PowerPoint® Lecture Presentations

CHAPTER 5

Microbial Growth and Its Control

General Microbiology II

Lecture- 1

5.1 Binary Fission, Budding, and Biofilms

- Growth: *increase in the number of cells*
- Binary fission: cell division following enlargement of a cell to twice its minimum size (Figure 5.1)
- *Septum:* partition between dividing cells, pinches off between two daughter cells
- Generation time: time required for microbial cells to double in number
  - depends on nutritional and genetic factors and temperature
  - example: *Escherichia coli* = 20 minutes
- During cell division, each daughter cell receives a chromosome and sufficient copies of all other cell constituents to exist as an independent cell.

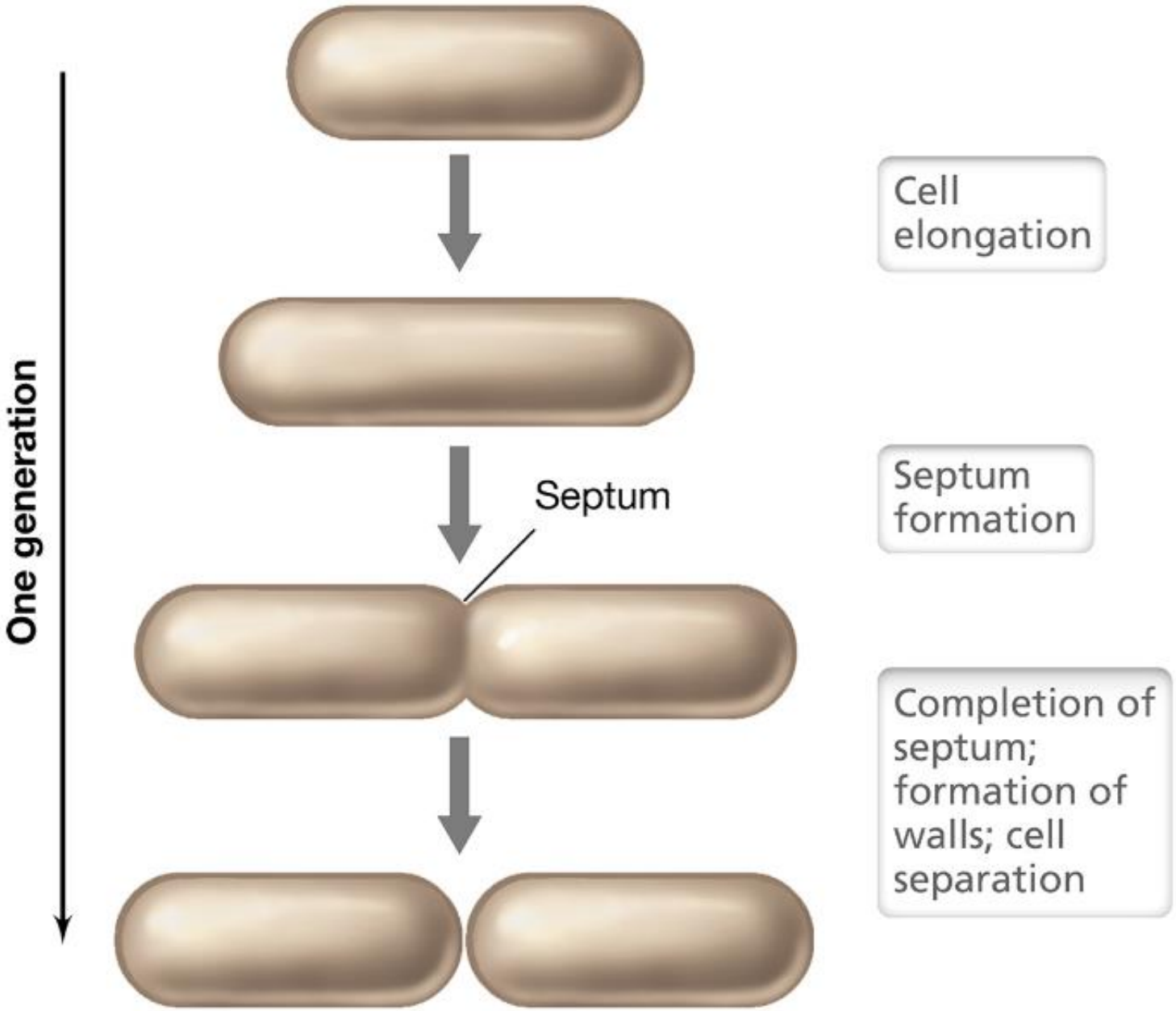
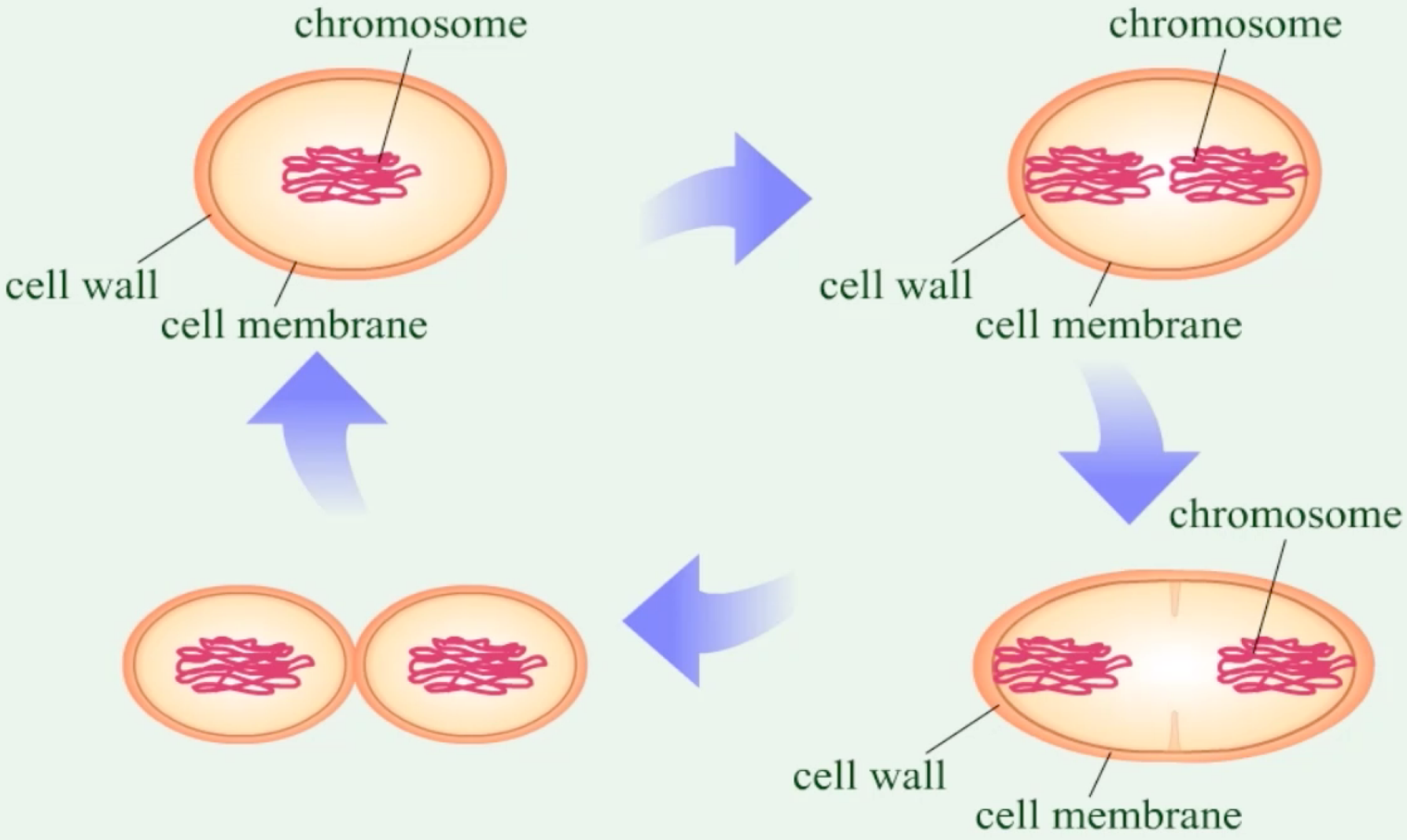


