Introduction to Microbiology

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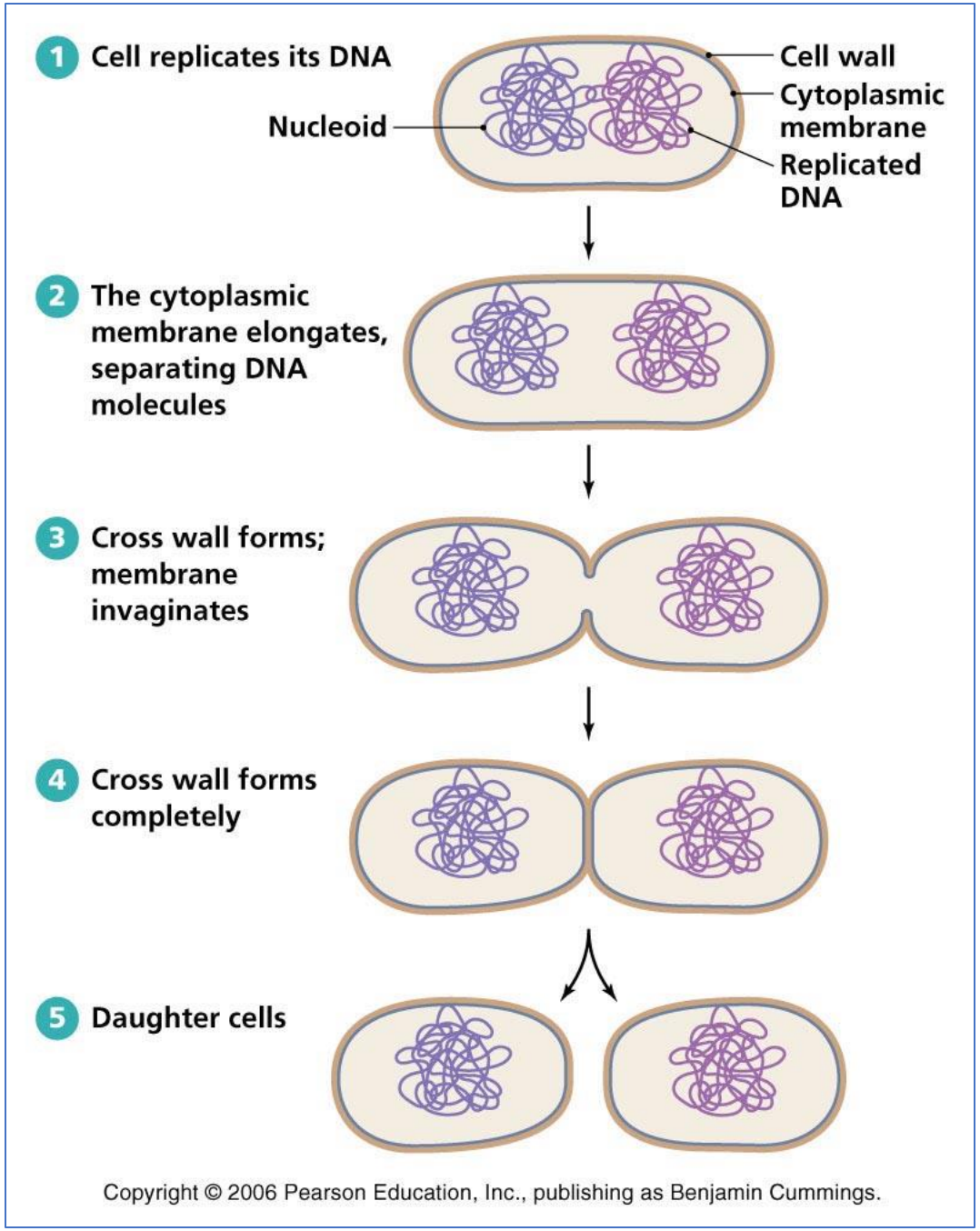
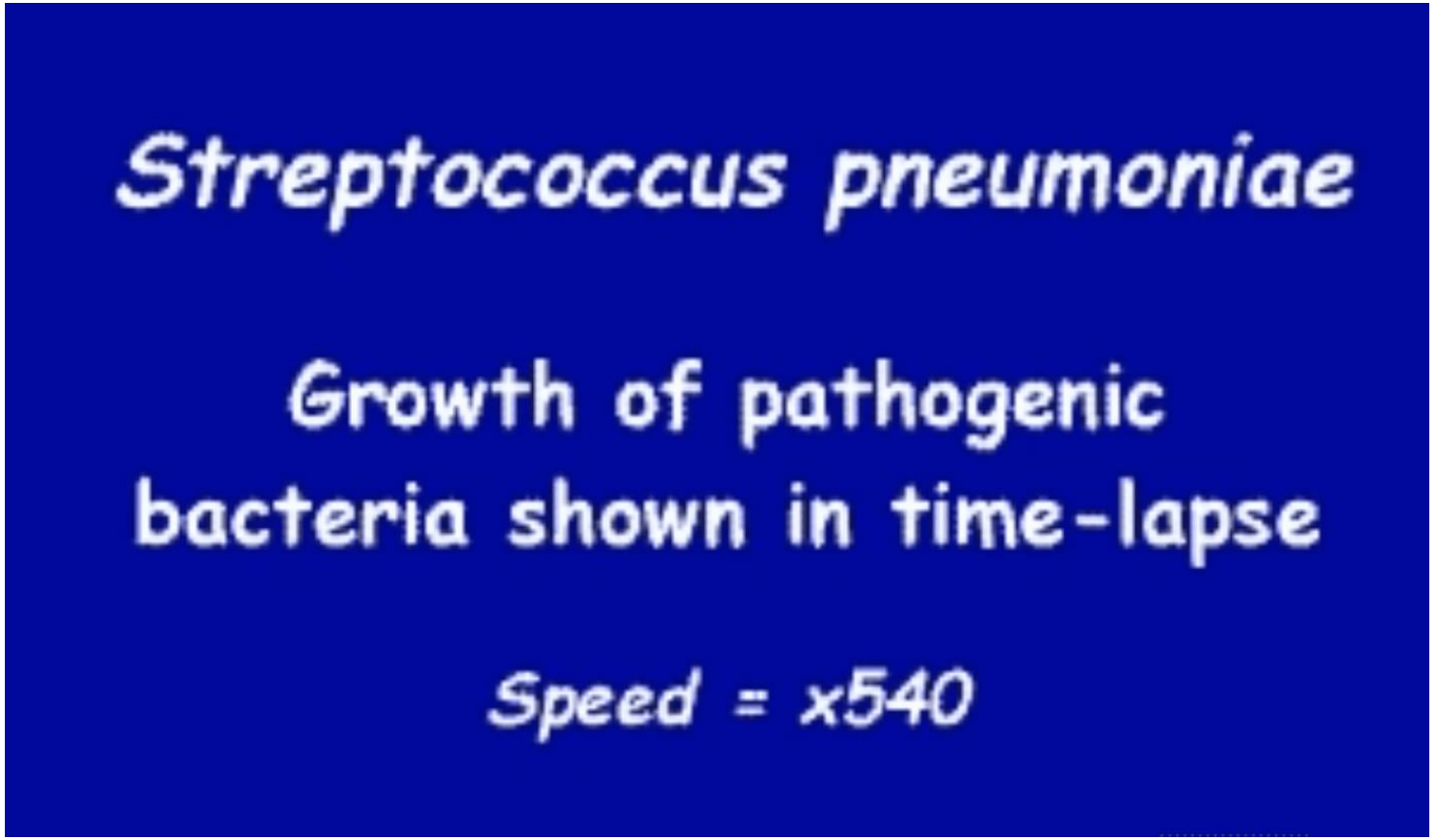
Lecture 3

