air it grows far more and makes little ethanol. Explain.** 4 marks

With O_2 yeast respires, getting much more ATP per glucose, so more biomass forms and pyruvate goes to the TCA cycle. Without O_2 it ferments: 2 ATP per glucose, and NADH is reoxidised by reducing acetaldehyde to ethanol. (At high sugar levels yeast can ferment even with air, the Crabtree effect.)

BEYOND THE SYLLABUS

Metabolic engineering redirects these pathways: E. coli strains are built to make succinate, isobutanol or 1,3-propanediol, and flux balance analysis models every reaction in a genome-scale network. Electroactive bacteria such as Geobacter respire onto electrodes, which is the basis of microbial fuel cells. Search "flux balance analysis COBRA" and "extracellular electron transfer". This is not for the exam.

4.6 Photophosphorylation and

secondary metabolism in microbes

Phototrophic microbes make ATP with light. **Photophosphorylation** uses light energy to drive electrons through a membrane chain, which pumps protons; ATP synthase then makes ATP as in respiration (see 4.5). The second half of this chapter turns to **secondary metabolism**: compounds such as antibiotics and pigments that a cell does not need to grow but makes mainly after growth slows. The other universities leave them to Biochemistry or Industrial Microbiology.

Cyclic and non-cyclic photophosphorylation

Light is captured by chlorophyll (cyanobacteria) or bacteriochlorophyll (purple and green bacteria) in a **reaction centre**. An excited electron leaves the reaction centre and passes along carriers such as quinones and cytochromes.

- **Non-cyclic** (oxygenic, in cyanobacteria): two photosystems in series. Photosystem II splits water, releasing O_2; electrons flow through the cytochrome b_6f complex to photosystem I and finally reduce $NADP^+$ to NADPH. Both ATP and NADPH are made.
- **Cyclic**: electrons from photosystem I return to the cytochrome complex and back to photosystem I. Only ATP is made, no NADPH and no O_2. Cells use it to top up ATP.
- **Anoxygenic** photosynthesis (purple and green sulfur bacteria, heliobacteria): only one photosystem, and electrons cycle, so ATP comes by cyclic photophosphorylation. Water is not split and no O_2 forms. For reducing power they take electrons from H_2S, H_2 or organic compounds, which is why purple sulfur bacteria deposit sulfur granules.

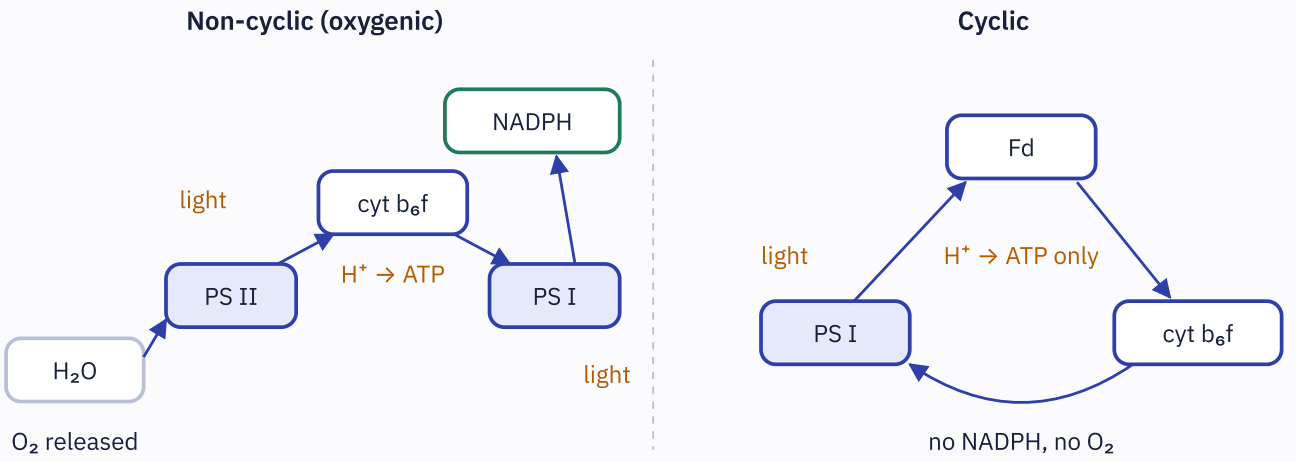


Figure 4.6.1 Electron flow in cyanobacterial photophosphorylation. Left, non-cyclic: water is the electron donor, NADPH is the end point, and O_2 is a by-product. Right, cyclic: electrons return to photosystem I, so only the proton gradient and ATP are produced. Anoxygenic bacteria work like the right panel with one photosystem (Fd = ferredoxin).

