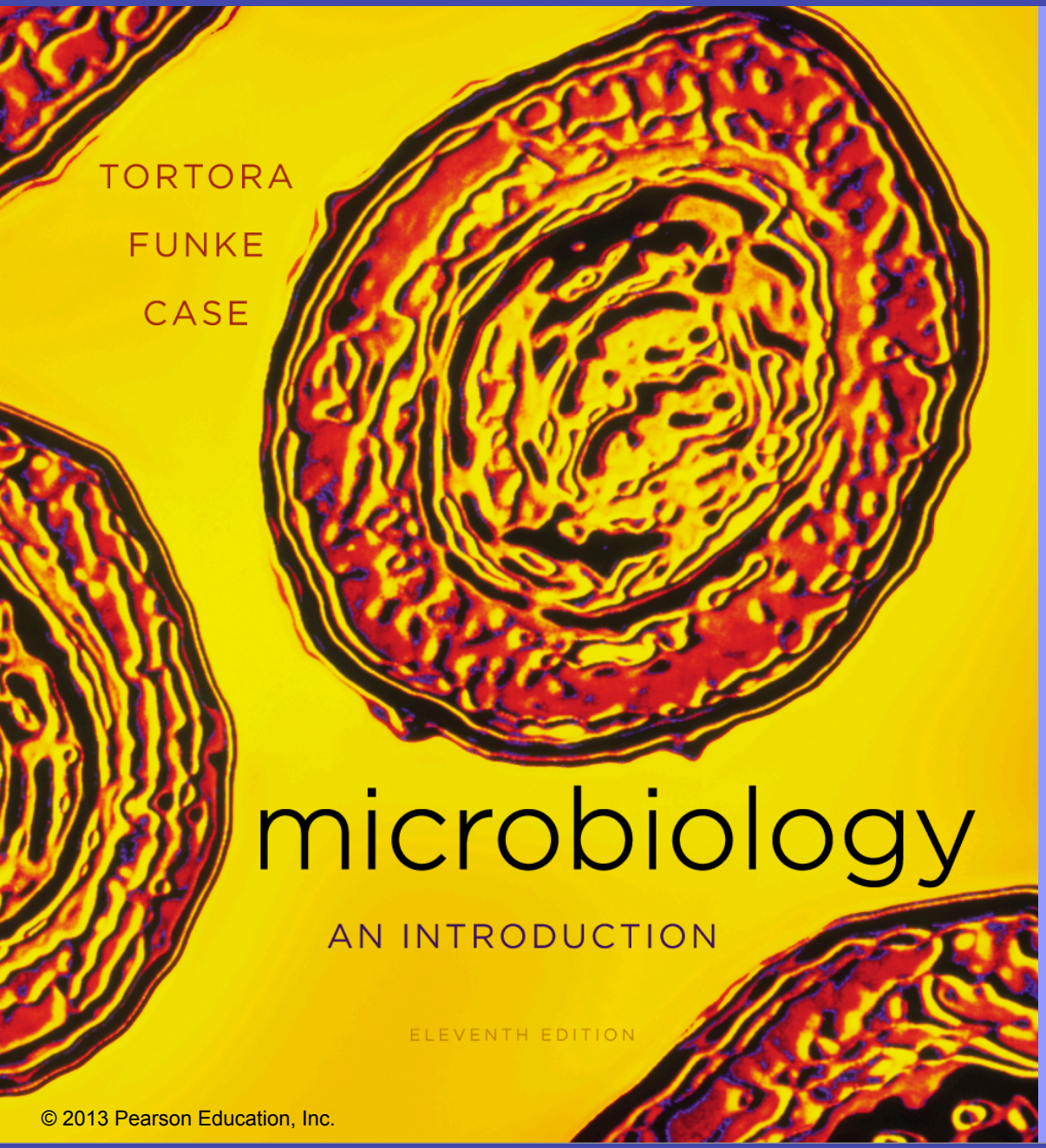
Module 4

Microbial Growth



Chapter 6

Microbial Growth

Lectures prepared by Helmut Kae

Microbial Growth

- Microbial growth is the increase in cell **number**, not cell **size**
  - Growing microbes means an increase in **population** size
- Important to understand conditions necessary for microbial growth
  - Limit growth of microbes that cause disease, food spoilage
  - Encourage growth of beneficial microbes

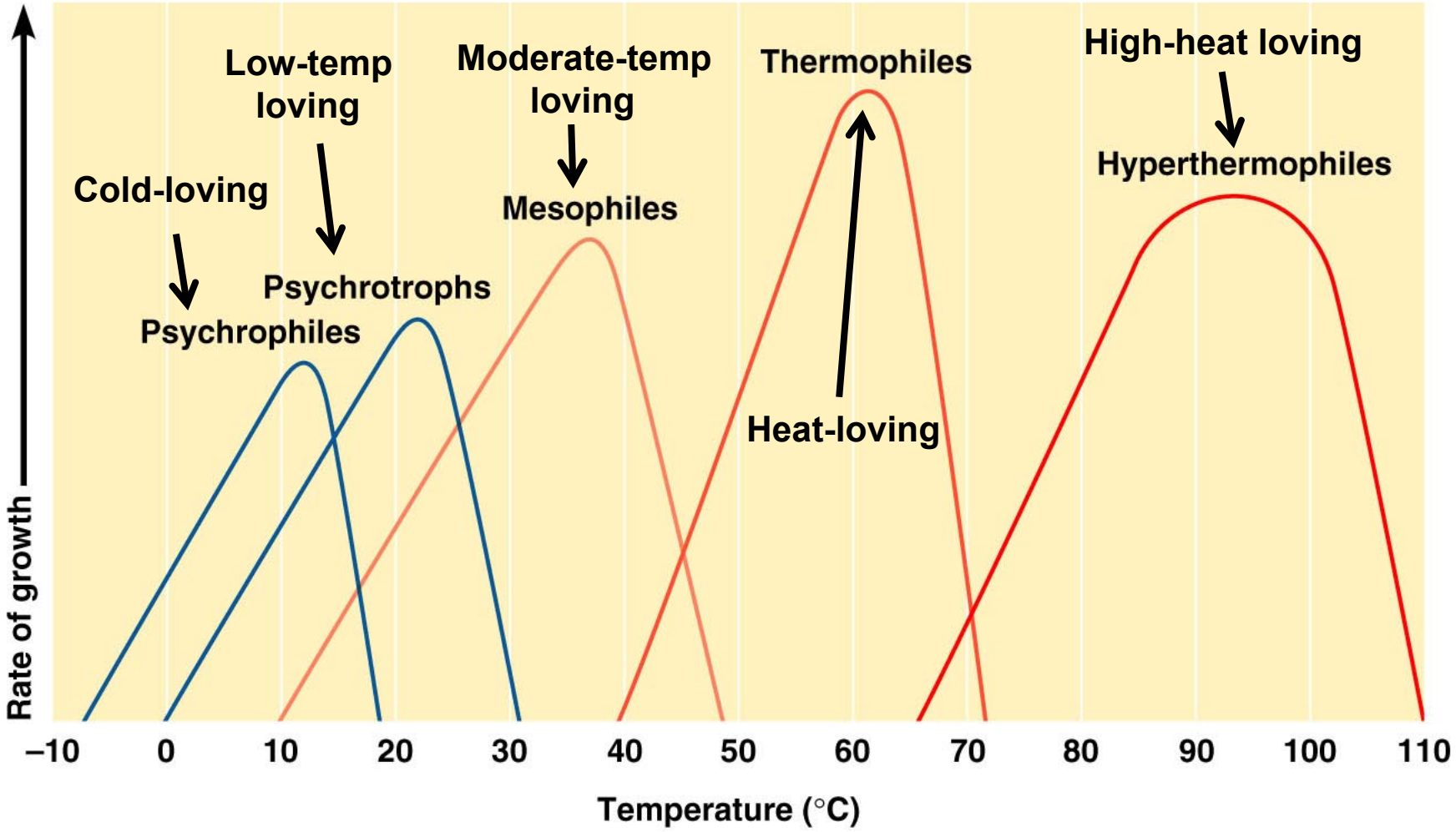
The Requirements for Growth

- Physical requirements
  - Temperature
  - pH
  - Osmotic pressure
- Chemical requirements
  - Carbon
  - Nitrogen, sulfur, and phosphorous
  - Trace elements
  - Oxygen
  - Organic growth factor

Temperature

- Microbes grow within limited temperature range
  - Low, high temp affect enzyme function, cell structure
- **Minimum growth temperature** - lowest temp at which a species will grow
- **Optimum growth temperature** - temp at which microbe grows best
- **Maximum growth temperature** - highest temp at which growth is possible

Microbes divided into 5 categories according to temperature range



Temperature

Psychrophiles

- Can grow below 0°C; optimum at 11°C
- Usually killed by temp above 20°C
  - Therefore, rarely problem with food spoilage

Psychrotrophs

- Can grow at 0°C; optimum around 20°C
- Cause problems with food spoilage, can grow in fridge
  - But grow slow → proper refrigeration helps prevent food spoilage

Temperature

Mesophiles

- Many human pathogens grow best at 37°C
  - Human body temp
- Mesophiles include most common pathogens, food spoilage microbes