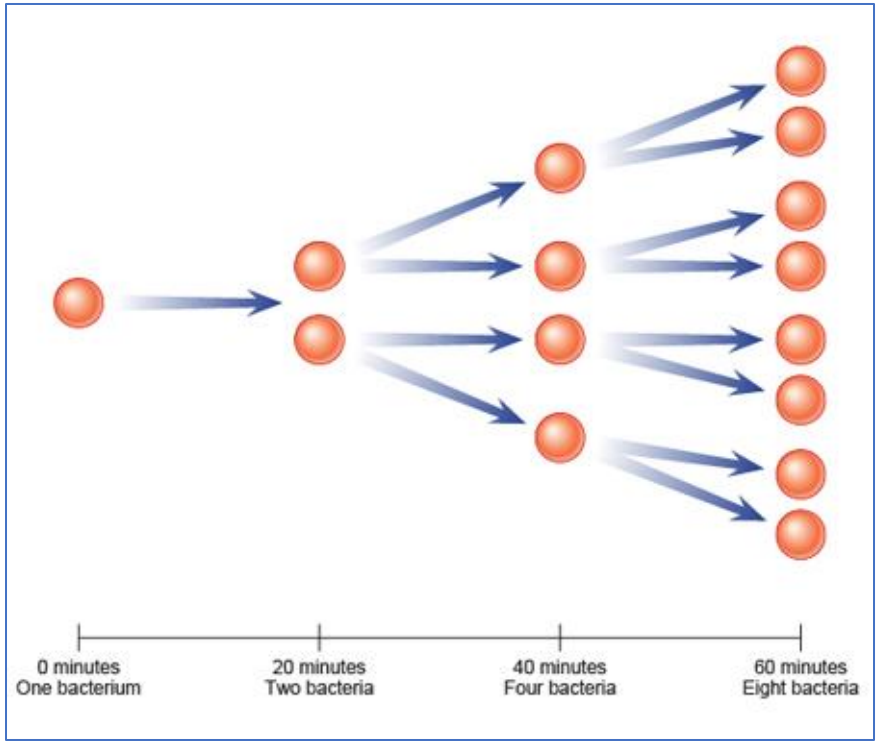
Bacterial survival and growth

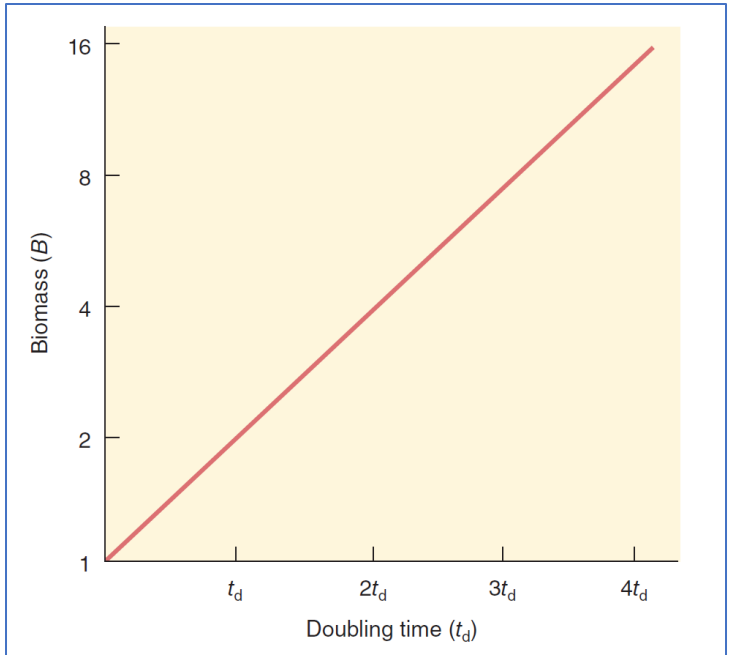
- Bacterial survival and growth depend on utilization of nutrients in its niche.
- Lab cultures provide optimum conditions for bacterial growth, defined as an increase in the sum of all the components of an organism.
- Bacteria divide by binary fission producing 2 identical offspring.



Bacterial survival and growth

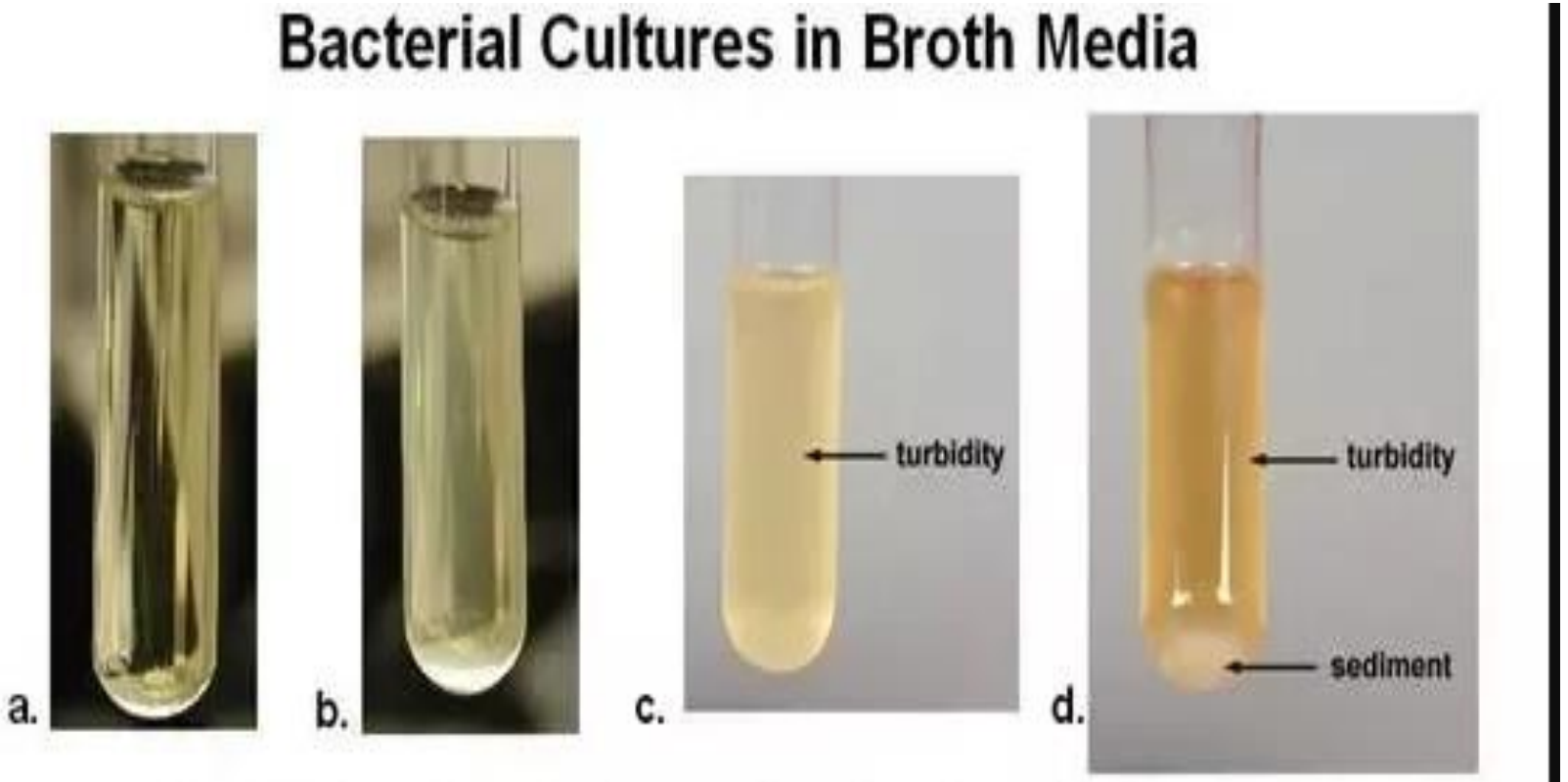






- Exponential growth. The biomass (B) doubles with each doubling time (td).