Primary and secondary metabolism

Primary metabolites are needed for growth and are made during the log phase: amino acids, nucleotides, ethanol, organic acids. **Secondary metabolites** are not needed for growth, are often species-specific, and are usually made in the late log and stationary phase. Industry calls the growth phase the **trophophase** and the production phase the **idiophase**.

Examples: penicillin (Penicillium chrysogenum), streptomycin (Streptomyces griseus), and many other antibiotics from Streptomyces; pigments such as prodigiosin (Serratia); toxins such as aflatoxin (Aspergillus flavus); and immunosuppressants such as cyclosporin (Tolypocladium). Secondary metabolites are made from primary building blocks (acetyl-CoA, amino acids) by long enzyme pathways, often encoded together in a **biosynthetic gene cluster**.

Production is switched on by signals of slowing growth. Rich, quickly used carbon such as glucose represses many antibiotic pathways, so penicillin fermentations feed lactose or sugar slowly. Phosphate and nitrogen limitation, and quorum sensing (cell density signals), also trigger production.

WORKED EXAMPLE

Question. A purple sulfur bacterium grows in light with H_2S and CO_2 but no O_2 forms. (a) Name its nutritional type. (b) How does it make ATP and reducing power? (c) Why does it not release O_2? (4 marks)

1. Energy from light, electrons from H_2S (inorganic), carbon from CO_2: photolithoautotroph (see 4.1).
2. ATP: cyclic photophosphorylation through one bacteriochlorophyll photosystem. Reducing power: electrons from H_2S, which is oxidised to sulfur.
3. It has no photosystem II and cannot split water, so no O_2 forms; this is anoxygenic photosynthesis.

EXAM TRAPS

- Cyclic flow makes only ATP; non-cyclic makes ATP and NADPH.
- Only cyanobacteria (among bacteria) make O_2; purple and green bacteria are anoxygenic.
- Secondary does not mean unimportant: antibiotics are secondary metabolites.
- Ethanol and citric acid are primary metabolites, even though they are big industrial products.

CHECK YOURSELF

- REMEMBER **Define photophosphorylation.** 1 mark

  ATP synthesis driven by light-powered electron flow and the resulting proton gradient.

- REMEMBER **Name two secondary metabolites and their producers.** 2 marks

  Penicillin from Penicillium chrysogenum; streptomycin from Streptomyces griseus.

- UNDERSTAND **Distinguish trophophase and idiophase.** 2 marks

  Trophophase: rapid growth, primary metabolites. Idiophase: slow or no growth, secondary metabolites made.

- APPLY **A cell has excess NADPH but needs ATP. Which flow should it use?** 2 marks

  Cyclic photophosphorylation, which makes ATP without more NADPH.

- ANALYZE **Why does a penicillin fermentation fed glucose all at once give low yield?** 3 marks

  Plentiful glucose keeps cells in fast growth and represses the antibiotic pathway (carbon catabolite repression). Slow feeding or lactose lets cells enter the idiophase.

BEYOND THE SYLLABUS

Genome mining with tools such as antiSMASH finds silent biosynthetic gene clusters that could encode new antibiotics; switching them on is a major research field. Cyanobacteria are being engineered to turn light and CO_2 directly into fuels and chemicals. Search "antiSMASH" and "cyanobacterial biofactories". This is not for the exam.

4.7 Bacterial recombination: transformation, transduction, conjugation

Bacteria reproduce asexually, yet they swap genes all the time. In **horizontal (lateral) gene transfer**, DNA moves from a donor cell to a recipient cell of the same generation, not from parent to daughter. There are three routes: naked DNA taken up from outside (**transformation**), DNA carried by a bacteriophage (**transduction**), and DNA passed through direct cell contact (**conjugation**). Incoming DNA survives only if it is a self-replicating plasmid or if it is joined into the chromosome by **homologous recombination**.

You need: bacteriophage lytic and lysogenic cycles from Unit 3, *Bacteriophages: structure and lytic and lysogenic cycles*.

Homologous recombination

Donor DNA that enters a cell is usually a fragment. The RecA protein coats a single strand of it and pairs it with the matching (homologous) stretch of the recipient chromosome. Two crossovers swap the donor segment in, and the replaced recipient piece is degraded. A recipient that has taken in a donor gene this way is a **recombinant**. A *recA* mutant cannot recombine, so it is used to keep cloned DNA stable.