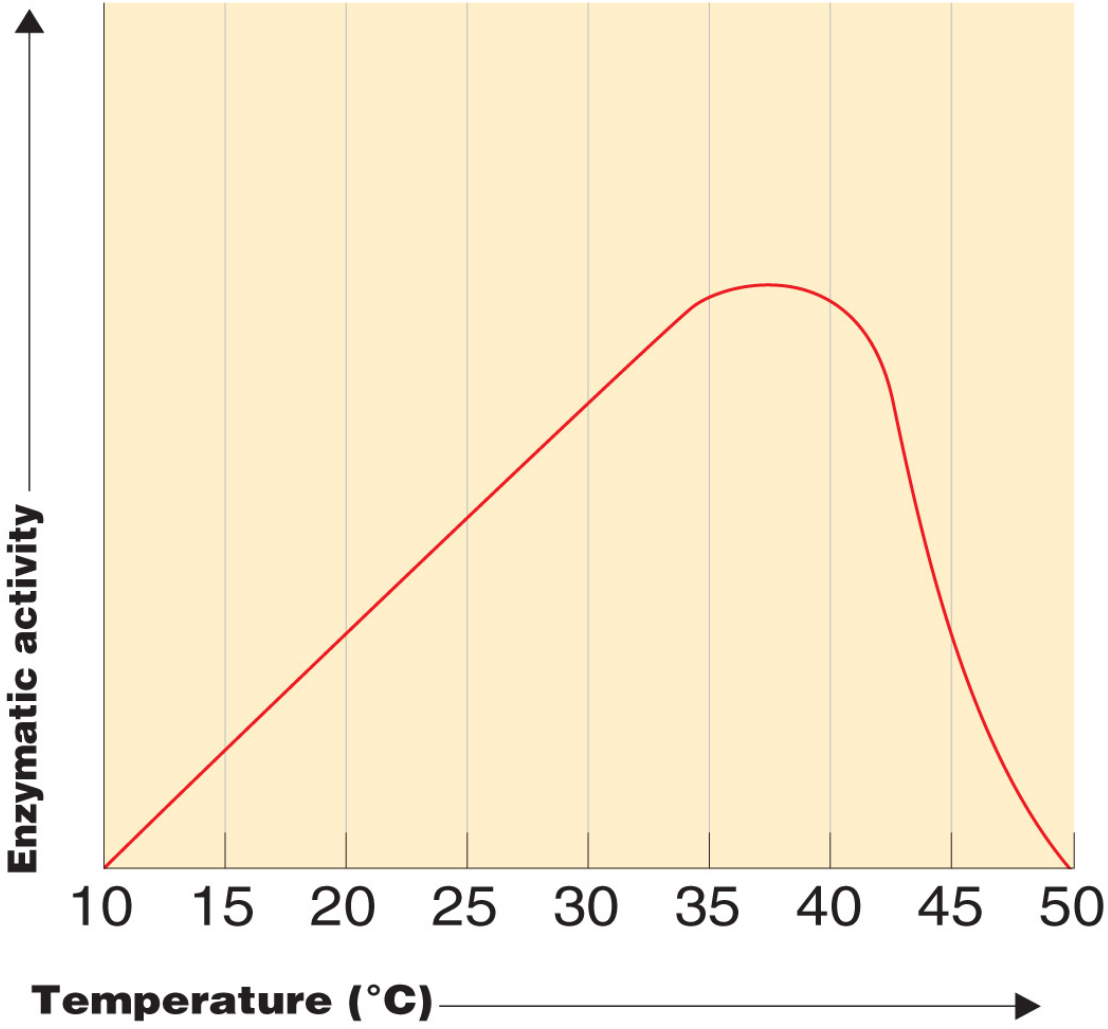
Thermophiles, **hyperthermophiles**

- Grow in hot water tank, volcanic hot springs
- Cannot grow below 45°C – usually not health problem

pH

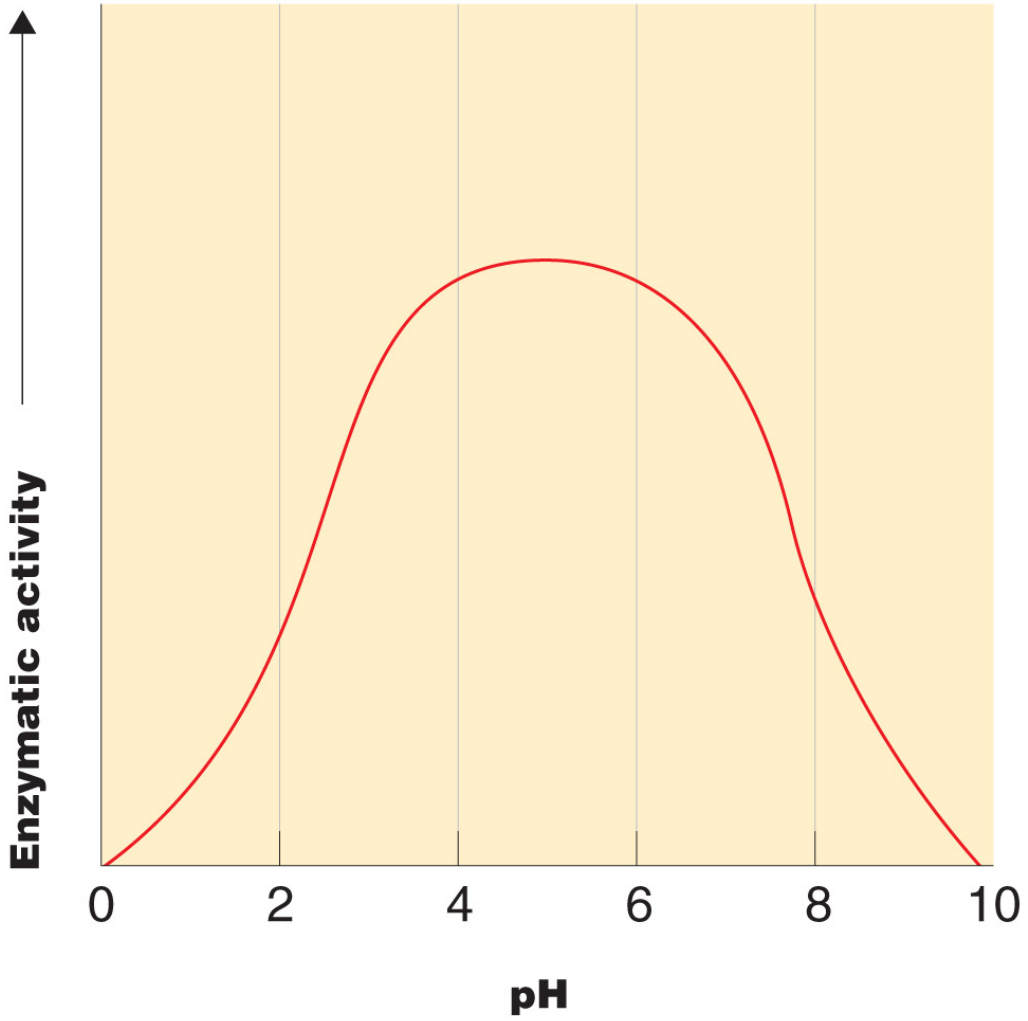
- pH refers to concentration of H+ ions
  - Low pH → high H+ → **acid**
  - High pH → low H+ → **alkaline**
  - Most bacteria grow near neutral pH, pH 7
- **Acidophiles** grow in acidic environments
  - Sauerkraut, yogurt products of acidophiles
  - Preserved from spoilage by bacterial fermentation
- Molds and yeasts can grow between pH 5 and 6

Figure 5.5a Factors that influence enzymatic activity, plotted for a hypothetical enzyme.



(a) Temperature. The enzymatic activity (rate of reaction catalyzed by the enzyme) increases with increasing temperature until the enzyme, a protein, is denatured by heat and inactivated. At this point, the reaction rate falls steeply.

Figure 5.5b Factors that influence enzymatic activity, plotted for a hypothetical enzyme.