Measuring bacterial growth

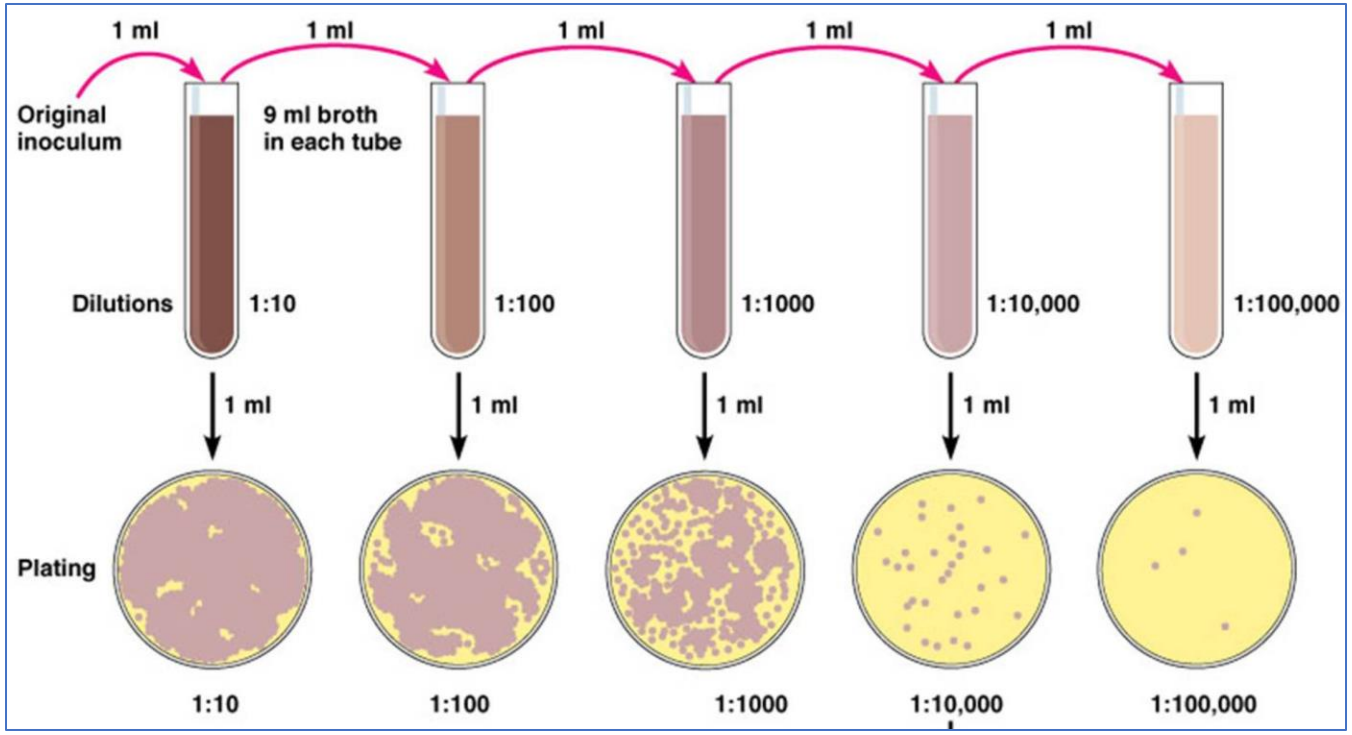


a. Sterile (uninoculated broth) - note how clear the media is
b. Broth showing slight turbidity (some bacterial growth)
c. Broth showing significant turbidity (a lot of bacterial growth)
d. Broth that hasn't been agitated (shaken)

Measuring bacterial growth

- To know how many microorganisms there are in a solution of bacteria or fungi, it's usually too time-consuming to count every cell individually under the microscope.

- By **diluting** a sample of microbes and **spreading** it across a **petri plate**, microbiologists can instead count groups of microbes, called colonies, **with the naked eye**. Each colony is assumed to have grown from a single **colony-forming unit**, or **CFU**.

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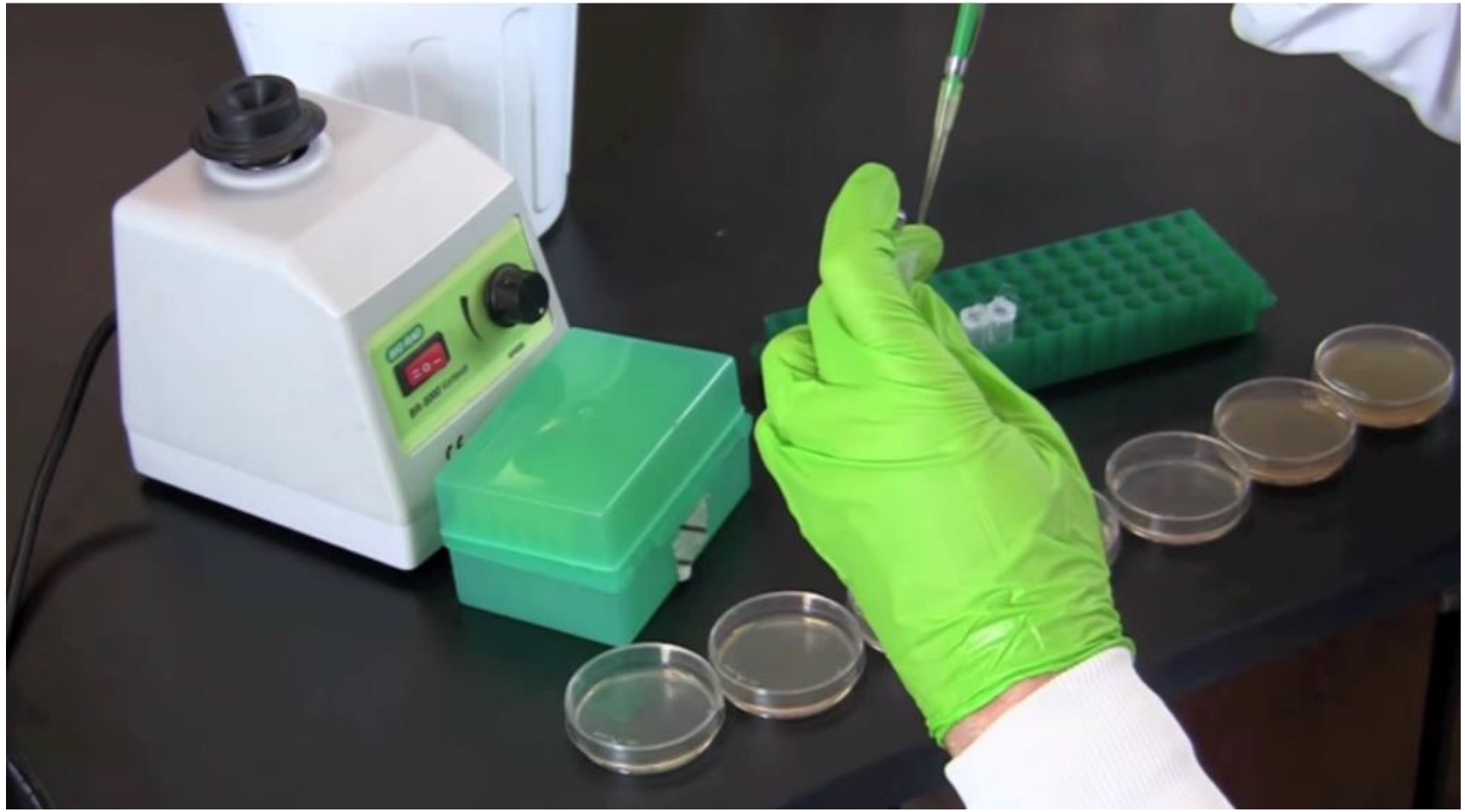
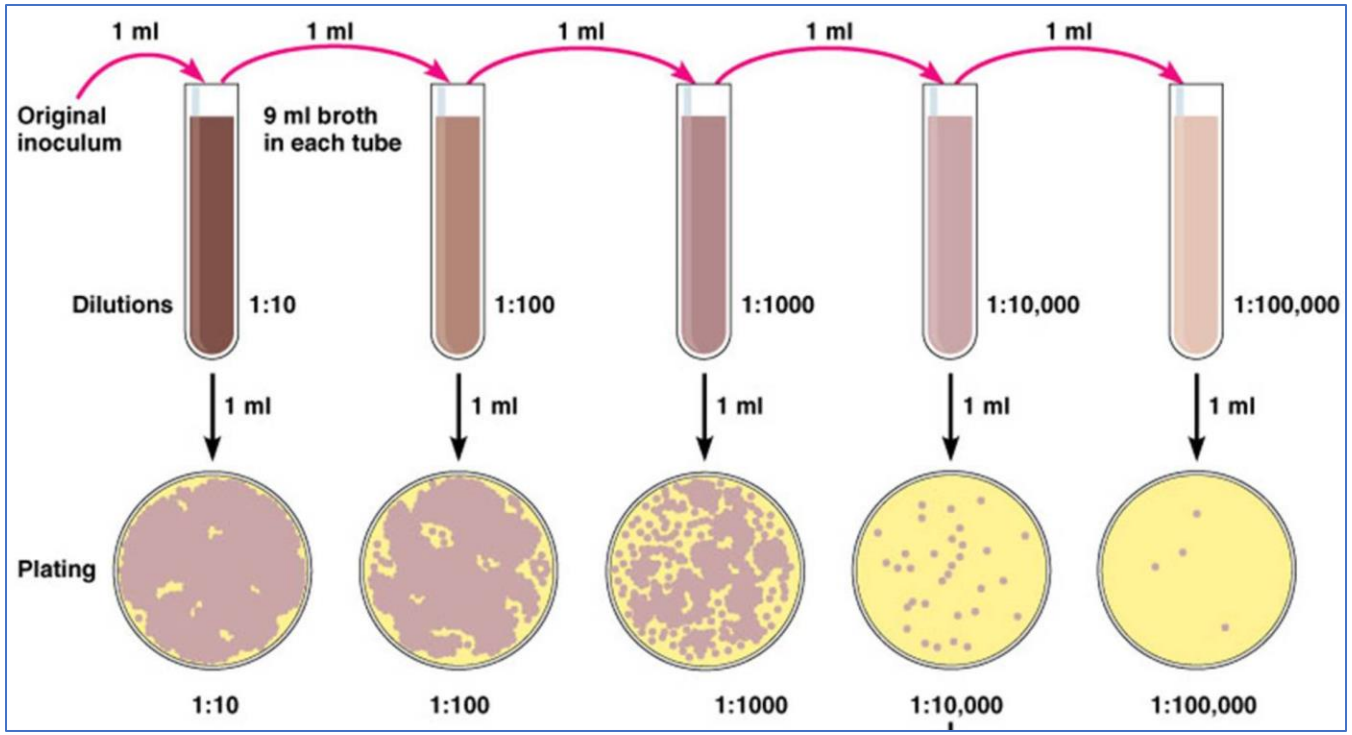


Measuring bacterial growth

- Cell concentration by viable counts and turbidometry, both are related through a standard curve.
- Turbidometry (e.g. measuring the turbidity of a culture using a spectrophotometer).
- Biomass density by dry weight of microbial culture, or through correlations to protein concentration.