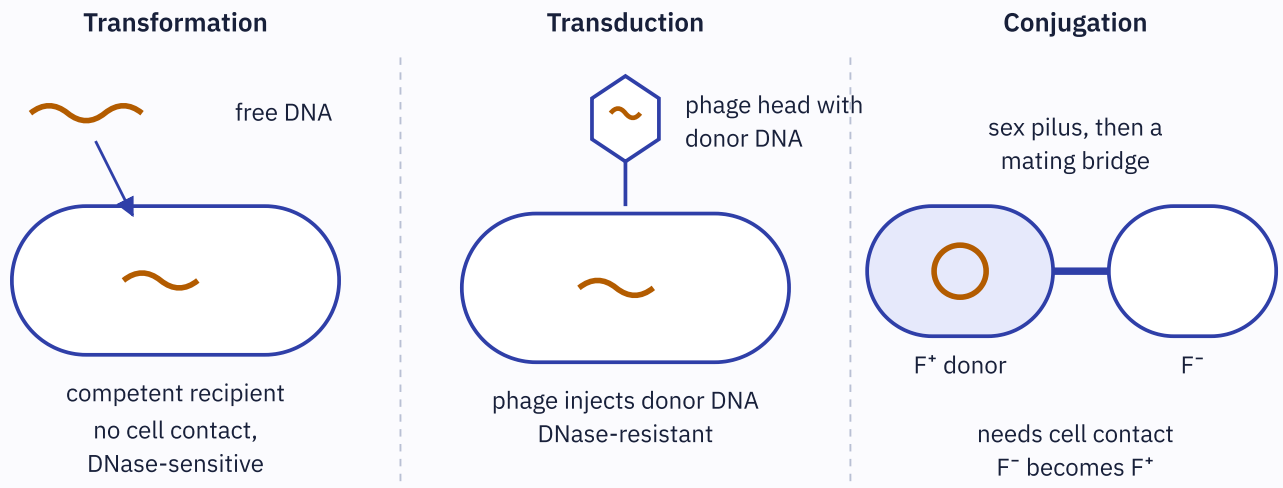


Figure 4.7.1 The three routes of gene transfer. The two tests that tell them apart: adding DNase to the medium blocks only transformation, and separating cells by a filter that lets phage through (the U-tube test) blocks only conjugation.

Transformation

In 1928 Frederick Griffith injected mice with live harmless (rough, R) *Streptococcus pneumoniae* plus heat-killed virulent (smooth, S) cells. The mice died, and live S bacteria were recovered: something from the dead cells had "transformed" the living ones. In 1944 Oswald Avery, Colin MacLeod and Maclyn McCarty showed the transforming principle was DNA, since only DNase destroyed it.

A cell able to take up DNA is **competent**. Natural competence is found in S. pneumoniae, Bacillus subtilis, Haemophilus influenzae and Neisseria. Usually one strand of the fragment enters and is recombined in. E. coli is not naturally competent, so labs make it so **artificially**: cold $CaCl_2$ treatment followed by a brief heat shock (about 42 °C), or **electroporation**, a short high-voltage pulse that opens pores. This is how plasmids are put into bacteria in genetic engineering.

Transduction

Zinder and Lederberg discovered transduction in Salmonella with phage P22 in 1952.

- **Generalized transduction.** During the lytic cycle, a phage such as P22 or P1 sometimes packs a piece of host DNA instead of its own. This "transducing particle" injects it into the next cell. Any gene can be carried.
- **Specialized transduction.** A temperate phage such as λ (lambda) leaves the chromosome imprecisely when induced, taking genes beside its attachment site (gal or bio for λ). Only those neighbouring genes are carried.

Two genes carried in the same particle are **cotransduced**; the more often it happens, the closer they lie. If the donor DNA is neither recombined nor degraded, it persists in one cell line without replicating: **abortive transduction**.

Conjugation

Joshua Lederberg and Edward Tatum showed gene exchange between E. coli strains in 1946; Bernard Davis's U-tube showed it needs contact. The donor carries the **F (fertility) plasmid**, which codes for the sex pilus and the transfer machinery.

1. **$F^+ \times F^-$.** The pilus contacts the F^- cell and pulls the cells together. One strand of F is nicked and passed across while the donor makes a new copy (rolling-circle replication). The recipient makes the complementary strand and becomes F^+. No chromosomal genes move.
2. **Hfr × F^-.** In an Hfr (high frequency of recombination) cell, F is integrated into the chromosome. Transfer starts inside F and drags chromosomal genes behind it in a fixed order. The bridge usually breaks before all of it crosses (the whole E. coli chromosome needs about 100 minutes), so the recipient gets donor genes but stays F^-.
3. **F′ × F^-.** When F leaves the chromosome imprecisely it takes nearby genes, giving an F′ plasmid. The recipient becomes F′ and partly diploid (a merodiploid) for those genes.

Interrupted mating (Wollman and Jacob) maps genes: Hfr and F^- cells are mixed, samples are shaken apart in a blender at set times, and each gene is recorded when its recombinants first appear. Map distance is given in minutes.

Implications

Recombination spreads antibiotic resistance: R plasmids carrying several resistance genes move by conjugation, even between species, which is why a resistant gut bacterium can hand resistance to a pathogen (Unit 5, *Antimicrobial resistance*). Pathogenicity islands and toxin genes (the cholera toxin gene arrived by phage) also move this way. The same tools let us map genes, build strains and clone DNA.