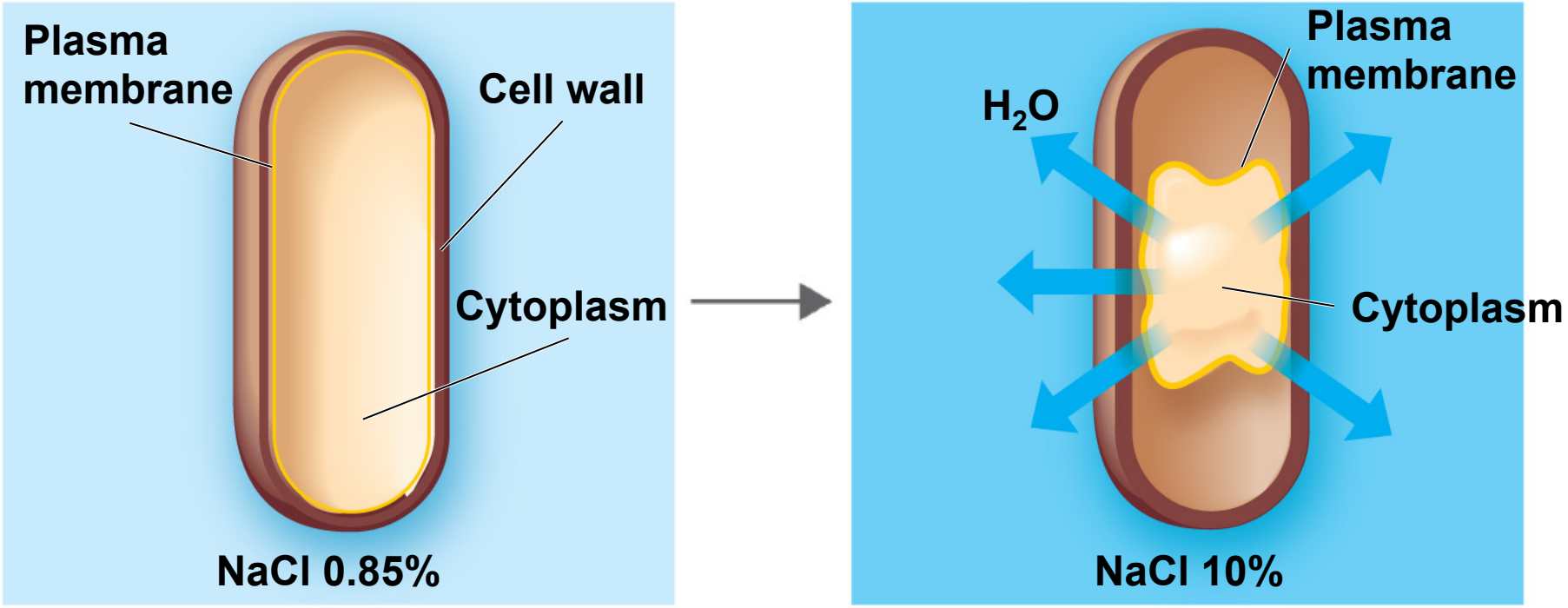


(b) pH. **The enzyme illustrated is most active at about pH 5.0.**

Osmotic Pressure

- Microbes dependent on water to carry nutrients
  - Microbes live in aqueous (water) environments
- Hypertonic environments causes water to leave cell
  - Growth inhibited due to plasmolysis
- Food preserved by high osmotic pressure - add solutes
- **Halophiles** tolerate high osmotic pressure
- Extreme halophiles *require* high salt conditions
  - Live in the Dead Sea, salt lakes

Figure 6.4 Plasmolysis.



(a) Cell in isotonic solution.

(b) Plasmolyzed cell in hypertonic solution.

Chemical Requirements

- Carbon
  - Structural organic molecules, energy source
  - Chemoheterotrophs use organic carbon sources
  - Autotrophs use CO_2

Chemical Requirements

- Nitrogen
  - In amino acids, proteins, nucleic acids
  - Most bacteria decompose proteins
  - Some bacteria use NH_4^+ or NO_3^-
  - A few bacteria use nitrogen gas (N2) from atmosphere
    - Called **nitrogen fixation**

Chemical Requirements

- Sulfur
  - In amino acids, thiamine, and biotin
  - Most bacteria decompose proteins
  - Some bacteria use SO_4^{2-} or H_2S
- Phosphorus
  - In DNA, RNA, ATP, and membranes
  - PO_4^{3-} is a source of phosphorus

Chemical Requirements

- Trace elements
  - Inorganic elements required in small amounts
  - Usually as enzyme cofactors

Organic Growth Factors

- Organic compounds obtained from the environment
- Vitamins, amino acids, purines, and pyrimidines

Oxygen

- Aerobic metabolism provides more energy than anaerobic metabolism
- BUT, Oxygen is toxic in high amounts to ALL organisms
  - Toxic forms of oxygen are highly reactive; damage cell components
  - Many metabolic pathways exist to detoxify oxygen

Toxic Oxygen

- **Singlet oxygen**: $^1O_2^-$ boosted to a higher-energy state
- **Superoxide free radicals**: $O_2^{-\bullet}$

$$O_2^{-\bullet} + O_2^{-\bullet} + 2\,H^+ \xrightarrow{\text{Superoxide dismutase}} H_2O_2 + O_2$$

- **Peroxide anion**: O_2^{2-}

$$2\,H_2O_2 \xrightarrow{\text{Catalase}} 2\,H_2O + O_2$$

$$H_2O_2 + 2\,H^+ \xrightarrow{\text{Peroxidase}} 2\,H_2O$$

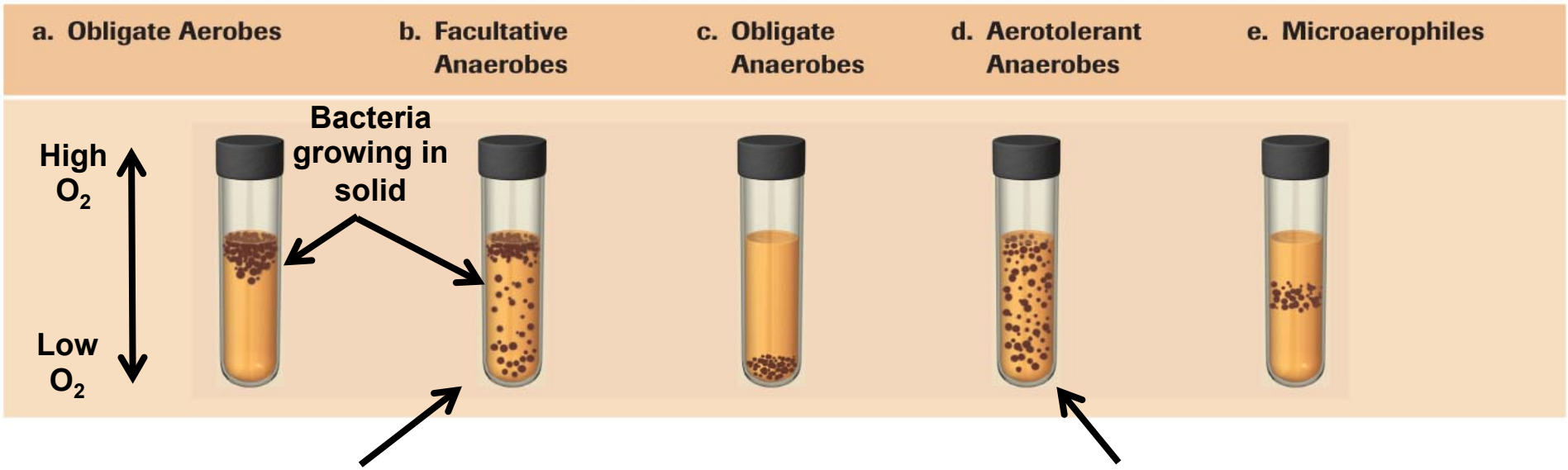
- **Hydroxyl radical** (OH•)

Chemical Requirements

- Grow where high O_2 occurs
- Have enzymes that detoxify O_2

- Lack enzymes to detoxify O_2
- Grow slower than aerobes

- Cannot detoxify high concentration of O_2

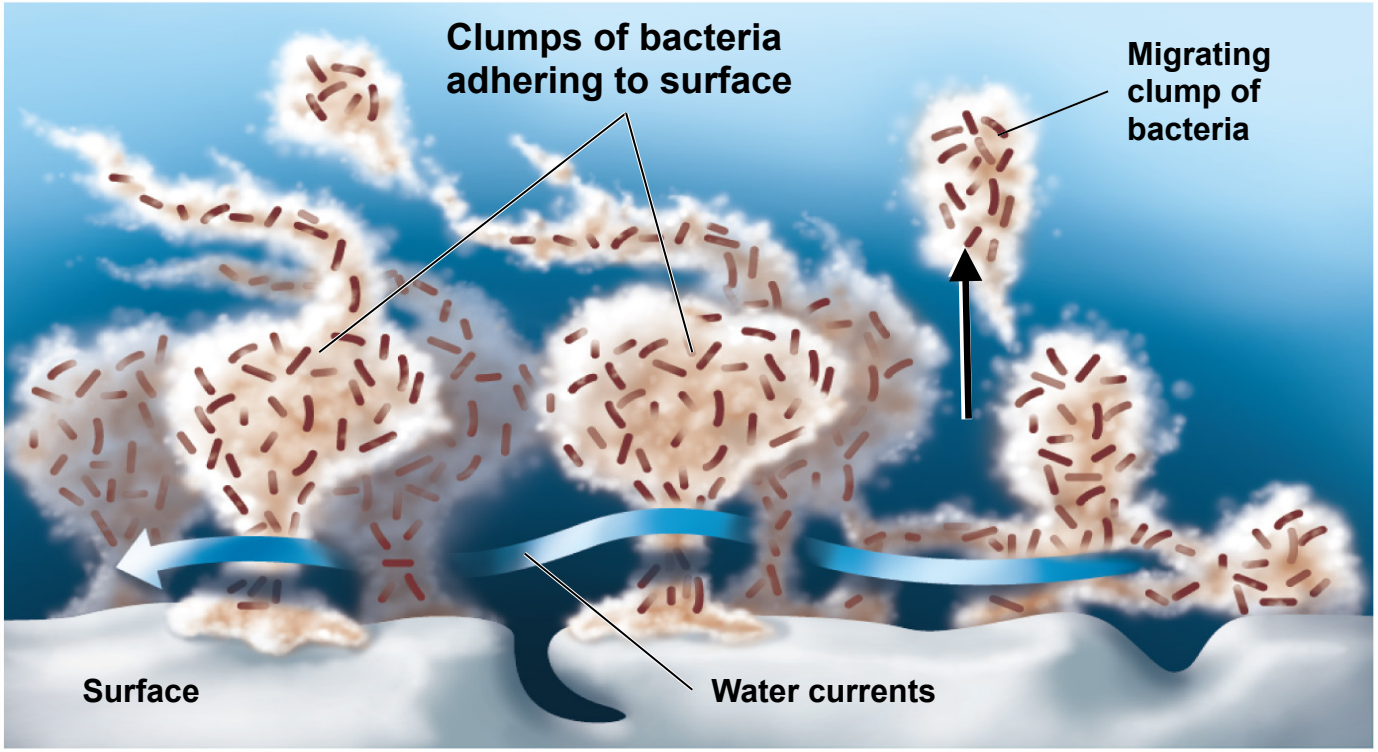


- Grow faster with O_2
- Anaerobic growth via fermentation or respiration

- Obligate fermenters, produce acids that inhibit competition from aerobes
- Posses enzymes to detoxify O_2