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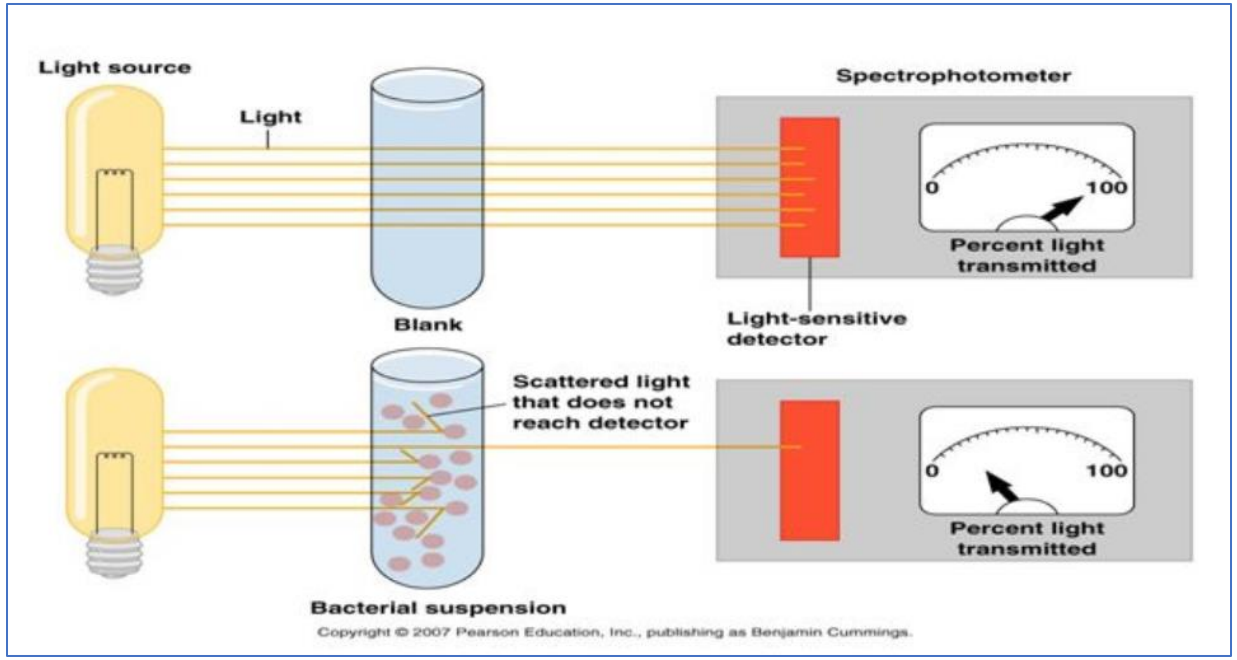
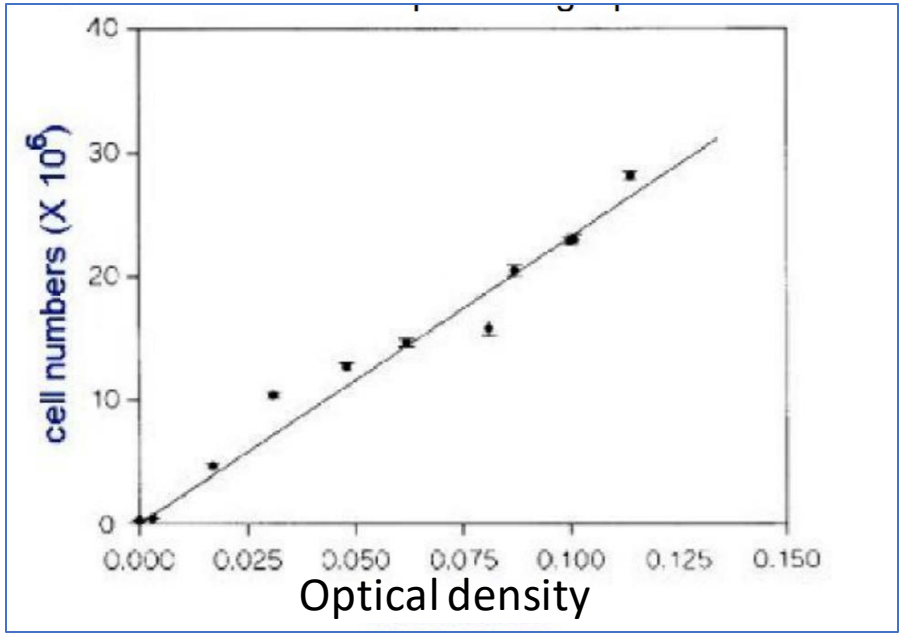
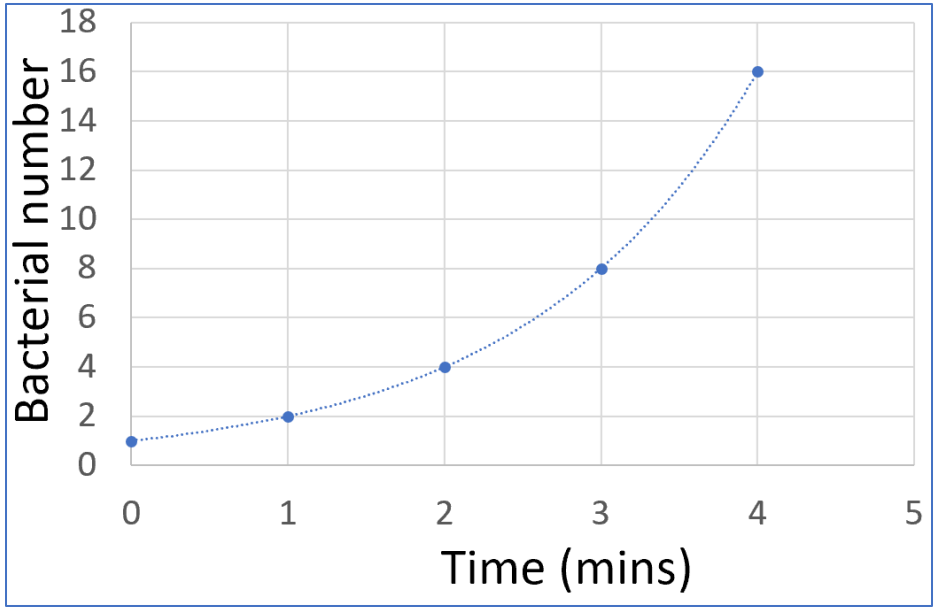
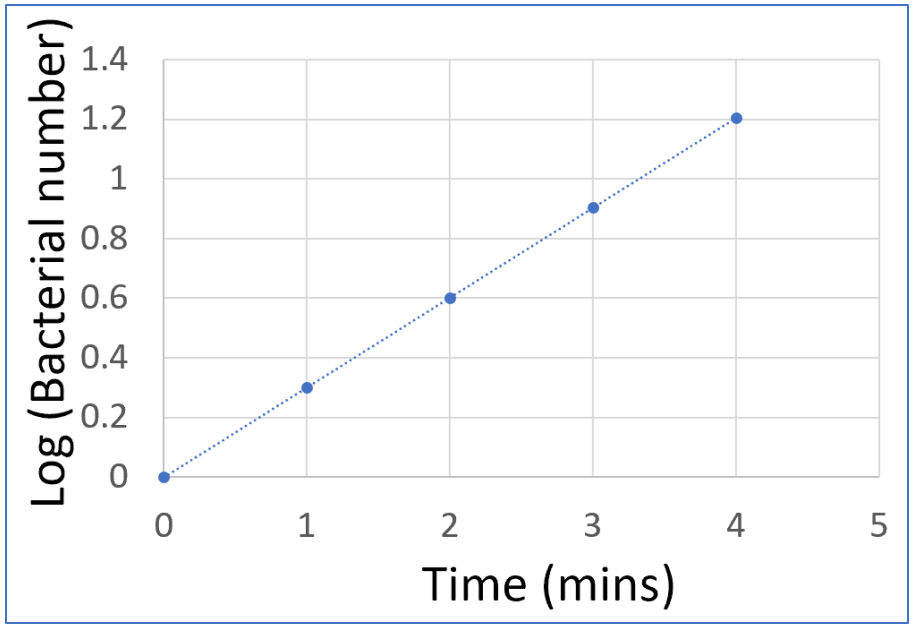




Calculating and predicting bacterial growth

- Bacteria have different doubling times (e.g. *E.coli* 20 mins, *M.tuberculosis* 100 hours) .
- By measuring bacterial numbers over time, we can calculate the doubling time (specific for each bacterial species under optimal growth conditions).
- We can then calculate number of bacteria following a period of time, or time needed to reach a certain number of bacteria.