WORKED EXAMPLE 1: TRANSFORMATION EFFICIENCY

Question. 2 µL of plasmid at 50 ng/µL is added to competent E. coli and the final volume after recovery is 1 mL. 100 µL is spread on ampicillin agar and 180 colonies grow. Find the transformation efficiency in transformants per µg. (4 marks)

1. DNA added = 2 µL × 50 ng/µL = 100 ng = 0.1 µg.
2. Fraction plated = 100 µL ÷ 1000 µL = 0.1, so DNA on the plate = 0.01 µg.
3. Efficiency = 180 ÷ 0.01 µg = 1.8×10^4 transformants per µg.

WORKED EXAMPLE 2: INTERRUPTED MATING

Question. In an Hfr × F^- cross, recombinants for genes a^+, b^+, c^+ and d^+ first appear at 25, 9, 18 and 11 minutes. Give the gene order and the distance between a and c. (3 marks)

1. Order by entry time: b (9), d (11), c (18), a (25). Gene order from the origin: b–d–c–a.
2. Distance a to c = 25 − 18 = 7 minutes.

EXAM TRAPS

- F^+ × F^- turns the recipient F^+; Hfr × F^- usually does not, because the F sequence enters last.
- Generalized carries any gene; specialized only genes next to the prophage site.
- DNase blocks transformation only; the U-tube blocks conjugation only.
- A chromosomal fragment must be recombined into the chromosome or it is lost; only a plasmid can replicate on its own.

CHECK YOURSELF

– REMEMBER **Who showed that the transforming principle is DNA, and when?** 1 mark

Avery, MacLeod and McCarty, 1944.

– REMEMBER **What does Hfr stand for?** 1 mark

High frequency of recombination: a strain with F integrated in the chromosome.

– UNDERSTAND **Why does specialized transduction carry only certain genes?** 2 marks

The phage DNA is integrated at one site; faulty excision takes only the host genes next to that site.

– UNDERSTAND **What makes a cell competent?** 2 marks

A physiological state with DNA-binding and uptake proteins on the surface; natural in some species, induced artificially by $CaCl_2$ and heat shock or electroporation.

– APPLY **0.5 µg of plasmid, all plated, gives 400 colonies. Efficiency?** 2 marks

400 ÷ 0.5 = 800 transformants per µg (8×10^2 /µg).

– APPLY **Gene transfer occurs between two strains in a U-tube with a filter, and DNase has no effect. Which mechanism?** 2 marks

Transduction: no contact needed and DNA is protected inside phage.

– ANALYZE **Why do genes near the Hfr origin appear in more recombinants than distant ones?** 3 marks

Transfer is linear from the origin and the bridge breaks at random times; early genes cross in most matings, late genes only in long ones.

BEYOND THE SYLLABUS

Integrative conjugative elements (ICEs) behave like plasmids that live in the chromosome. CRISPR–Cas systems are bacteria's defence against incoming phage and plasmid DNA, later turned into gene-editing tools. Search "resistome" and "integrative conjugative element". This is not for the exam.

4.8 Genetics of viruses and bacteria

Microbial genetics asks how a microbe's genes are organised and how they change. Bacteria have small genomes, one copy of each gene (they are haploid) and a new generation every half hour, so a mutation shows up at once and can be selected from billions of cells. Viruses change even faster. That speed made E. coli and its phages the organisms in which much of molecular biology was worked out. It is tagged advanced because few syllabi give it a unit of its own.

The bacterial genome

Most bacteria have one circular chromosome, packed in the **nucleoid** without a nuclear membrane; E. coli K-12's is about 4.6 million base pairs. Genes are close together with little non-coding DNA, and related genes are often grouped in **operons** under one promoter (the lac operon is the classic case, covered in Biochemistry and Molecular Biology). Extra genes sit on **plasmids**: small, usually circular DNA that replicates on its own and carries optional traits such as antibiotic resistance (R plasmids), toxins or the F factor (see 4.7). **Transposons** ("jumping genes") move from place to place in the genome, and insertion sequences are the simplest of them.

Mutation

A **mutation** is a heritable change in DNA sequence. **Spontaneous** mutations come from replication errors; **induced** ones from mutagens such as UV light (thymine dimers), base analogues (5-bromouracil) and alkylating agents. Point mutations can be silent, missense (one amino acid changed) or nonsense (a stop codon); insertions or deletions that are not a multiple of three cause **frameshifts**. A **prototroph** (wild type) grows on minimal medium; an **auxotroph** needs a supplement because it has lost a biosynthetic gene. Conditional mutants such as temperature-sensitive ones work only at the permissive temperature.