Biofilms

- Glycocalyx that holds community of bacteria together
  - Share nutrients
  - Sheltered from harmful factors



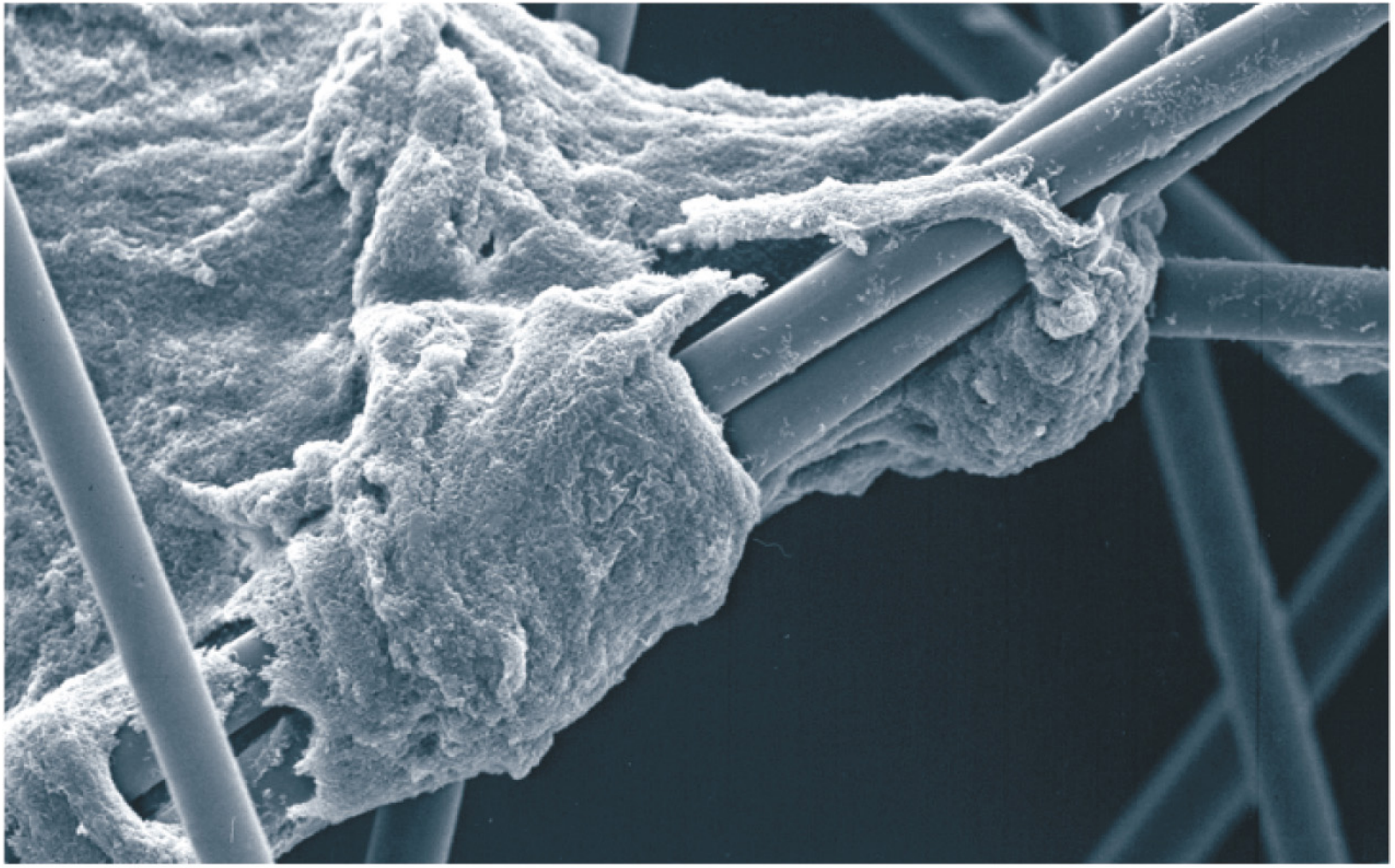
Biofilms and Human Health

- Dental plaque is a biofilm created by an extracellular polysaccharide
- Formed by *Streptococcus* species in mouth
  - Only when sucrose is present
- Plaque allows other microbes to join and survive
  - Form acids that lead to tooth decay, gum disease

Biofilms and Human Health

- Biofilms often form on catheters and other tubing
- Numbers are often too low to detect
  - Biofilm protects bacteria from antimicrobial treatments
- Can grow rapidly once inside body, causing UTIs and other infections

Applications of Microbiology 3.2 *Pseudomonas aeruginosa* biofilm.



Growing Microbes in the Lab

- **Culture medium**: nutrients prepared for microbial growth
- **Sterile**: no living microbes
- **Inoculation**: introduction of microbes (the **inoculum**) into sterile medium
- **Culture**: microbes growing in/on culture medium

Agar

- Complex polysaccharide
- Used as solidifying agent for culture media in Petri plates, slants, and deeps
- Generally not metabolized by microbes
- Liquefies at 100°C
- Solidifies at ~40°C

Culture Media

- **Chemically defined media**: exact chemical composition is known
- **Complex media**: extracts and digests of yeasts, meat, or plants
  - Undefined mixture of nutrients

Table 6.2 A Chemically Defined Medium for Growing a Typical Chemoheterotroph, Such as *Escherichia coli*

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Constituent	Amount
Glucose	5.0 g
Ammonium phosphate, monobasic ($NH_4H_2PO_4$)	1.0 g
Sodium chloride (NaCl)	5.0 g
Magnesium sulfate ($MgSO_4 . 7H_2O$)	0.2 g
Potassium phosphate, dibasic (K_2HPO_4)	1.0 g
Water	1 liter

Table 6.4 Composition of Nutrient Agar, a Complex Medium for the Growth of Heterotrophic Bacteria

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Constituent	Amount
Peptone (partially digested protein)	5.0 g
Beef extract	3.0 g
Sodium chloride	8.0 g
Agar	15.0 g
Water	1 liter

Biosafety Levels

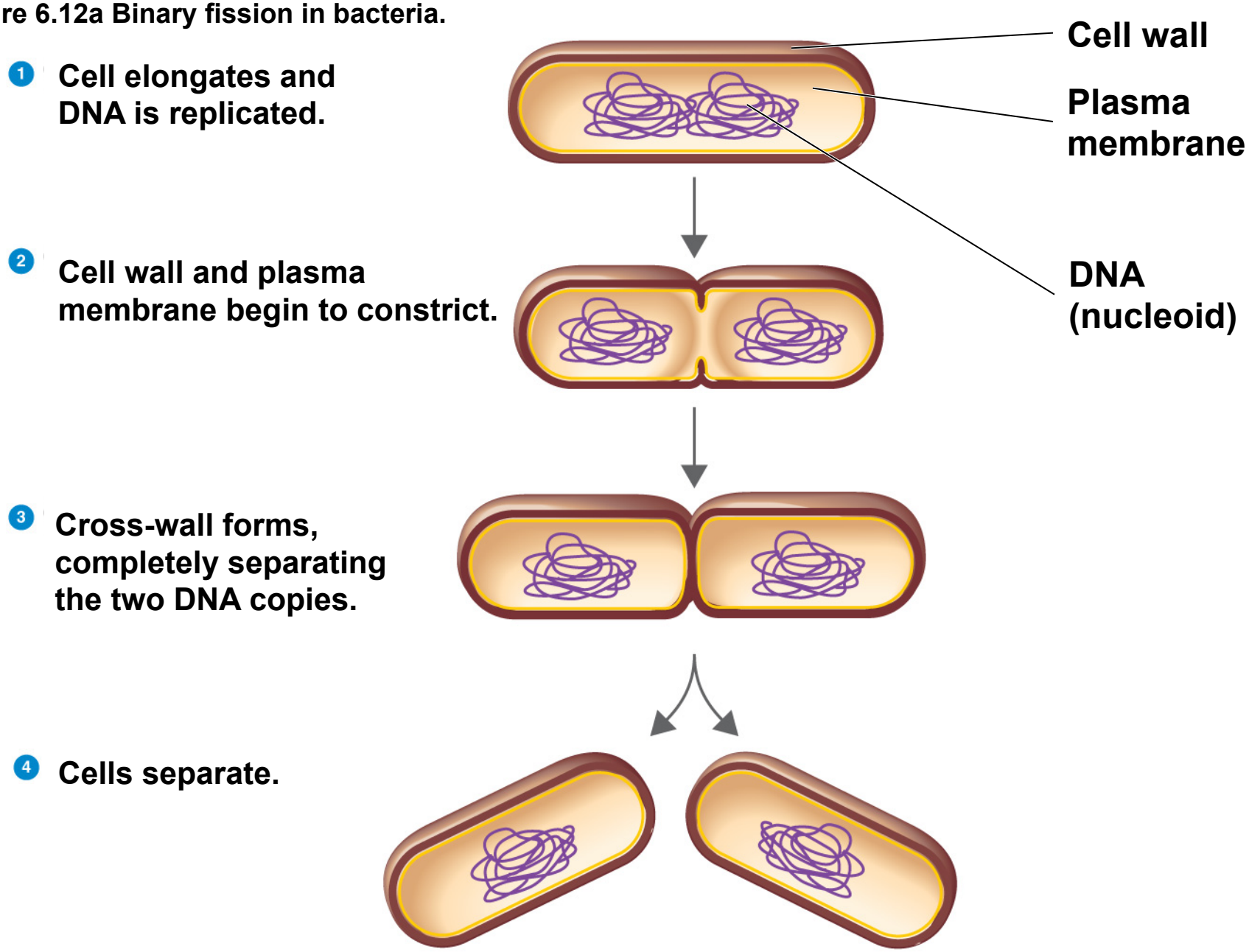
- BSL-1: no special precautions
- BSL-2: lab coat, gloves, eye protection
- BSL-3: biosafety cabinets to prevent airborne transmission
- BSL-4: sealed, negative pressure
  - Exhaust air is filtered twice

Figure 6.8 Technicians in a biosafety level 4 (BSL-4) laboratory.