Calculating and predicting bacterial growth

- If growth would begin with any cell number (N0), after a certain time (t) the number of cells (N) can be calculated by knowing the doubling time (td) as the following : $\mathbf{N = N0 * 2^{t / td}}$

- Example: In a bacterial culture of *E.coli* (doubling time 20 mins) inoculated with 2 bacterial cells, how many bacterial cells are expected to be found in the culture after 2 hours?

Calculating and predicting bacterial growth rate

- If growth would begin with any cell number (N0), after a certain time (t) the number of cells (N) can be calculated by knowing the doubling time (td) as the following : $\mathbf{N = N0 * 2^{t / td}}$

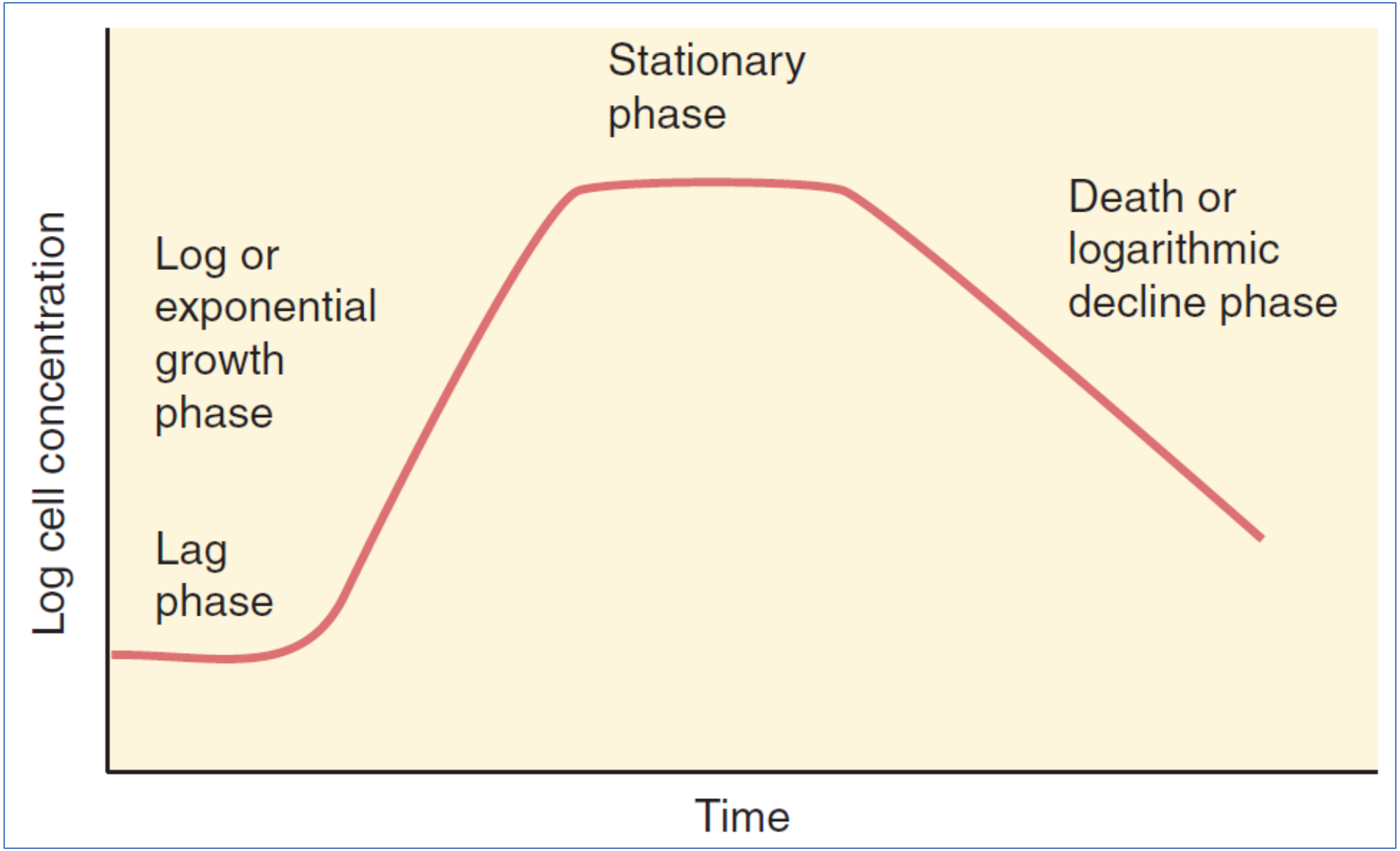
- Example: In a bacterial culture of *E.coli* (doubling time 20 mins) inoculated with 2 bacterial cells, how many bacterial cells are expected to be found in the culture after 2 hours?

- Answer: $\mathbf{N = 2 * 2^{120 / 20}}$

  $\mathbf{N = 2 * 2^{6} \quad = 2^{7} = 128}$

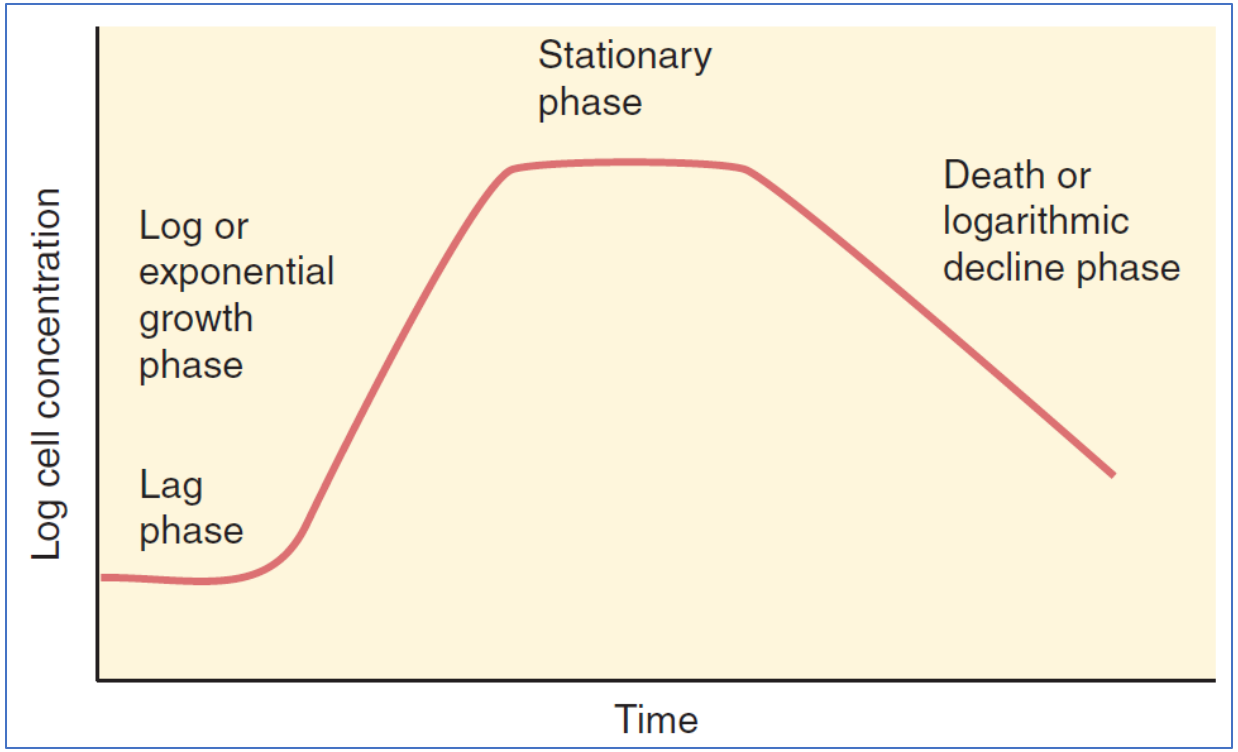
- After 4 hours = 8192
- After 24 hours = 4,722,366,482,869,645,213,696