Two classic experiments showed that mutations arise at random, before selection, not in response to it. The **Luria–Delbrück fluctuation test** (1943) found that the number of phage-resistant E. coli varied hugely between parallel cultures, which fits random early mutations. **Replica plating** (Joshua and Esther Lederberg, 1952) showed resistant colonies were already present on a plate never exposed to the agent.

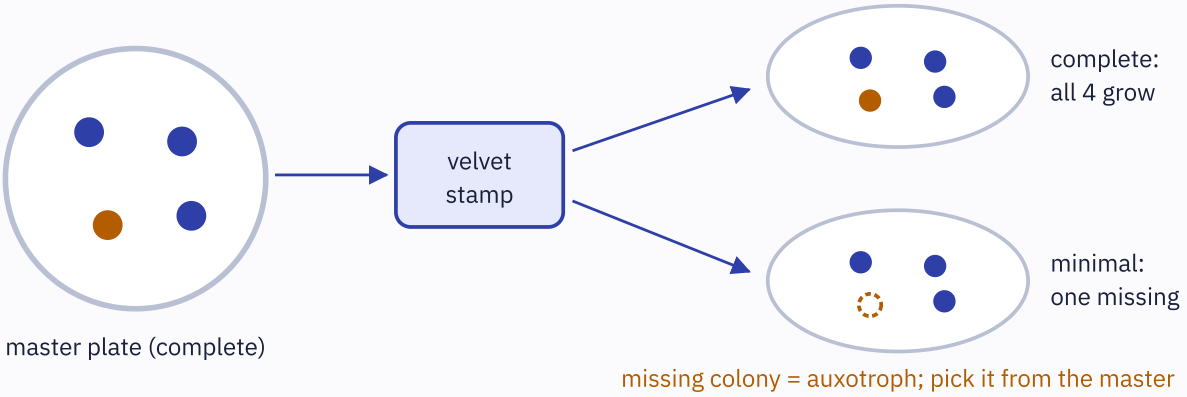


Figure 4.8.1 Replica plating. One velvet press copies every colony, in the same positions, onto two plates. The colony that grows on complete medium but not minimal medium (orange) is an auxotroph, and it is recovered from the master plate.

The **Ames test** uses histidine-requiring Salmonella mutants: a chemical that raises the rate of reversion to his^+ is a mutagen and a possible carcinogen. Liver enzyme extract is added because some chemicals become mutagenic only after the body processes them.

Viral genetics

Viral genomes are DNA or RNA, single- or double-stranded, and the Baltimore scheme sorts them by how they make mRNA (Unit 3 covers structure and classification). Key genetic features:

- **High mutation rates** in RNA viruses, because RNA polymerases do not proofread. Small changes in surface proteins (antigenic drift) are why flu vaccines are updated.
- **Reassortment**: viruses with segmented genomes, such as influenza, swap whole segments when two strains infect one cell, giving antigenic shift and pandemic strains.
- **Recombination and complementation** in phages: when two mutant phages infect one cell, recombination can give wild-type progeny, and the frequency maps the mutations. If two mutants help each other grow without recombining, they are in different genes (complementation). Seymour Benzer used phage T4 rII mutants this way to map a gene at fine scale.

WORKED EXAMPLE

Question. In an Ames test, a plate without the chemical shows 25 his^+ revertant colonies; with the chemical and liver extract it shows 300; with the chemical but no liver extract it shows 28. Interpret. (4 marks)

1. 25 colonies is the spontaneous reversion rate (background).
2. With liver extract the count is 300 ÷ 25 = 12 times background: the chemical is mutagenic.
3. Without extract, 28 is about background, so the chemical itself is not mutagenic; a product made by liver enzymes is.
4. Conclusion: a pro-mutagen, and a suspected carcinogen needing further animal testing.

EXAM TRAPS

- Mutations are random; selection only reveals them (replica plating, fluctuation test).
- Auxotroph needs a supplement; prototroph does not. Do not swap them.
- Antigenic drift (point mutation) differs from antigenic shift (reassortment).
- Plasmids are optional; the chromosome carries the essential genes.

CHECK YOURSELF

– REMEMBER **What is an auxotroph?** 1 mark

A mutant that cannot make a needed nutrient and must be given it.

– REMEMBER **Name two mutagens and their effect.** 2 marks

UV light, thymine dimers; 5-bromouracil, a base analogue that mispairs.

– UNDERSTAND **Why do RNA viruses evolve faster than DNA viruses?** 2 marks

Their RNA polymerases lack proofreading, so many more errors occur per copy.

– APPLY **How would you find a tryptophan auxotroph among 200 colonies?** 3 marks

Replica-plate onto minimal medium and minimal plus tryptophan; a colony growing only with tryptophan is the auxotroph.

– ANALYZE **What did the wide spread of resistant counts in the fluctuation test prove?** 3 marks

If phage induced resistance, every culture would show similar counts. Large jackpots mean mutations arose at random times before phage exposure, early ones giving many resistant descendants.