Figure 5.1

Binary Fission



5.1 Binary Fission, Budding, and Biofilms

- Budding division results from unequal cell growth and forms totally new daughter cell. (Figure 5.3)
- Some budding bacteria form cytoplasmic extensions such as stalks (*Caulobacter*), hyphae (*Hyphomicrobium*), and appendages (*Ancalomicrobium*).

I. **Equal products of cell division:**

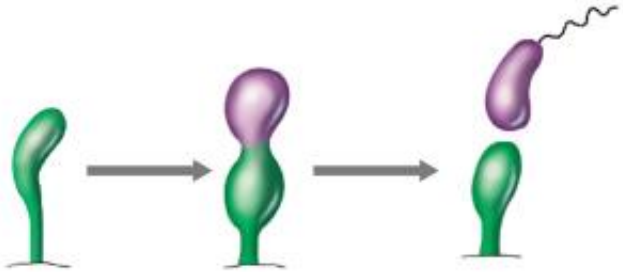
Binary fission: most bacteria

II. **Unequal products of cell division:**

1. Simple budding: *Pirellula, Blastobacter*

2. Budding from hyphae: *Hyphomicrobium*, *Rhodomicrobium*, *Pedomicrobium*

3. Cell division of stalked organism: *Caulobacter*



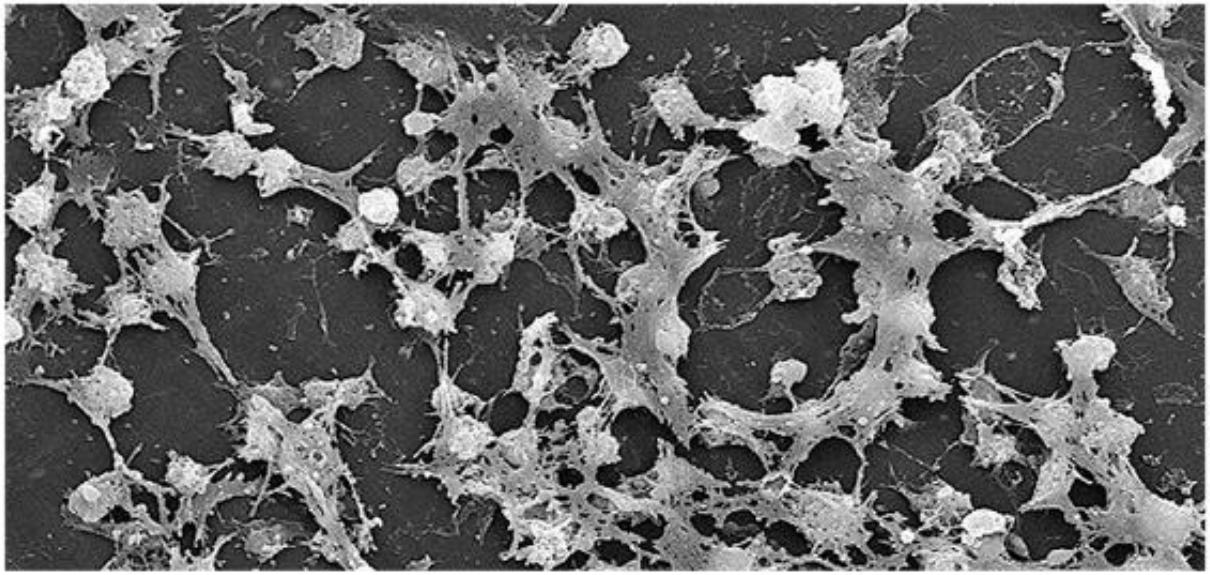
4. Polar growth without differentiation of cell size:

Rhodopseudomonas, *Nitrobacter*, *Methylosinus*

Figure 5.3

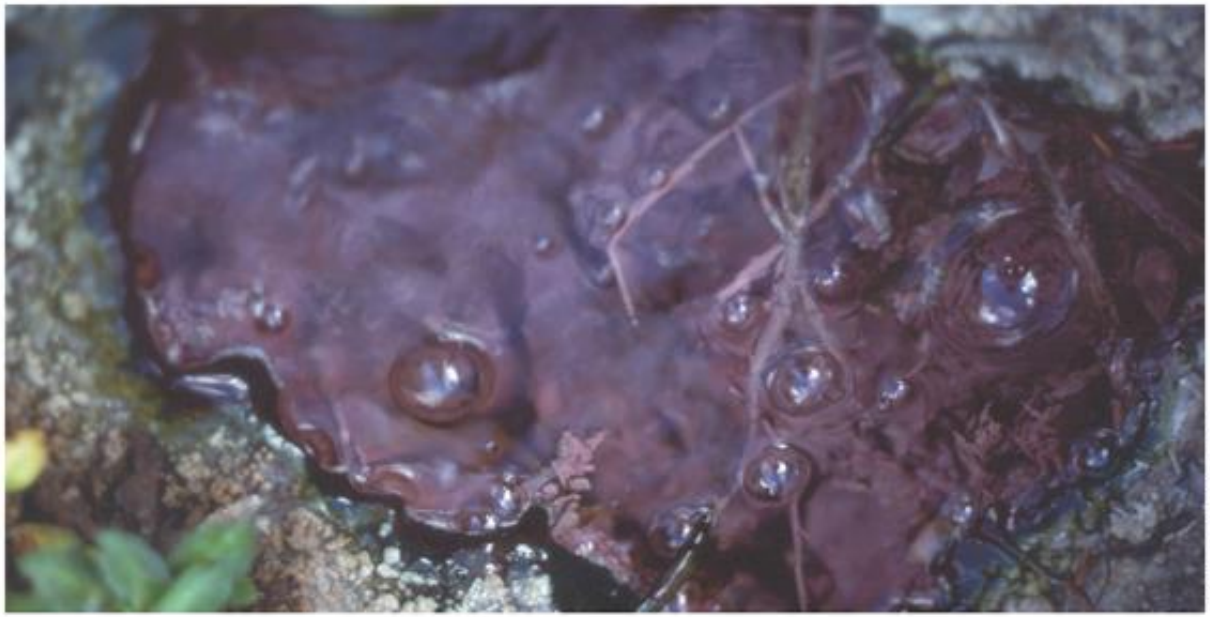
5.1 Binary Fission, Budding, and Biofilms