Bacterial growth curve



Bacterial growth curve

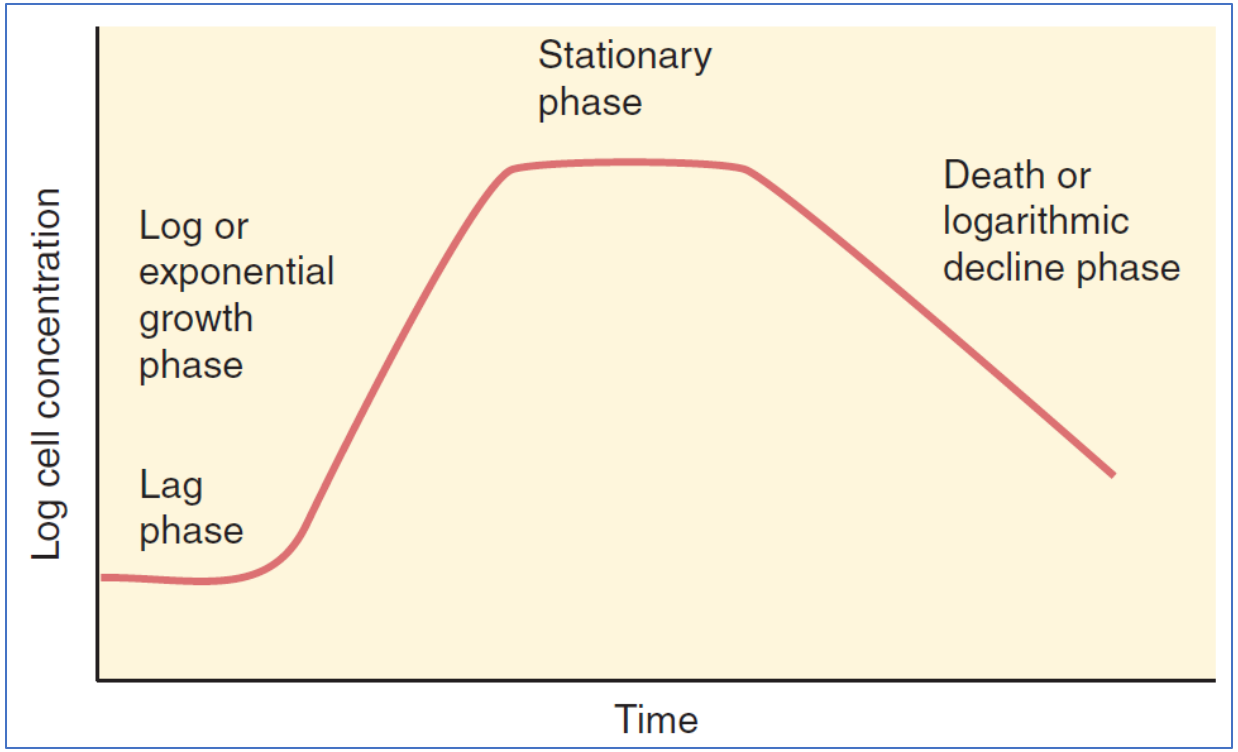
- Bacteria in culture follow a growth curve related to nutrient supply and waste production.
- The curve varies with the organism and culture medium.
- In the Lag phase, enzymes and intermediates are formed and accumulate until they are present in concentrations that permit growth to resume.



Phase	Growth Rate
Lag	Zero
Exponential	Constant
Maximum stationary	Zero
Decline	Negative (death)

Bacterial growth curve

- For aerobic organisms, oxygen limitation usually results in stopping the exponential growth phase.
- In the stationary phase, loss of cells is balanced by cell division.
- Decline phase is much slower than the exponential growth phase, and some bacteria tend to survive for prolonged periods after death of the majority of bacteria.



Phase	Growth Rate
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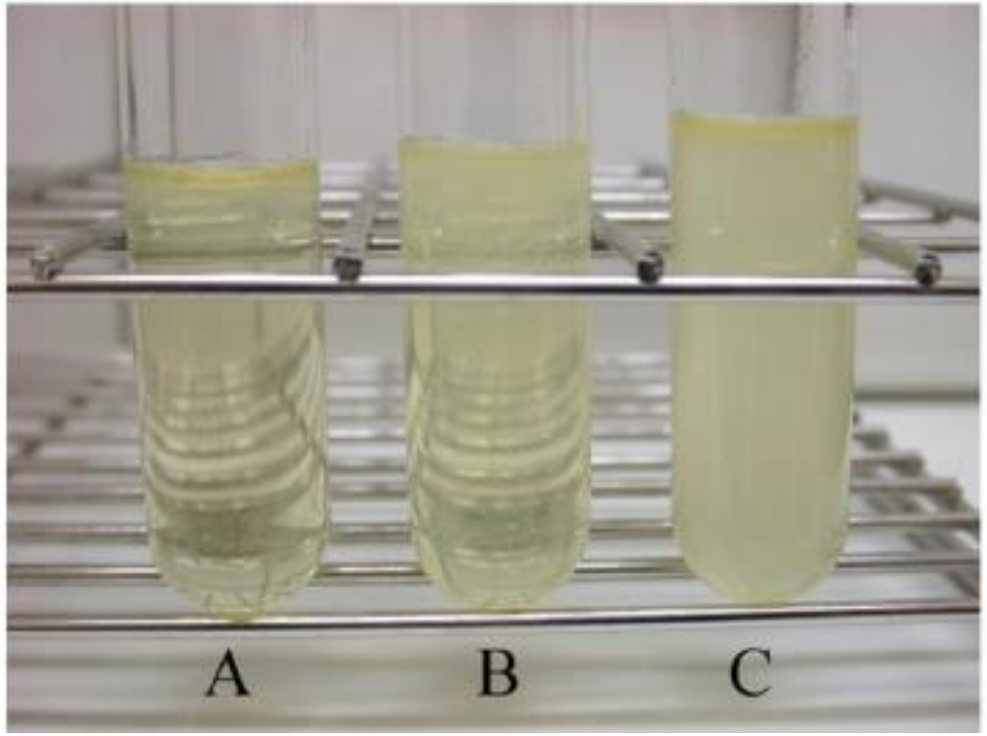
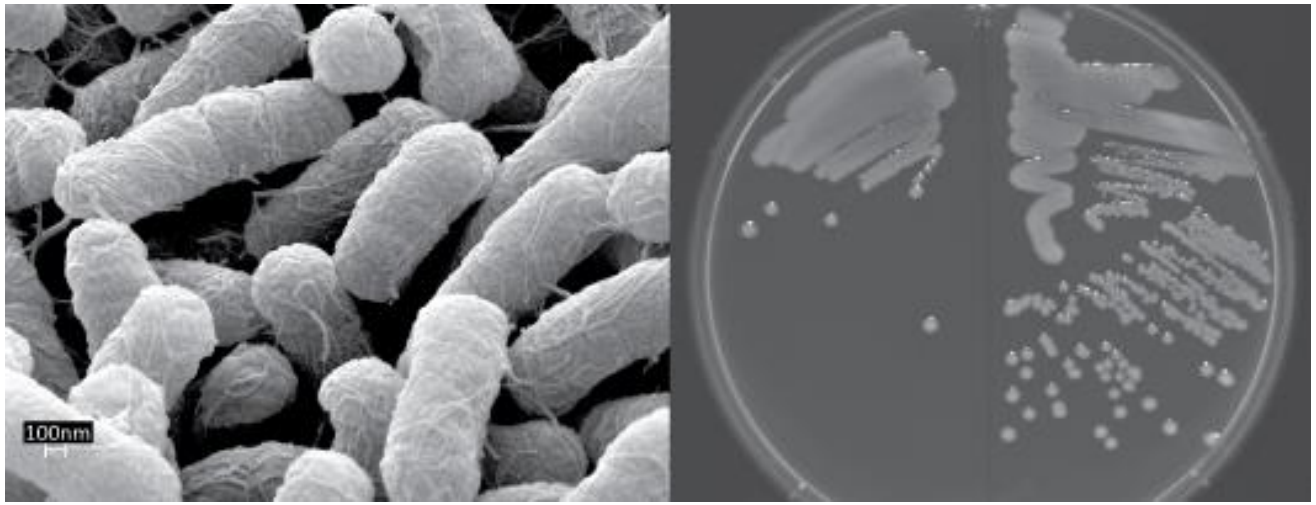
Cultivation of bacteria

- The medium for growth can be either in **liquid** or **gelled medium using Agar**.