BEYOND THE SYLLABUS

Whole-genome sequencing now tracks outbreaks mutation by mutation, as was done for SARS-CoV-2 variants. Tn-seq floods a genome with transposon insertions to find which genes are essential. Search "genomic epidemiology" and "Tn-seq". This is not for the exam.

Unit summary

TERM	MEANING OR FORMULA	CHAPTER
Nutritional type	energy (photo/chemo) + electrons (litho/organo) + carbon (auto/hetero) + "troph"	4.1
Selective / differential medium	decides who grows / makes groups look different (MacConkey is both)	4.1
Pure culture	one kind of organism from one CFU; streak, spread or pour plate	4.2
Preservation	subculture at 4 °C; oil overlay; glycerol at −80 °C; liquid N_2 at −196 °C; lyophilisation	4.2
Generations	$n = 3.32\ (\log N - \log N_0)$; $g = t / n$	4.3
Specific growth rate	$dX/dt = \mu X$; $t_d = 0.693 / \mu$	4.3
Monod equation	$\mu = \mu_{max}\ S / (K_s + S)$	4.3
Chemostat	$D = F/V$; $\mu = D$; $\bar{S} = K_s\ D/(\mu_{max} - D)$; $\bar{X} = Y(S_0 - \bar{S})$	4.3
Viable count	CFU/mL = colonies ÷ (mL plated × dilution); use 30–300	4.4
Free energy from redox	$\Delta G^{\circ\prime} = -n\ F\ \Delta E^{\circ\prime}$	4.5
ATP per glucose	aerobic up to 38 (textbook); homolactic fermentation 2	4.5
Cyclic / non-cyclic	ATP only / ATP + NADPH + O_2 (oxygenic)	4.6
Idiophase	stationary-phase production of secondary metabolites	4.6
Transfer routes	transformation (free DNA), transduction (phage), conjugation (contact, F plasmid)	4.7
Transformation efficiency	colonies ÷ µg DNA plated	4.7
Random mutation	fluctuation test (1943), replica plating (1952)	4.8

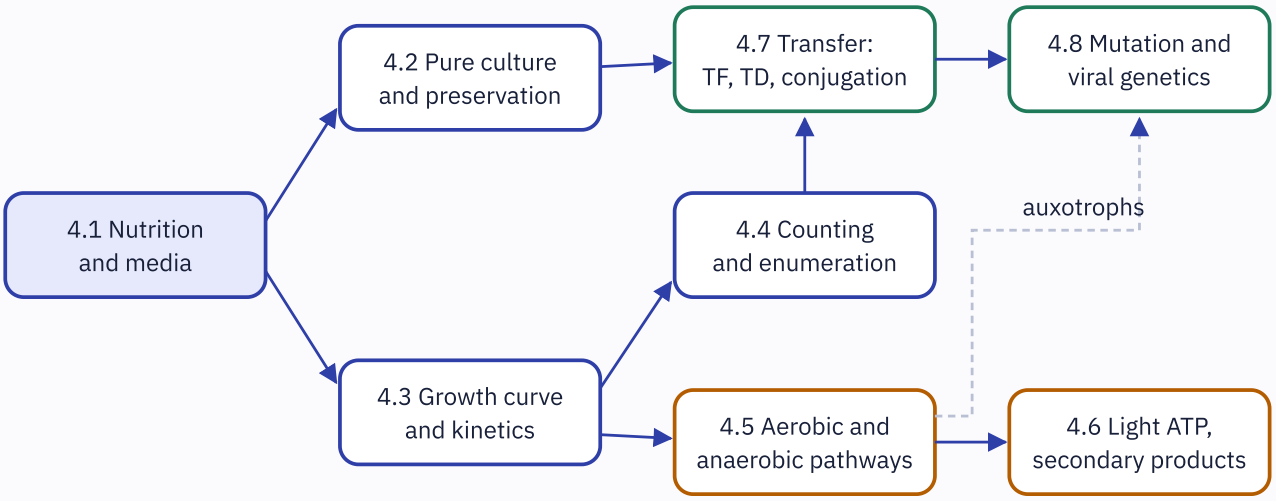


Figure 4.S.1 How the chapters connect. Media (4.1) make pure cultures and growth possible; growth is measured by counting (4.4) and powered by metabolism (4.5, 4.6). Gene transfer (4.7) is detected by counting recombinant colonies on selective media, and auxotrophs link metabolism to genetics (4.8).