Reproduction in Prokaryotes

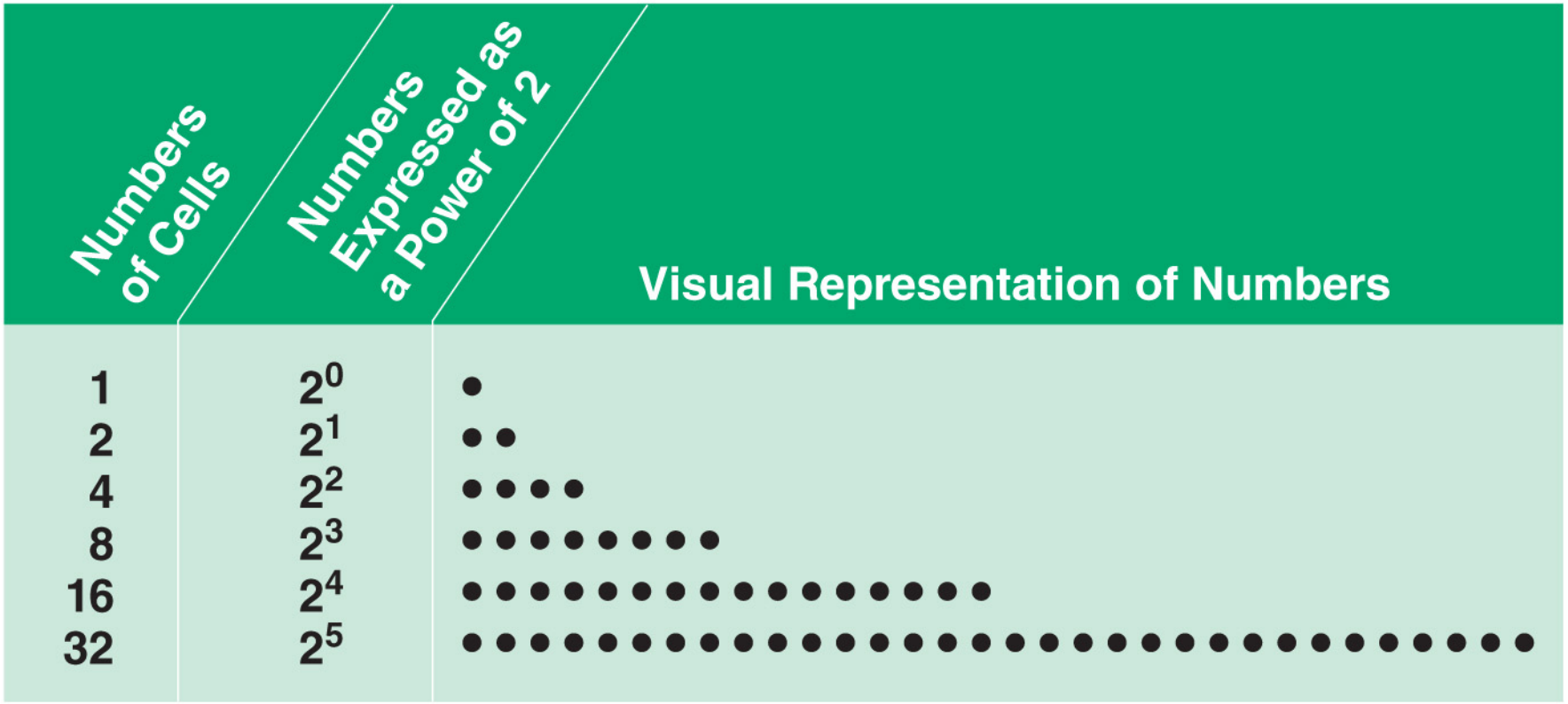
- Recall, microbial growth is increase in cell number
- Bacteria reproduce by **binary fission**
  - A single cell splits into two identical cells
- Some microbes reproduce by budding
  - Small growth (bud) gets larger, and finally separates

Figure 6.12a Binary fission in bacteria.



(a) A diagram of the sequence of cell division

Figure 6.13a Cell division.



(a)

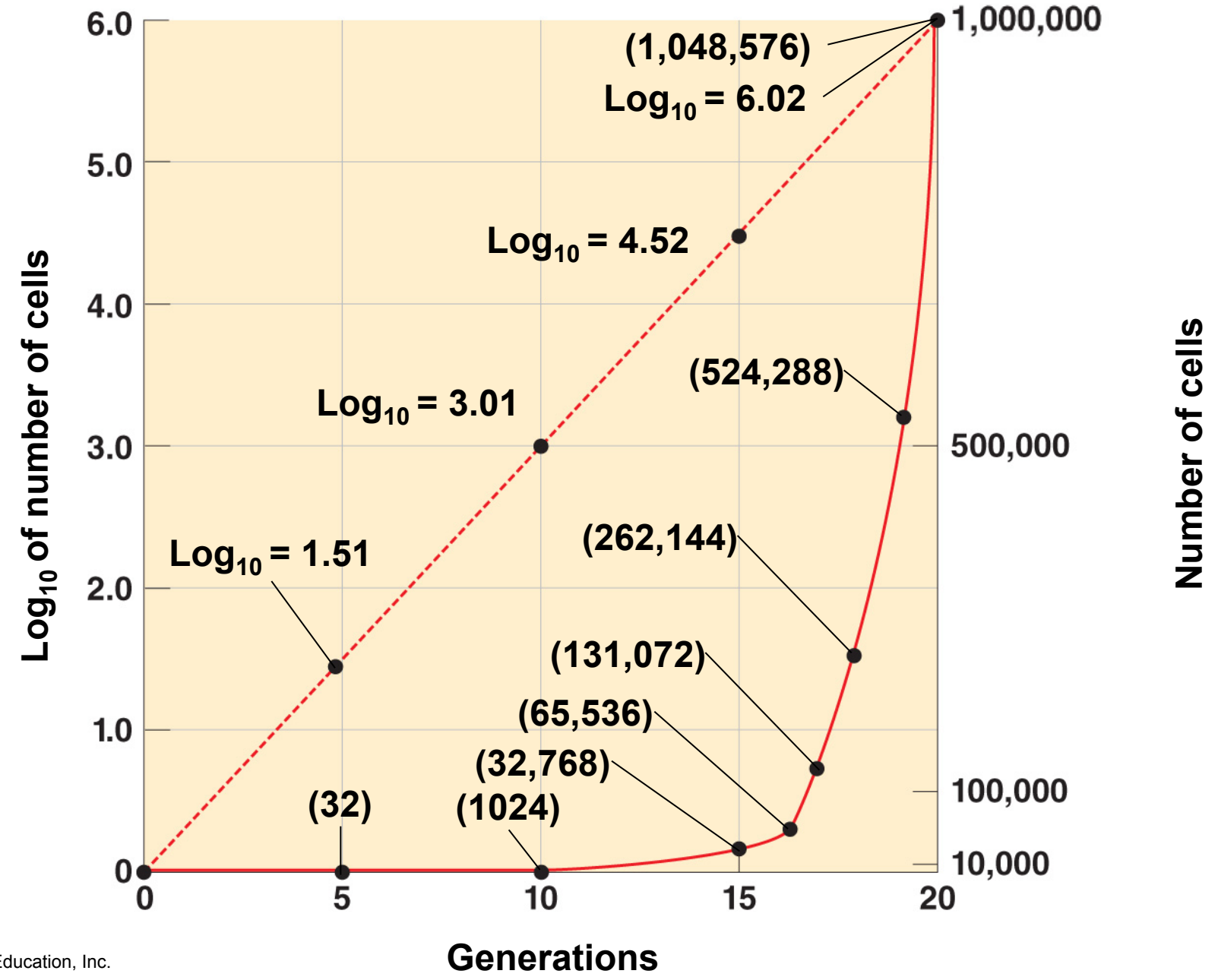
The Growth of Bacterial Cultures

- **Generation time, g** – the time it takes for a cell to divide
  - Essentially, time it takes for population to double
- Varies among species
  - Can be 20 mins, can be 20 days
- Microbes can grow fast in ideal conditions
  - Eg, if g=20 mins, then:
    - 1 cell → 1 million+ in 20 generations, ~7hrs
    - 1 cell → 1 billion+ in 30 generations, ~10 hrs

The Growth of Bacterial Cultures

- Bacterial growth plotted on **logarithmic** graph
  - Numbers too high for linear or arithmetic graph
- Logarithmic scale increases in increments of 10
  - 10, 100, 1000, 10000, 100000, etc …
- Converts rapidly increasing exponential growth from curved line into **straight** line