- *Planktonic growth:* growth as suspension
- *Sessile growth:*
  - attached to surface
  - can develop into biofilms
    - attached polysaccharide matrix containing embedded bacteria (Figure 5.4a)
- Biofilms form in stages:
  - Planktonic cells attach.
  - Sticky matrix forms.
- *Microbial mats:* multilayered sheets with different organisms in each layer (*e.g.,* hot springs, intertidal regions)



Janice Carr, CDC

(a)



Michael T. Madigan

(b)

Figure 5.4

5.1 Binary Fission, Budding, and Biofilms

- Biofilms prevent harmful chemicals (*e.g.*, antibiotics) from penetrating, prevent protists from grazing, and prevent washing away of cells.
- Biofilms affect human health, water distribution systems, and fuel storage.

5.2 Quantitative Aspects of Microbial Growth

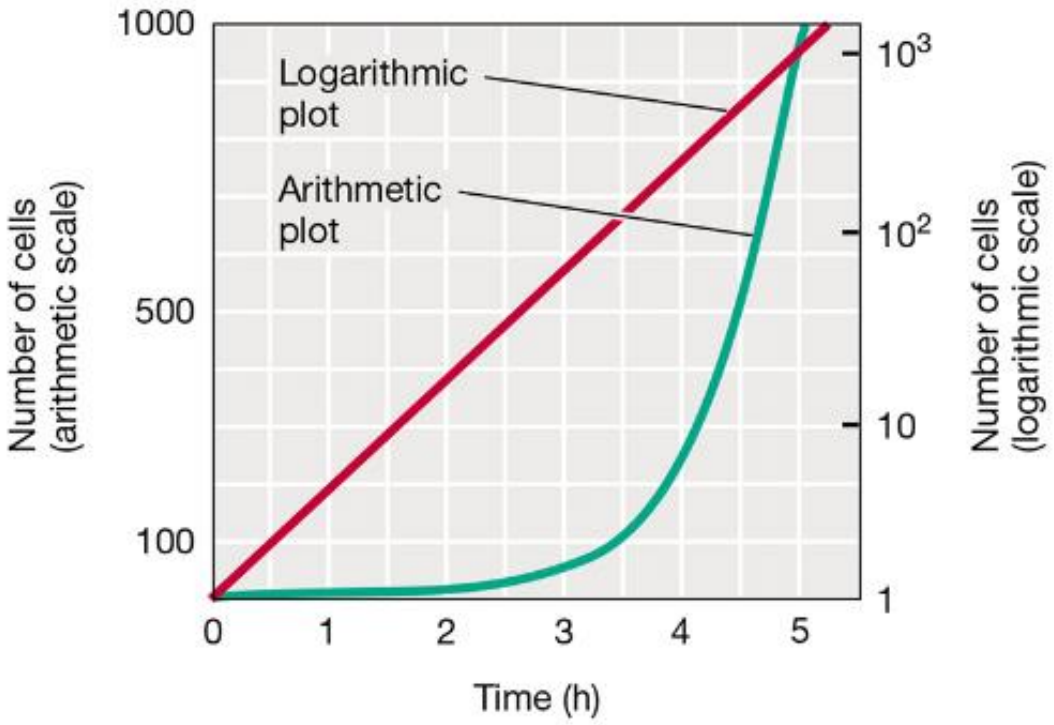
- Exponential growth: growth of a microbial population in which cell numbers double within a specific time interval (Figure 5.5)
- A relationship exists between the initial number of cells present in a culture and the number present after a period of exponential growth:

$$N = N_0 2^n$$

  - N is the final cell number.
  - N_0 is the initial cell number.
  - n is the number of generations during the period of exponential growth.