1. Microbes are named by their energy, electron and carbon sources; most lab and industrial microbes are chemoorganoheterotrophs.
2. Media are chosen by composition (defined or complex), state (broth, semi-solid, solid) and purpose (selective, differential, enriched, enrichment, reducing).
3. A pure culture comes from one well-isolated colony; colder and drier storage keeps it longer and more stable.
4. A batch culture shows lag, log, stationary and death phases; generation time is read from the straight log phase on a log plot.
5. Monod links growth rate to substrate; in a chemostat μ equals the dilution rate until washout.
6. Plate counts give living CFU (30 to 300 per plate); chamber counts and turbidity include dead cells.
7. After pyruvate, the electron acceptor decides the yield: O_2 gives the most ATP, other acceptors less, fermentation only 2 per glucose.
8. Light drives cyclic (ATP only) or non-cyclic (ATP, NADPH, O_2) photophosphorylation; antibiotics are secondary metabolites of the idiophase.
9. Bacteria exchange genes by transformation, transduction and conjugation, and this spreads antibiotic resistance.
10. Mutations arise at random before selection; RNA viruses mutate fast and segmented viruses reassort.

Exam question bank

Remember and understand

- UNDERSTAND **Classify microorganisms by nutritional type with one example each.** 8 marks

  Outline: three criteria (energy, electron, carbon source); photoautotroph (cyanobacteria), photoheterotroph (purple non-sulfur), chemolithoautotroph (Nitrosomonas), chemoorganoheterotroph (E. coli); macro- and micronutrients and growth factors; a 2 × 2 table.

- UNDERSTAND **Describe the types of culture media with examples.** 10 marks

  Outline: by composition (defined, complex), state (agar levels), purpose: selective (mannitol salt), differential (MacConkey), enriched (blood, chocolate), enrichment (selenite F), reducing (thioglycollate), transport; haemolysis types on blood agar.

- UNDERSTAND **Draw and explain the bacterial growth curve.** 8 marks

  Outline: log-scale y-axis; reasons for each phase; generation time formula; diauxie and synchronous growth as extras; batch versus continuous culture.

- REMEMBER **Explain methods of preserving microbial cultures.** 8 marks

  Outline: subculture, oil overlay, glycerol freezing (cryoprotectant), liquid nitrogen, lyophilisation steps; advantages and limits; culture collections (MTCC, NCIM, ATCC).

- UNDERSTAND **Explain conjugation, including F^+, Hfr and F' cells.** 12 marks

  Outline: F plasmid and pilus; rolling-circle transfer; outcomes of each cross; interrupted mating and map minutes; R plasmids and AMR.

Apply

- APPLY **A culture grows from 5×10^4 to 3.2×10^6 cells/mL in 3 h of log phase. Find n, g and μ, and sketch the curve.** 8 marks

  Outline: log values 4.699 and 6.505; n = 3.32 × 1.806 = 6.0; g = 180/6 = 30 min; μ = 0.693/0.5 = 1.39 h^{-1}; a straight log phase on a log plot.

– APPLY **A 5 L chemostat with μmax = 0.6 h^{-1}, Ks = 0.1 g/L, Y = 0.45, S_o = 20 g/L is run at 1.5 L/h. Find steady-state S, X and productivity. Will 3.5 L/h work?** 10 marks

Outline: D = 0.3 h^{-1}; $\bar{S}$ = 0.1 × 0.3/0.3 = 0.1 g/L; $\bar{X}$ = 0.45 × 19.9 = 8.96 g/L; productivity 2.69 g/L/h. At 3.5 L/h, D = 0.7 h^{-1} > μmax: washout.

– APPLY **Describe the standard plate count of a milk sample with a dilution scheme and calculation.** 10 marks

Outline: 1 mL in 9 mL steps; spread or pour plating; 30–300 rule; worked CFU/mL; why plate count underestimates (clumps, non-culturable cells); compare MPN and direct count; indicator organisms.

– APPLY **Calculate ATP yield from glucose by aerobic respiration and by two fermentations, and use redox potentials to explain the difference.** 12 marks

Outline: EMP, link reaction and TCA tallies; 38 (or about 32) ATP; homolactic 2, heterolactic 1; $\Delta G^{\circ\prime} = -nF\Delta E^{\circ\prime}$ for NADH to O_2 (−220 kJ/mol) versus nitrate (−143 kJ/mol); fermentation has no ETC.

– APPLY **Design an experiment to isolate a streptomycin-resistant, leucine-requiring mutant of E. coli and keep it for five years.** 12 marks

Outline: mutagenesis (UV); plate on streptomycin agar with leucine; replica plate to minimal medium to find leu^- colonies; streak to purity; confirm; store as glycerol stock at −80 °C or lyophilise. Spans 4.1, 4.2 and 4.8.

Analyze and evaluate

– ANALYZE **Compare transformation, transduction and conjugation, and explain how each could spread a resistance gene in a hospital.** 12 marks

Outline: table of DNA source, contact needed, DNase sensitivity, vector, size transferred; Griffith and Avery; generalized versus specialized; R plasmids by conjugation as the main route; DNase and U-tube tests.