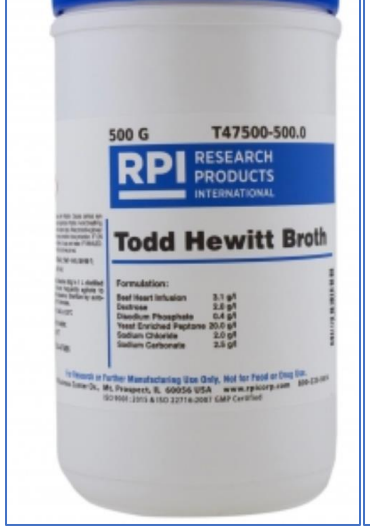




Cultivation of bacteria/ nutrients

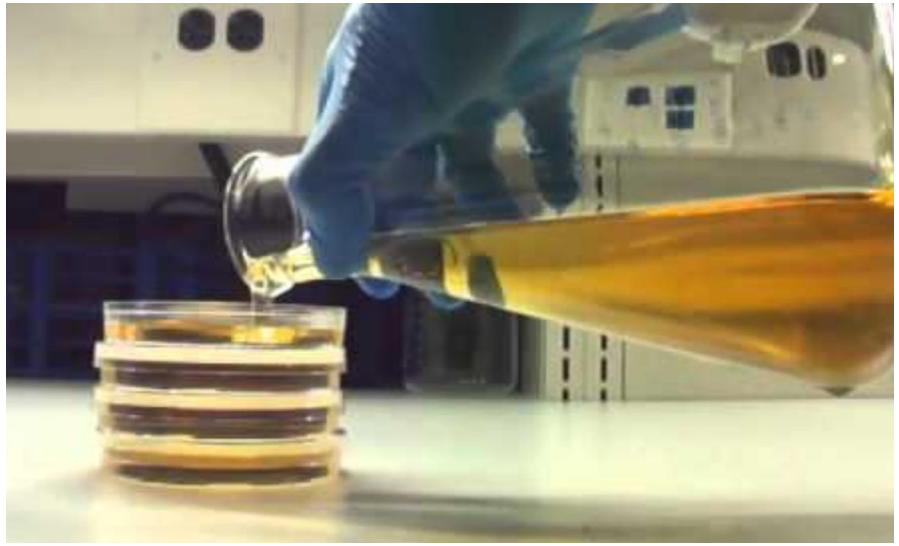
- To propagate bacteria in culture, **nutrients in the medium must provide the building blocks as well as energy for growth of the specific bacteria**. (Carbon, sulphur, nitrogen, phosphorus, minerals, growth factors).

- Other than nutrients, several factors affect growth as well, for example: **pH**, **temperature**, **aeration**, **salt concentration** must be controlled.



Components
Components (g/L):
Beef Heart Infusion 500.00
Peptic Digest of Animal Tissue 20.00
Dextrose 2.00
Sodium Chloride 2.00
Sodium Phosphate 0.40
Sodium Carbonate 2.50

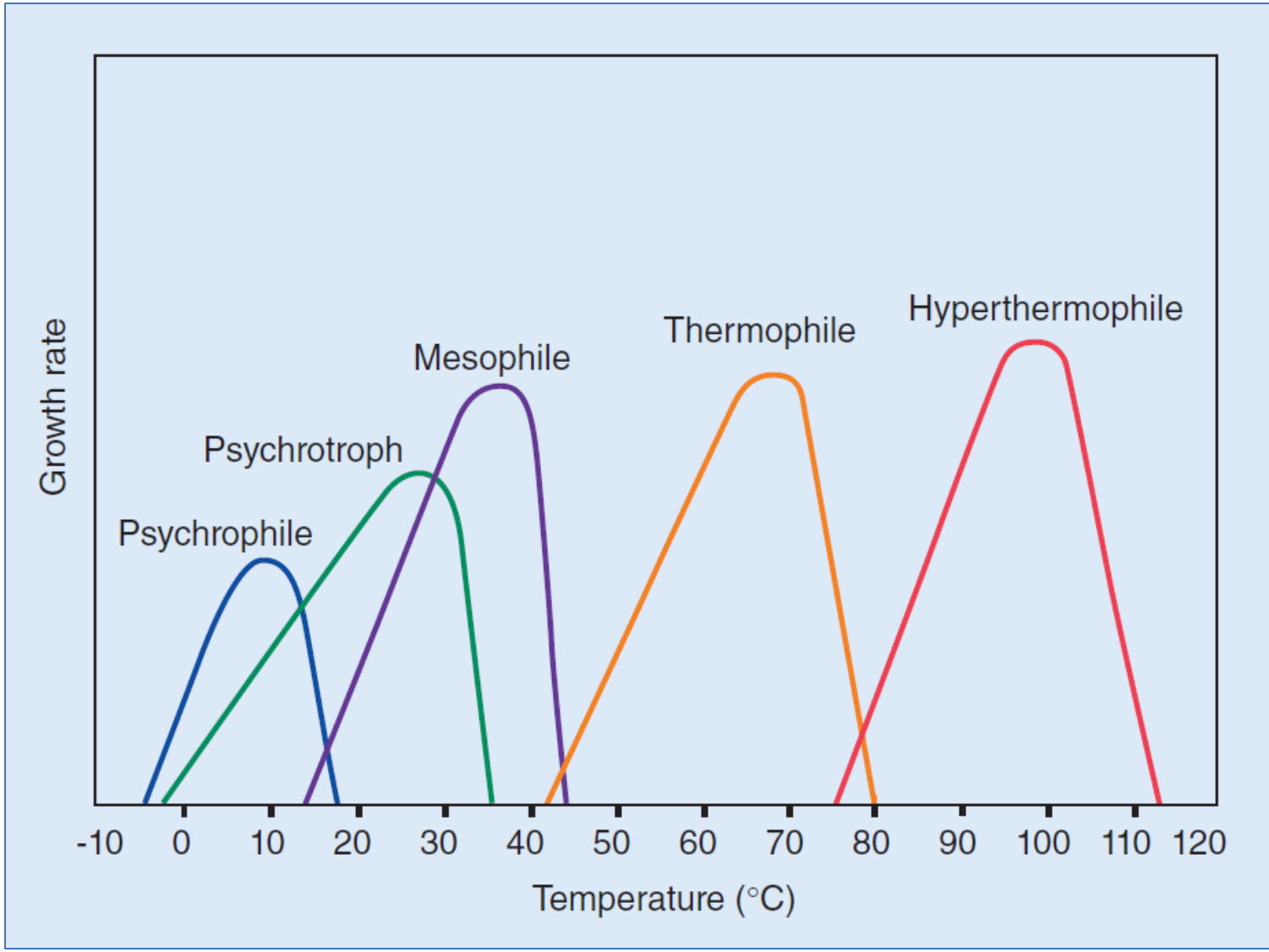
An example of components of a media used to grow many types of bacteria.



Making agar plates. When the temperature cools down the liquid medium turns solid in petri dishes

Cultivation of bacteria/ temperature

- Different temperatures fit growth of different bacteria.
- Most medically relevant bacteria grow in a temperature of 37 degrees celcius, which can be controlled in incubators in the lab.



Cultivation of bacteria/ aeration

- Different O2 and CO2 concentrations fit growth of different bacteria.
- Some bacteria cannot survive **without O2** and are called **obligate aerobes.**
- Others cannot survive in the **presence of O2,** and are called **obligate anaerobes.**
- Some will survive **in the absence or presence of O2,** called **facultative anaerobes.**