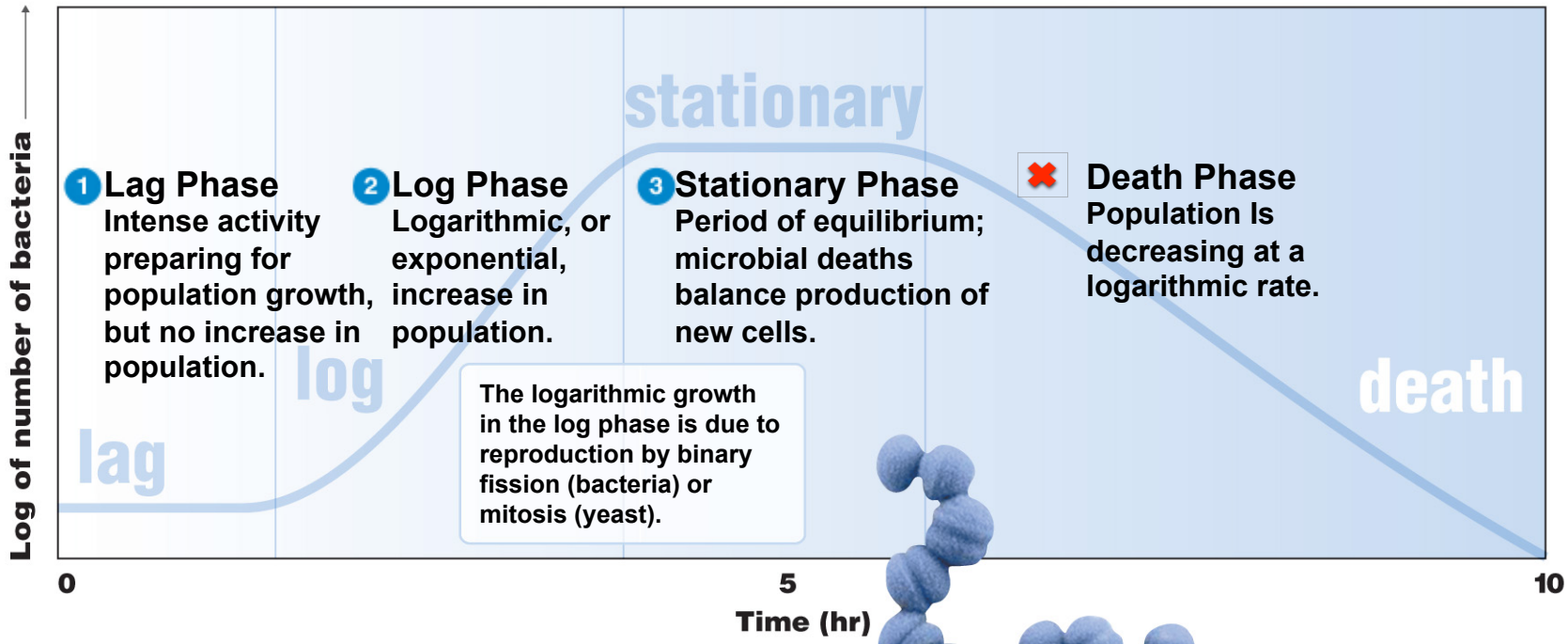
Figure 6.14 A growth curve for an exponentially increasing population, plotted logarithmically (dashed line) and arithmetically (solid line).

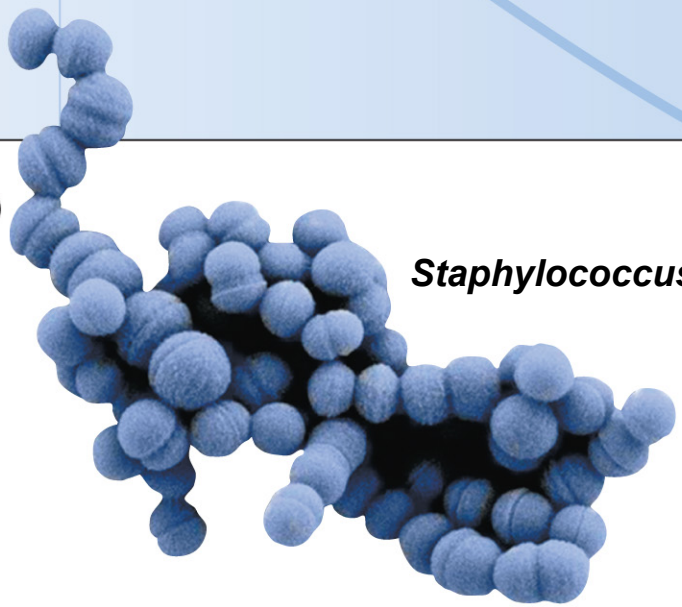


Phases of Growth

- Bacteria growing in liquid medium have characteristic growth pattern
  - When plotted on logarithmic graph – **bacterial growth curve**
- The **lag** phase
- The **log** phase
- The **stationary** phase
- The **death** phase

Figure 6.15 Understanding the Bacterial Growth Curve.





***Staphylococcus* spp.**

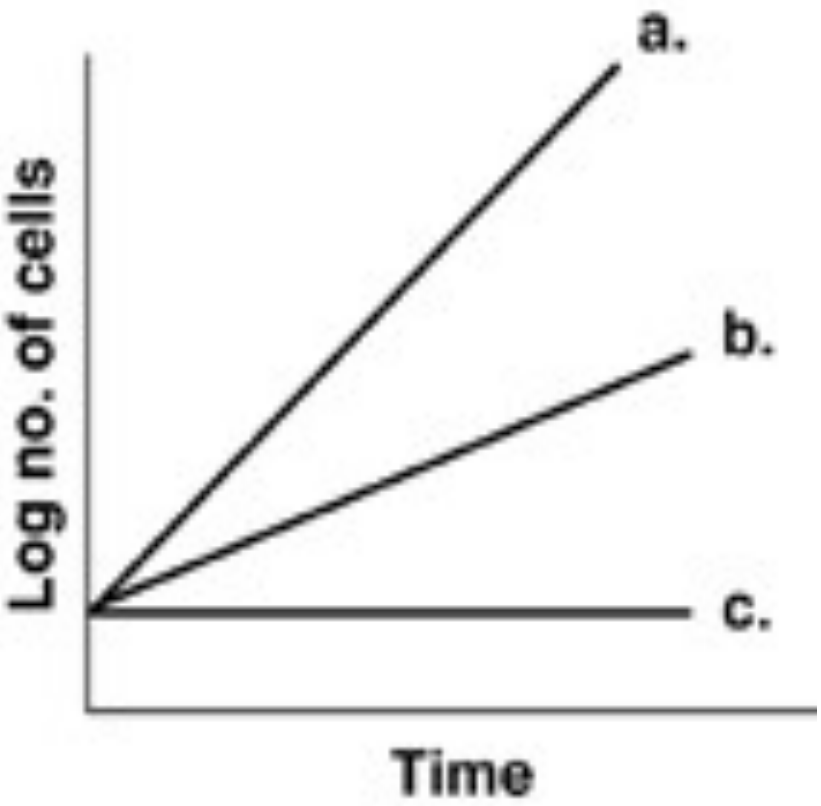
KEY CONCEPTS

- Bacterial populations follow a sequential series of growth phases: the lag, log, stationary, and death phases.
- Knowledge of the bacterial growth curve is critical to understanding population dynamics and population control in the course of infectious diseases, in food preservation and spoilage, as well as in industrial microbiology processes, such as ethanol production.

Bacterial Growth

1. Draw and label the bacterial growth curve
   - List the characteristics at each phase
2. If a population of 3000 cells growing in _____ phase has a generation time of 45 minutes, how many cells will there be in 3 hours?
3. A population contains 100 cells. 2 hours later there are 1600 cells. What is the generation time?

Which growth curve best represents …



1. a mesophile grown at room temperature?
2. a mesophile grown at body temperature?
3. a psychrotroph grown at room temperature?
4. a psychrophile grown at room temperature?
5. an obligate aerobe grown aerobically?
6. an obligate aerobe grown anaerobically?
7. a facultative anaerobe grown aerobically?
8. a facultative anaerobe grown anaerobically?
9. an obligate anaerobe grown aerobically?

