Microbiology

Unit 4 · Lesson 2 · Growth curve and counting cells

The notes for Lesson 2 of Unit 4: chapters 4.3 to 4.4 of the unit book, with their figures, worked examples, exam traps and quizzes, in one file. The full unit book, with every chapter, the exam question bank and the glossary, is linked at the end.

Lesson 2 of 4 | 2 chapters | Chapters 4.3 to 4.4

About these notes

These are the notes for **Lesson 2 · Growth curve and counting cells** of the University free n easy video lessons on Microbiology Unit 4, Microbial Nutrition, Growth and Genetics. They are chapters 4.3 to 4.4 of the unit book, unchanged, so a page here is the same page in the full book.

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.

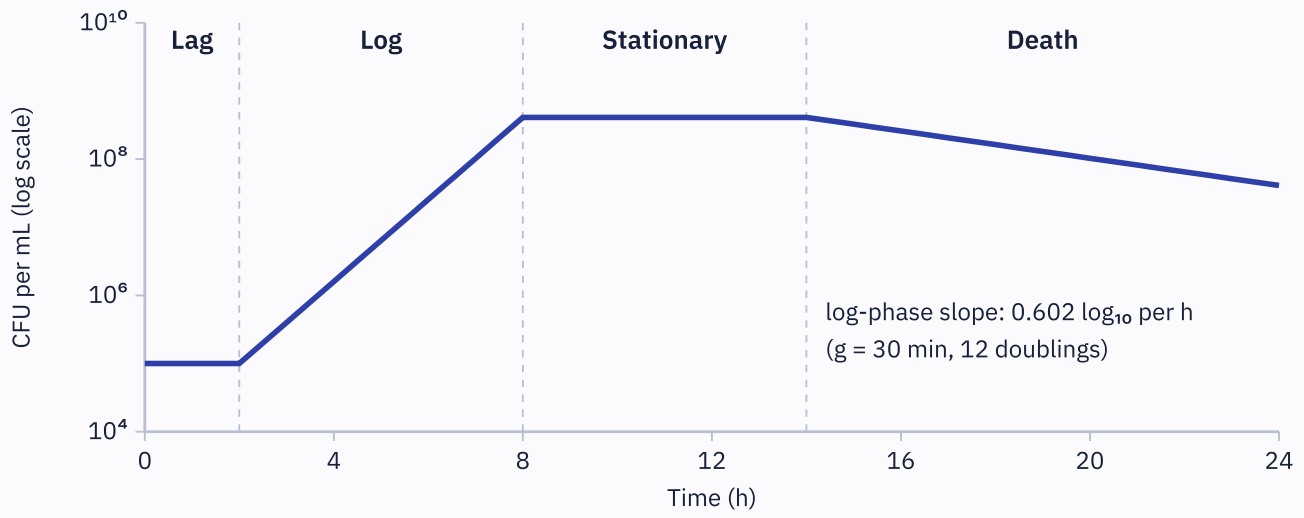


Figure 4.3.1 A batch growth curve computed for 10^5 CFU/mL inoculated with a 2 h lag, a 30 min generation time for 6 h, a stationary phase, then a slow death of 0.1 $\log_{10}$ per hour. On a log axis the log phase is a straight line; its slope gives the growth rate.

The arithmetic of doubling

Start with N_0 cells. After n generations there are

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N = N₀ × 2ⁿ
log N = log N₀ + n log 2
n = (log N − log N₀) / log 2 = 3.32 (log N − log N₀)
```

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
```

Engineers prefer the **specific growth rate** μ (mu), defined from biomass X: dX/dt = μX, so X = X_0 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 $Y_{X/S} = \Delta X / \Delta S$ 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 $\bar{S} = Ks\ D / (\mu max - D)$, 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. $\log N_o = \log (2 \times 10^3) = 3.301$; $\log N = \log (6.4 \times 10^4) = 4.806$.
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. $\mu = \ln 2 / t_d = 0.693 / 0.5 \text{ h} = 1.39\ h^{-1}$.

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 h^{-1}. Since D < μmax, there is no washout, and μ = 0.4 h^{-1}.
2. $\bar{S}$ = Ks D / (μmax − D) = 0.2 × 0.4 / (0.8 − 0.4) = 0.08 / 0.4 = 0.2 g/L.
3. $\bar{X}$ = Y (S_0 − $\bar{S}$) = 0.5 × (10 − 0.2) = 4.9 g/L.
4. Productivity = D × $\bar{X}$ = 0.4 × 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: μ = 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

μ = μmax S/(Ks + 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 × 2^{12} = 100 × 4096 = 4.1 × 10^5 cells.

– APPLY **μ = 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 μmax = 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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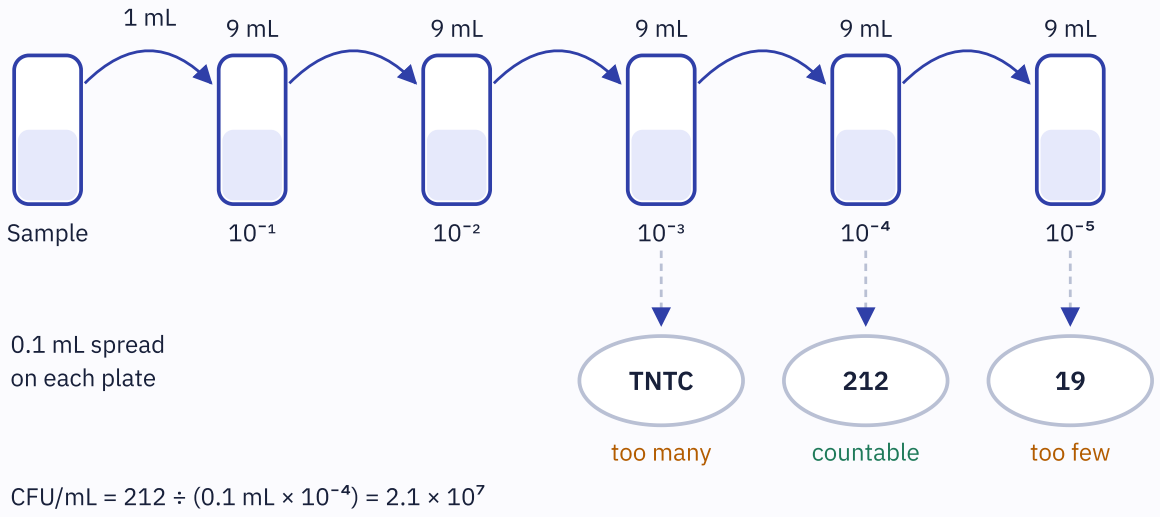


Figure 4.4.1 A tenfold serial dilution with 0.1 mL spread from the last three tubes. Only the 10^{-4} plate falls in the 30 to 300 range, so only it is used; the 10^{-5} plate agrees roughly ($19 \div 10^{-6} = 1.9 \times 10^{7}$) but is too few to trust. TNTC means too numerous to count.

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.

Key terms of this lesson

TERM	MEANING OR FORMULA	CHAPTER
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

Glossary (terms used in this lesson)

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

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.

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

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.

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.

Selective medium Medium that lets some organisms grow and suppresses others.

The full unit book

Every chapter of Unit 4, the unit summary, the exam question bank with model answers and the full glossary: Microbiology · Unit 4 · Microbial Nutrition, Growth and Genetics (one file, works offline).

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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