Culturing microorganisms and measuring their growth

Aseptic culture techniques, ways of measuring microbial growth, the bacterial growth curve and growth rate constants, and Core Practical 13.

Culturing microorganisms

Microorganisms are grown in a laboratory in **liquid broth** or on **solid agar** containing a suitable nutrient medium. **Aseptic technique** prevents contamination by unwanted microbes and prevents the culture escaping.

- **Sterilise** equipment and media by **autoclaving** (steam under pressure at 121 °C for 15 minutes), and the inoculating loop by **flaming**.
- Work near a **Bunsen flame** (the updraught carries airborne microbes away), and keep lids on as much as possible.
- Wash hands, disinfect the bench, and wear gloves and a lab coat.
- **Seal** dishes with tape (not fully), **incubate** inverted at a safe temperature (25 °C in schools), and **autoclave** cultures before disposal.

Measuring growth

Method	What it measures	Notes
Cell counts (haemocytometer, under a microscope)	The total number of cells, **living and dead**	Quick, but cannot tell live from dead (unless a stain is used)
Dilution plating (viable count)	The number of **viable** cells, as **colonies** on agar	Serially dilute the sample, spread a known volume on agar, incubate and count the colonies. Number per cm^3 = colonies × dilution factor / volume plated. Each colony is assumed to come from one cell
Mass (dry mass)	The **biomass** of the culture	Filter, dry and weigh. Destroys the sample
Turbidity (optical methods)	The **cloudiness** of a liquid culture using a **colorimeter** or **spectrophotometer**	Quick, but includes dead cells and is not sensitive at low densities

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Serial dilution

A **serial dilution** reduces the concentration by the same factor at each step (e.g. 1 cm^3 of culture into 9 cm^3 of sterile water gives a 10-fold dilution each time). If a plate prepared from the 10^{-4} dilution has 45 colonies from 0.1 cm^3, the original has $45 \times 10^4 \times 10 = 4.5 \times 10^6$ cells per cm^3.

The bacterial growth curve

A graph of **log (number of viable cells)** against time shows four phases:

Phase	What is happening
Lag	Cells adapt to the medium and synthesise enzymes, and growth is **slow** (few cells divide)
Exponential (log)	Cells divide at the **maximum rate**; the population doubles at regular intervals, because nutrients are abundant
Stationary	Growth rate **equals** death rate. Nutrients run low and **toxic waste** accumulates
Death (decline)	Death rate **exceeds** growth, as nutrients are used up and waste has built up

A **logarithmic** scale is used because the numbers cover a very large range. The exponential phase is a **straight line**.

Exponential growth rate constant

If a population has N_0 cells at the start and N cells after n generations:

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

$$\text{growth rate constant, } k = \frac{n}{t} \qquad \text{generation time} = \frac{t}{n}$$

$$\text{where } n = \frac{\log N - \log N_0}{\log 2}.$$

> **TRY IT**
>
> A culture grows from 1×10^3 to 1×10^6 cells in 5 hours of exponential growth. Calculate the number of generations, and the growth rate constant.

$n = \dfrac{\log 10^6 - \log 10^3}{\log 2} = \dfrac{3}{0.301} = 9.97$ generations. $k = 9.97/5 = 2.0$ generations per hour (generation time 30 minutes).

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Core Practical 13: growth in a liquid culture

1. Prepare a **sterile broth** and **inoculate** with a known volume of bacteria or yeast using aseptic technique.
2. **Incubate** at a constant temperature.
3. At **regular intervals**, take a sample and measure its growth by **turbidity** (colorimeter, with a blank of sterile broth) or by dilution plating.
4. Plot the results and identify the phases.

Take into account the **safe and ethical use** of microorganisms: use a non-pathogenic strain (e.g. *E. coli* K12 or yeast), keep the culture away from the face, and autoclave afterwards. Control the volume, temperature, aeration and starting concentration.

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