Measurements of Bacterial Growth

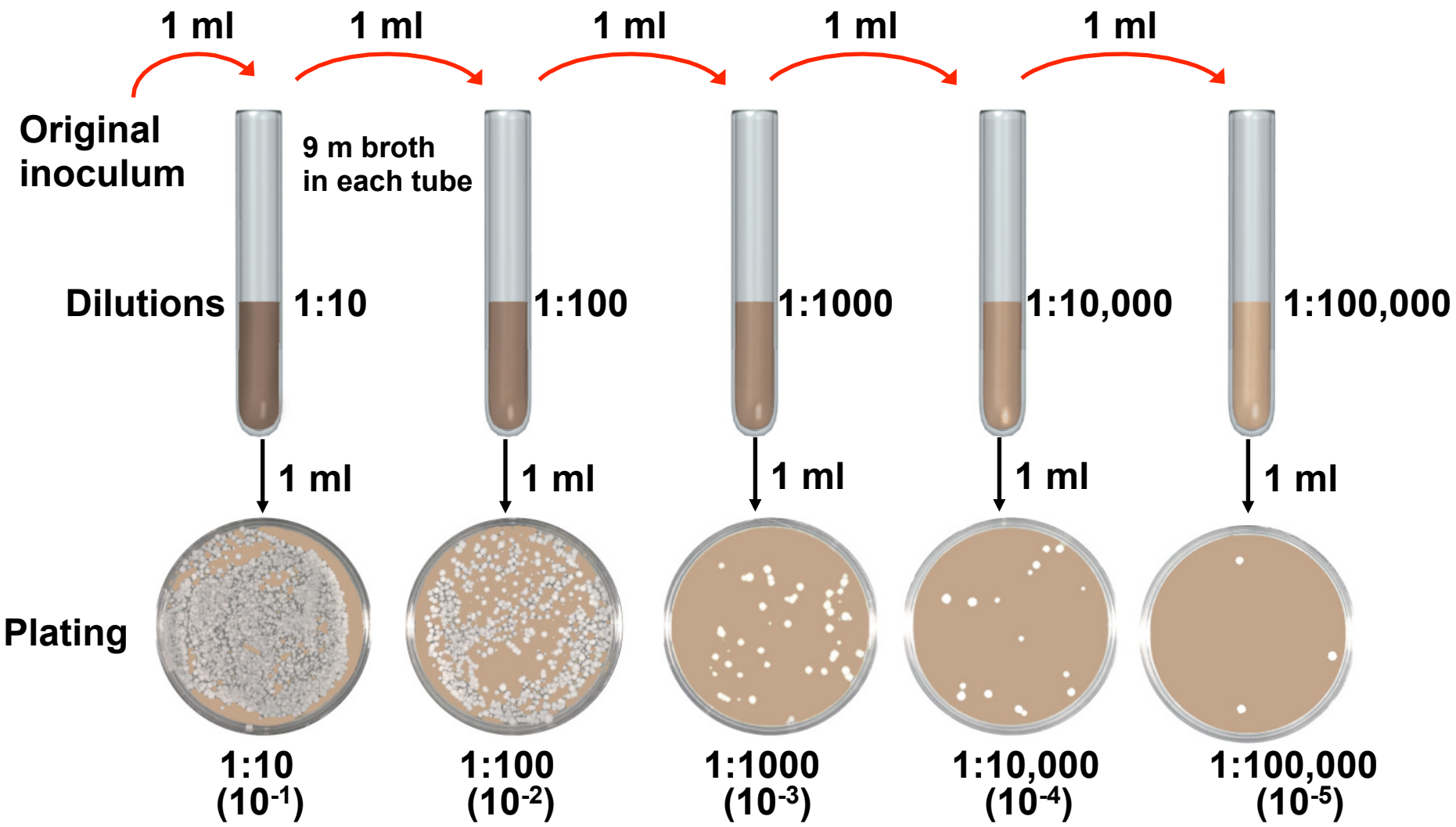
- Bacterial cultures and populations are quantified by two general types of measurements
  - **Direct** Measurements – measure cells or cell growth
  - **Indirect** Measurements – use alternative measures to determine population size

Direct Measurements of Microbial Growth

Standard Plate counts

- Grow microbial sample on agar plate
  - Count resulting colonies
  - 1 colony = 1 cell
- Advantages
  - Only viable (live) cells counted
  - Obtain real cell #
- Disadvantage
  - Takes time for colonies to form
  - Labor intensive

Figure 6.16 Serial dilutions and plate counts.

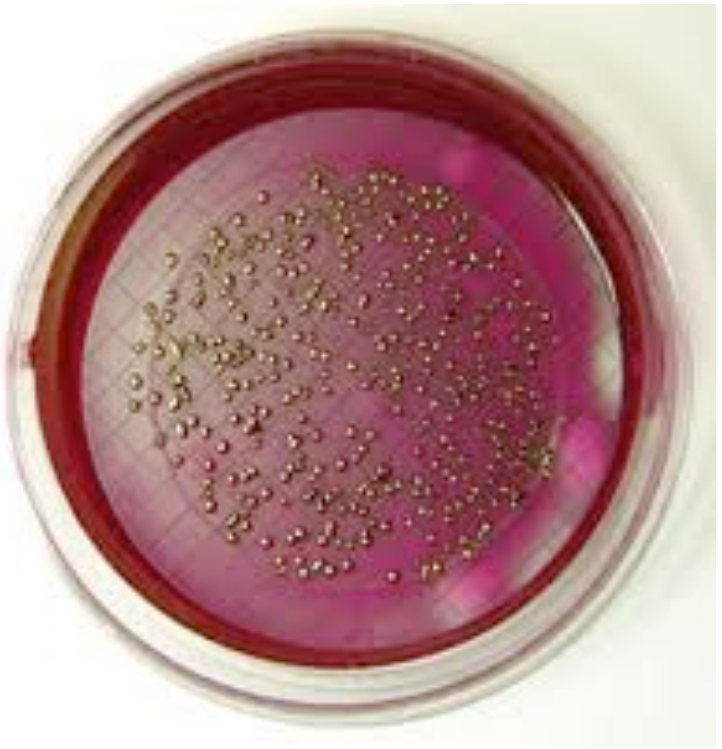


Calculation: Number of colonies on plate × reciprocal of dilution of sample = number of bacteria/ml
(For example, if 54 colonies are on a plate of 1:1000 dilution, then the count is 54 × 1000 = 54,000 bacteria/ml in sample.)

Direct Measurements of Microbial Growth

Filtration

- Liquid sample is passed through filter
  - Microbes retained on filter
- Filter is transferred to nutrient medium
- Useful when quantities of microbes in sample are small
- Often used to detect microbial contamination in food, water



Direct Measurements of Microbial Growth

Most Probable Number

- Multiple tube MPN test
- Dilute sample
  - Count tubes with growth
- Useful when bacteria do not grow on media
- But, numbers are an approximation
  - ~95% accurate

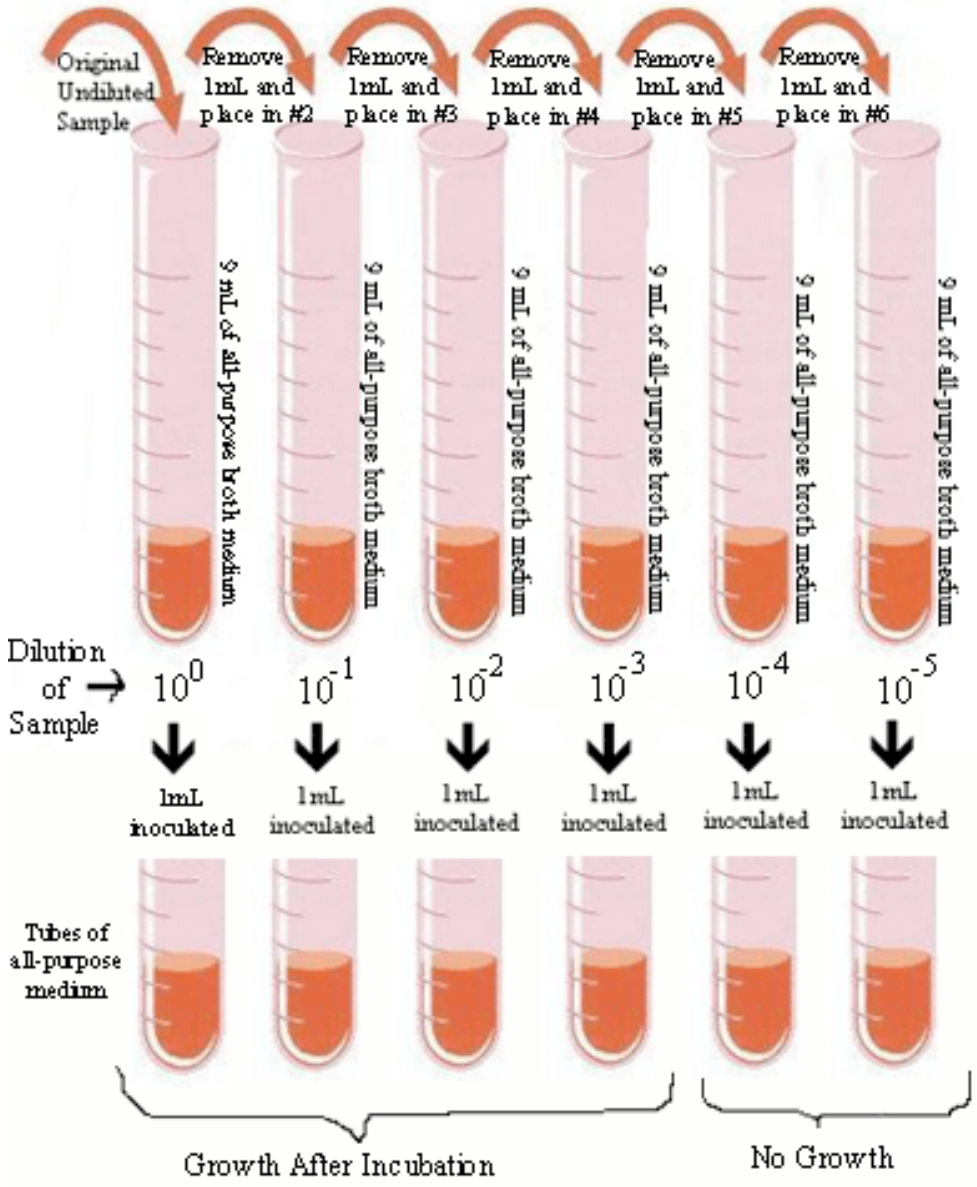


Figure 6.19b The most probable number (MPN) method.