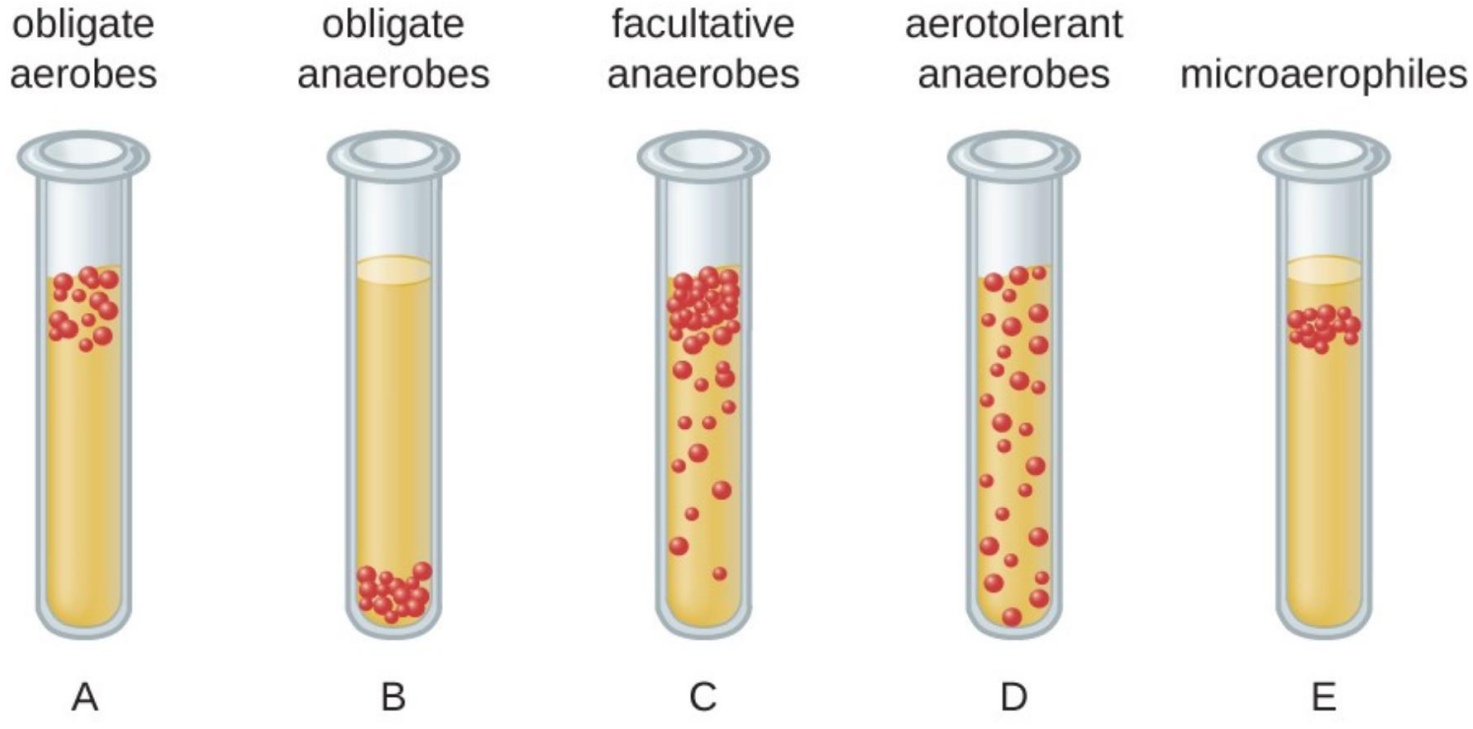
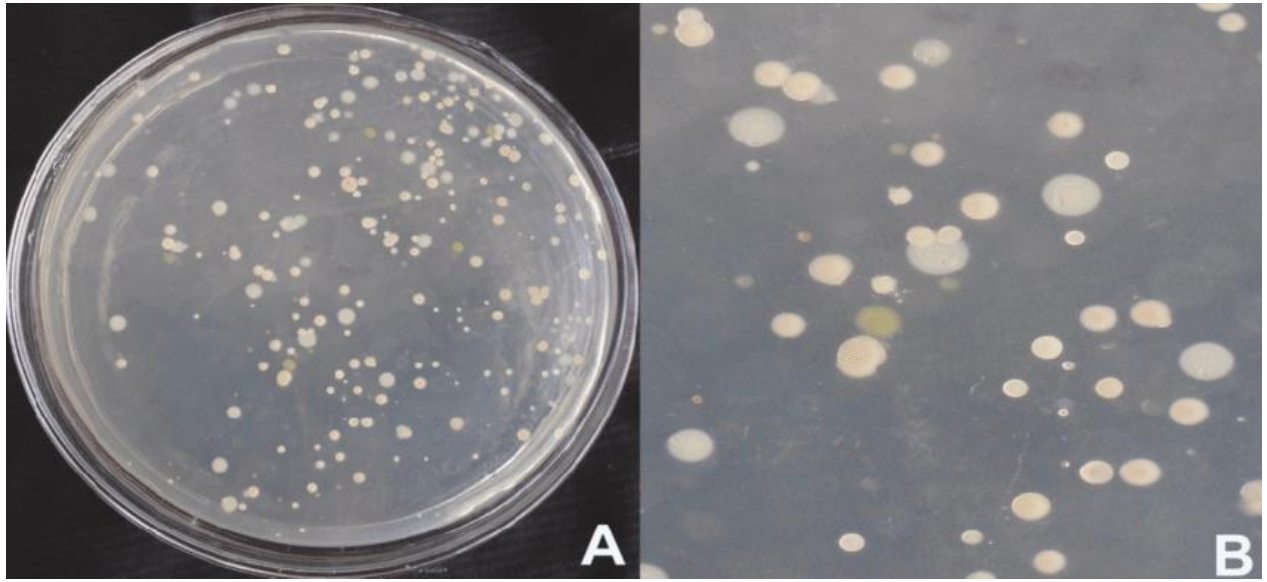


Figure 2. Diagram of bacterial cell distribution in thioglycolate tubes.

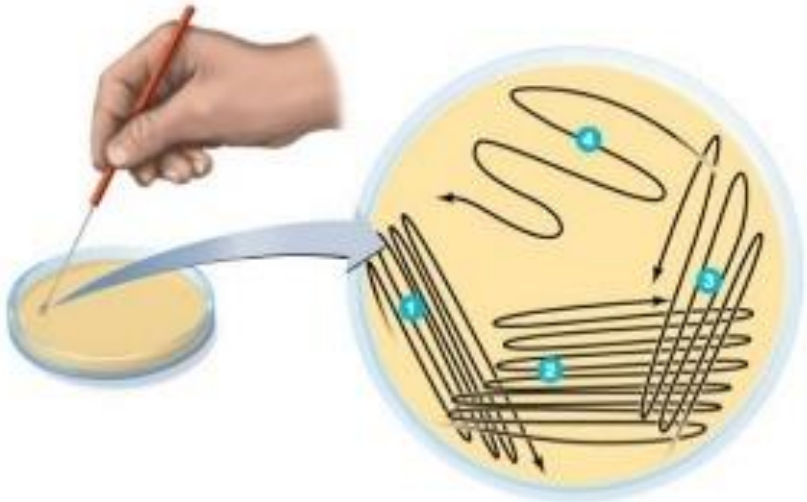
Isolation of bacteria in pure culture

- Mixed populations of bacteria are commonly found in tested samples.
- In order to study the properties of an organism (e.g. antibiotic sensitivity), **it must be handled separately**.
- Several methods (such as pour-plate, and streak-plate) aim to isolate single colonies, which will be used to propagate a pure cultures.



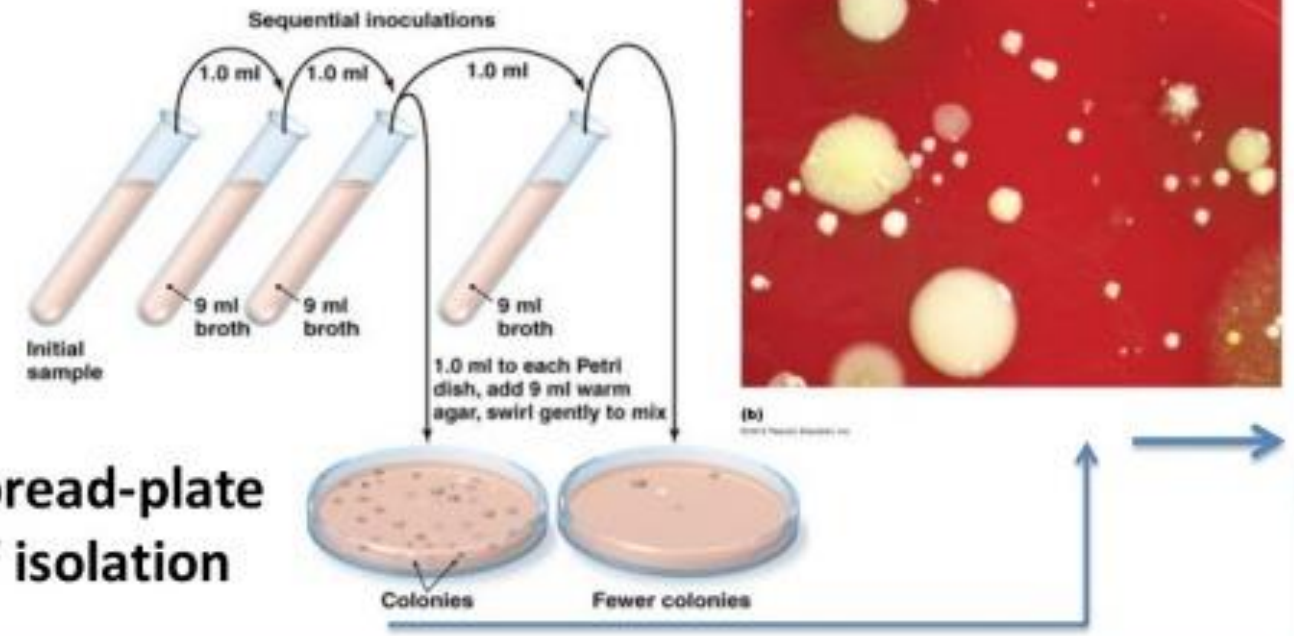
Techniques to isolate microorganisms in pure cultures or axenic cultures

- Streak-plate technique of isolation



(a)

(b)



- The method of serial dilutions

- Pour-plate/spread-plate techniques of isolation

Sample to use for streaking or dilution. E.g. oral swaps, urine, sputum, feces,.

Further reading:

- Jawetz, Melnick & Adelberg's Medical Microbiology, 26th edition-
  Section 1: Fundamentals of Microbiology-
    Chapter 4: The Growth, Survival, and death of Microorganisms
    Chapter 5: Cultivation of Microorganisms

Agar art !