– ANALYZE **Compare the methods of measuring microbial growth for a yeast fermenter, drinking water and a slow-growing soil bacterium.** 10 marks

Outline: fermenter: OD and dry weight with periodic plate counts; water: membrane filtration or MPN for coliforms; soil: plate counts miss non-culturable cells, so use microscopy, flow cytometry or qPCR; viable versus total counts.

– ANALYZE **Explain why a penicillin fermentation is run differently from an ethanol fermentation, using growth phases and metabolism.** 10 marks

Outline: ethanol is a primary metabolite made with growth, by yeast under anaerobic conditions; penicillin is a secondary metabolite of the idiophase, repressed by excess glucose, so fed slowly with aeration. Spans 4.3, 4.5 and 4.6.

– ANALYZE **Evaluate the evidence that bacterial mutations are random rather than induced by selection.** 8 marks

Outline: the two hypotheses; Luria–Delbrück fluctuation test logic and results; replica plating experiment; meaning for antibiotic use.

– ANALYZE **Contrast oxygenic and anoxygenic photosynthesis in bacteria.** 8 marks

Outline: pigments; one versus two photosystems; electron donors (H_2O versus H_2S, H_2); cyclic versus non-cyclic; O_2 and sulfur products; examples.

Glossary

Agar — Seaweed polysaccharide used at about 1.5% to solidify media; few microbes digest it.

Amphibolic — A pathway that serves both breakdown and synthesis, such as the TCA cycle.

Auxotroph — Mutant that needs a nutrient the wild type can make.

Batch culture — Growth in a closed vessel with no added medium; shows the four-phase curve.

Chemostat — Continuous culture where the dilution rate sets the growth rate.

Colony-forming unit (CFU) — A cell or clump that grows into one colony; the unit of viable counts.

Competence — The state in which a bacterium can take up free DNA.

Complex medium — Medium with ingredients of unknown exact composition, such as peptone.

Conjugation — DNA transfer through direct contact, driven by a conjugative plasmid such as F.

Defined medium — Medium whose every component and amount is known.

Diauxic growth — Two-step growth curve as a cell uses one sugar after another.

Differential medium	Medium that makes different organisms look different.
Dilution rate (D)	Flow rate divided by culture volume in a chemostat (h^{-1}).
Fermentation	Energy metabolism with no electron transport chain, in which an organic product accepts electrons from NADH.
Generation time	Time for a population to double.
Hfr	Strain with the F plasmid integrated into the chromosome.
Idiophase	Production phase of secondary metabolites, after rapid growth.
Lyophilisation	Freeze-drying: freezing, then removing water by sublimation under vacuum.
Monod equation	$\mu = \mu_{max} S/(K_s + S)$, linking growth rate to substrate.
MPN	Most probable number: a statistical count from growth in replicate tubes.
Oxidative phosphorylation	ATP synthesis driven by a proton gradient made by an electron transport chain.
Photophosphorylation	ATP synthesis driven by light-powered electron flow.
Plasmid	Small, self-replicating DNA separate from the chromosome.
Prototroph	Strain that grows on minimal medium, like the wild type.
Pure culture	Culture of one kind of organism from a single CFU.
Replica plating	Copying colonies by velvet onto several media to find mutants.
Selective medium	Medium that lets some organisms grow and suppresses others.
Specific growth rate (μ)	Rate of biomass increase per unit biomass (h^{-1}).
Substrate-level phosphorylation	Direct transfer of phosphate from an intermediate to ADP.
Transduction	Transfer of bacterial DNA by a bacteriophage.
Transformation	Uptake of free DNA from the surroundings.

Sources and further reading

Textbooks

- Willey, Sandman and Wood, *Prescott's Microbiology*, McGraw-Hill: microbial nutrition, growth, metabolism and genetics chapters (from memory; check).
- Madigan and others, *Brock Biology of Microorganisms*, Pearson: reduction potentials, anaerobic respiration and phototrophy (from memory; check).

- Pelczar, Chan and Krieg, *Microbiology*, McGraw-Hill (Indian edition widely used) (from memory; check).
- Tortora, Funke and Case, *Microbiology: An Introduction*, Pearson (from memory; check).
- Shuler and Kargi, *Bioprocess Engineering: Basic Concepts*, for Monod and chemostat kinetics (from memory; check).

Open resources

- OpenStax, *Microbiology*, section 9.1 "How Microbes Grow": growth phases, 30–300 plate rule, direct and indirect counts. openstax.org/books/microbiology/pages/9-1-how-microbes-grow
- OpenStax, *Microbiology*, section 11.6 "How Asexual Prokaryotes Achieve Genetic Diversity": Griffith and Avery, Hfr and F′ cells, 100-minute E. coli transfer. openstax.org/books/microbiology/pages/11-6-how-asexual-prokaryotes-achieve-genetic-diversity

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