Combination of Positives	MPN Index/ 100 m	95% Confidence Limits	
		Lower	Upper
4-2-0	22	6.8	50
4-2-1	26	9.8	70
4-3-0	27	9.9	70
4-3-1	33	10	70
4-4-0	34	14	100
5-0-0	23	6.8	70
5-0-1	31	10	70
5-0-2	43	14	100
5-1-0	33	10	100
5-1-1	46	14	120
5-1-2	63	22	150
5-2-0	49	15	150
5-2-1	70	22	170
5-2-2	94	34	230
5-3-0	79	22	220
5-3-1	110	34	250
5-3-2	140	52	400

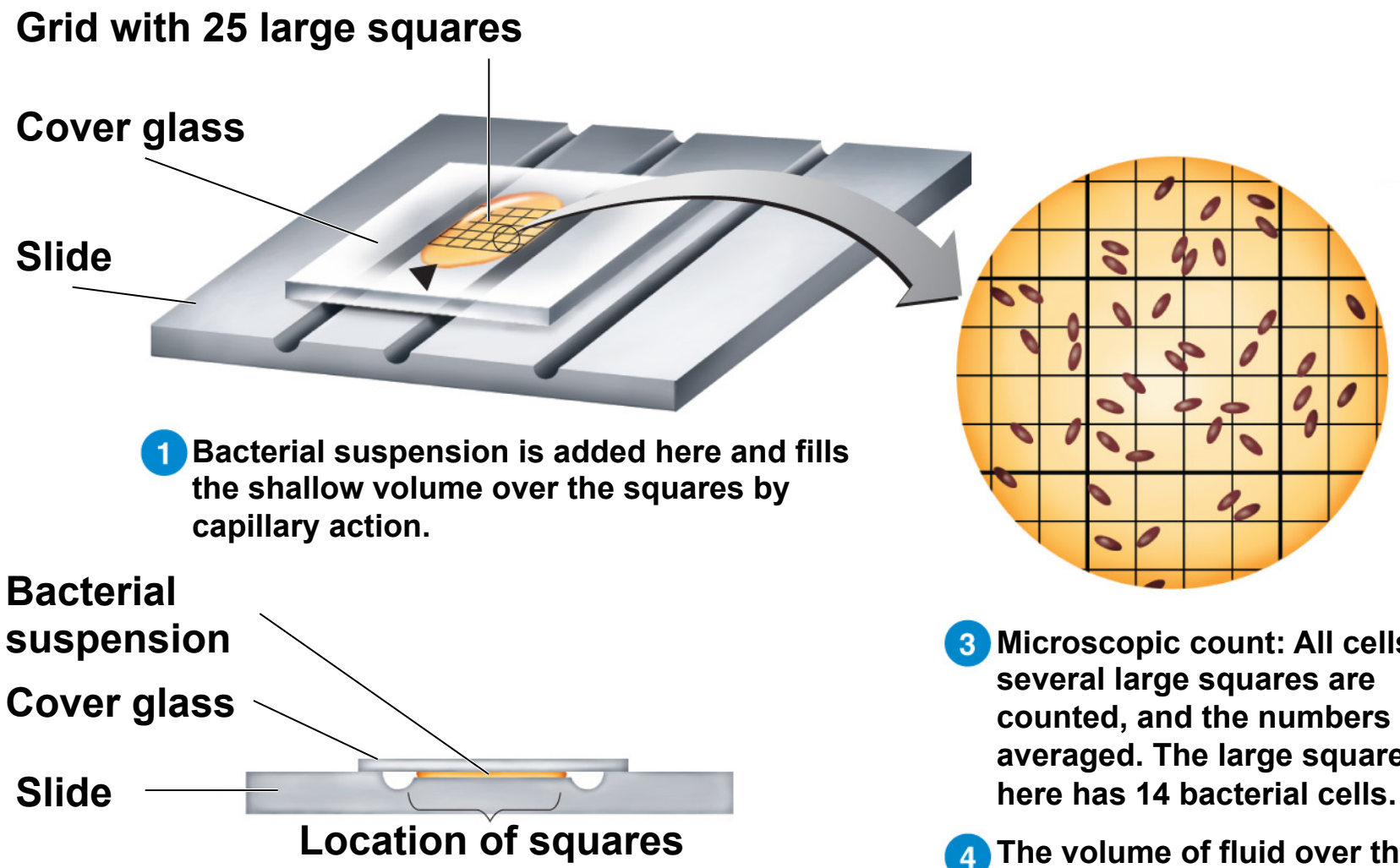
(b) MPN table.

Direct Measurements of Microbial Growth

Direct microscopic count

- Number of microbes counted in microscope
- Instant results, but …
  - Motile cells difficult to count
  - Dead cells look like live cells
  - Need high cell numbers to count accurately

Figure 6.20 Direct microscopic count of bacteria with a Petroff-Hausser cell counter.



1. Bacterial suspension is added here and fills the shallow volume over the squares by capillary action.

2. Cross section of a cell counter. The depth under the cover glass and the area of the squares are known, so the volume of the bacterial suspension over the squares can be calculated (depth × area).

3. Microscopic count: All cells in several large squares are counted, and the numbers are averaged. The large square shown here has 14 bacterial cells.

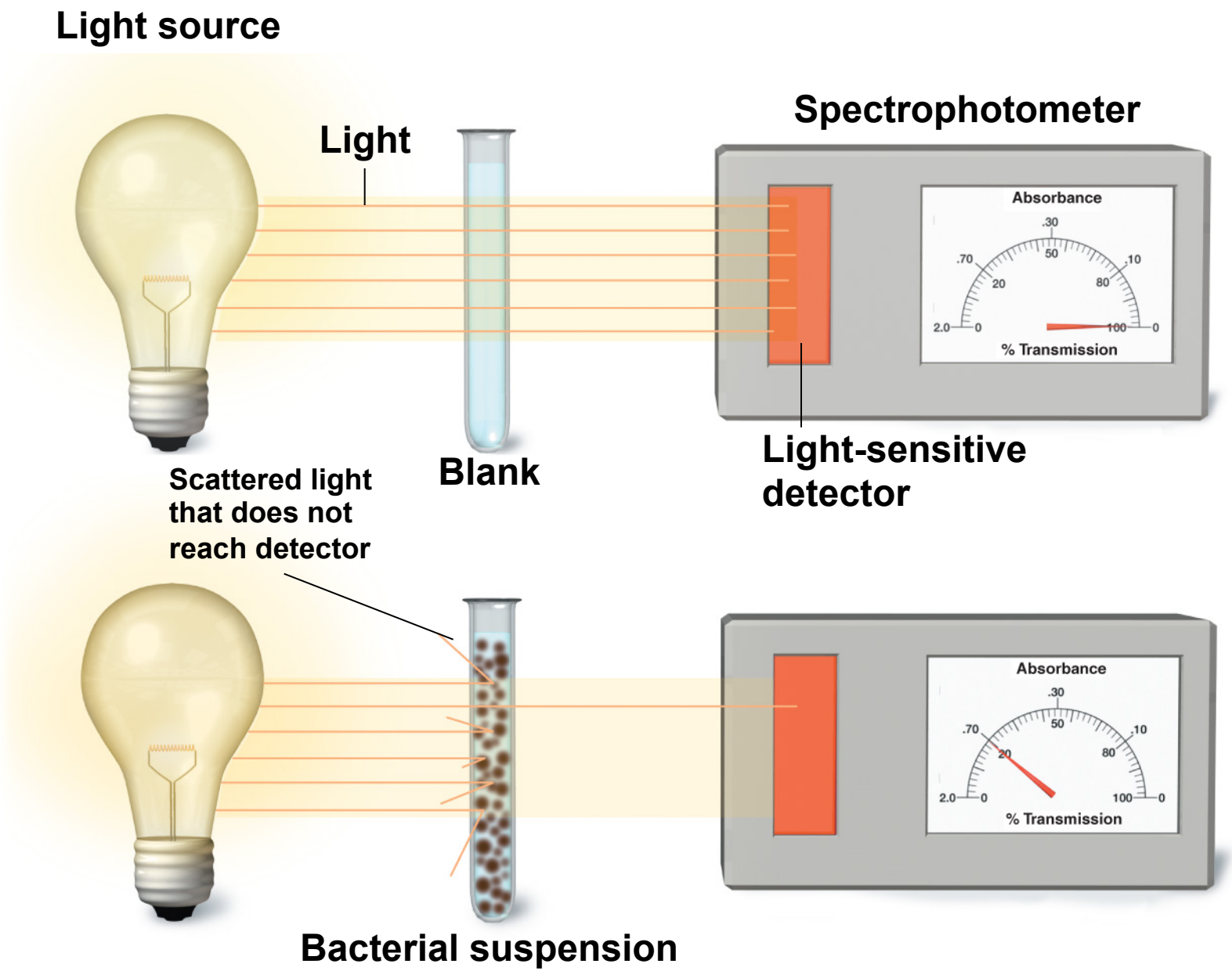
4. The volume of fluid over the large square is 1/1,250,000 of a milliliter. If it contains 14 cells, as shown here, then there are 14 × 1,250,000 = 17,500,000 cells in a milliliter.

Indirect Measurements of Bacterial Growth

Turbidity

- Cloudiness, or density, of a liquid culture
  - Detected using a spectrophotometer
- Higher cell number = increased cloudiness
- Fast and easy method of obtaining quantity, but …
  - Do not obtain cell # - values are only meaningful when compared to each other
  - Dead cells contribute to turbidity light just like live cells

Figure 6.21 Turbidity estimation of bacterial numbers.



Indirect Measurements of Bacterial Growth

Metabolic activity

- Assumes higher number of bacteria produces higher amount of metabolic product
  - Eg, measure CO_2 build-up
- Can be useful when cells can't be cultured
- Can be performed "on site" without needing to culture microbes

Indirect Measurements of Bacterial Growth

Dry Weight

- Removal of microbes from growth medium, dried, and weighed
- Useful for filamentous bacteria, molds

Measuring Microbial Growth

Direct Methods

- Plate counts
- Filtration
- MPN
- Direct microscopic count

Indirect Methods

- Turbidity
- Metabolic activity
- Dry weight