Time (h)	Total number of cells	Time (h)	Total number of cells
0	1	4	256 (2^8)
0.5	2	4.5	512 (2^9)
1	4	5	1,024 (2^{10})
1.5	8	5.5	2,048 (2^{11})
2	16	6	4,096 (2^{12})
2.5	32	.	.
3	64	.	.
3.5	128	10	1,048,576 (2^{20})

(a)



(b)

Figure 5.5

5.2 Quantitative Aspects of Microbial Growth

- Generation time (*g*) (Figure 5.6) of the exponentially growing population is:

$$g = t/n$$

  - *t* is the duration of exponential growth (days/hours/minutes).
  - *n* is the number of generations during the period of exponential growth.

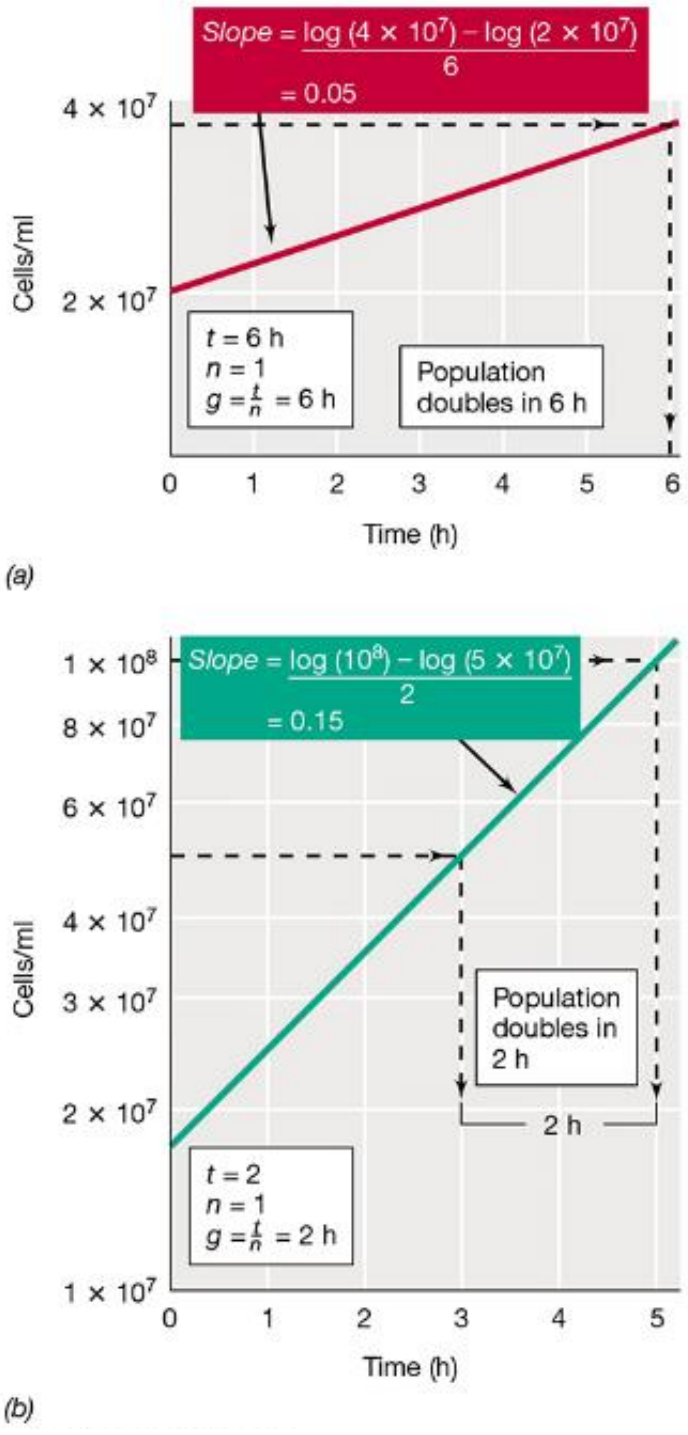


Figure 5.6

5.2 Quantitative Aspects of Microbial Growth

- *Instantaneous growth rate constant* (*k*) expresses rate of growth at any instant.
- Specific growth rate (*k*) is calculated as

$$k = 0.693/g$$

- Initial increase is slow but increases, resulting in huge increase in cell numbers (*e.g.,* lactic acid bacteria spoiling milk).

5.3 The Microbial Growth Cycle

- *Batch culture:* a closed-system microbial culture of fixed volume
- Typical growth curve for population of cells grown in a closed system is characterized by four phases. (Figure 5.7)
  - *lag phase*
  - *exponential phase*
  - *stationary phase*
  - *death phase*

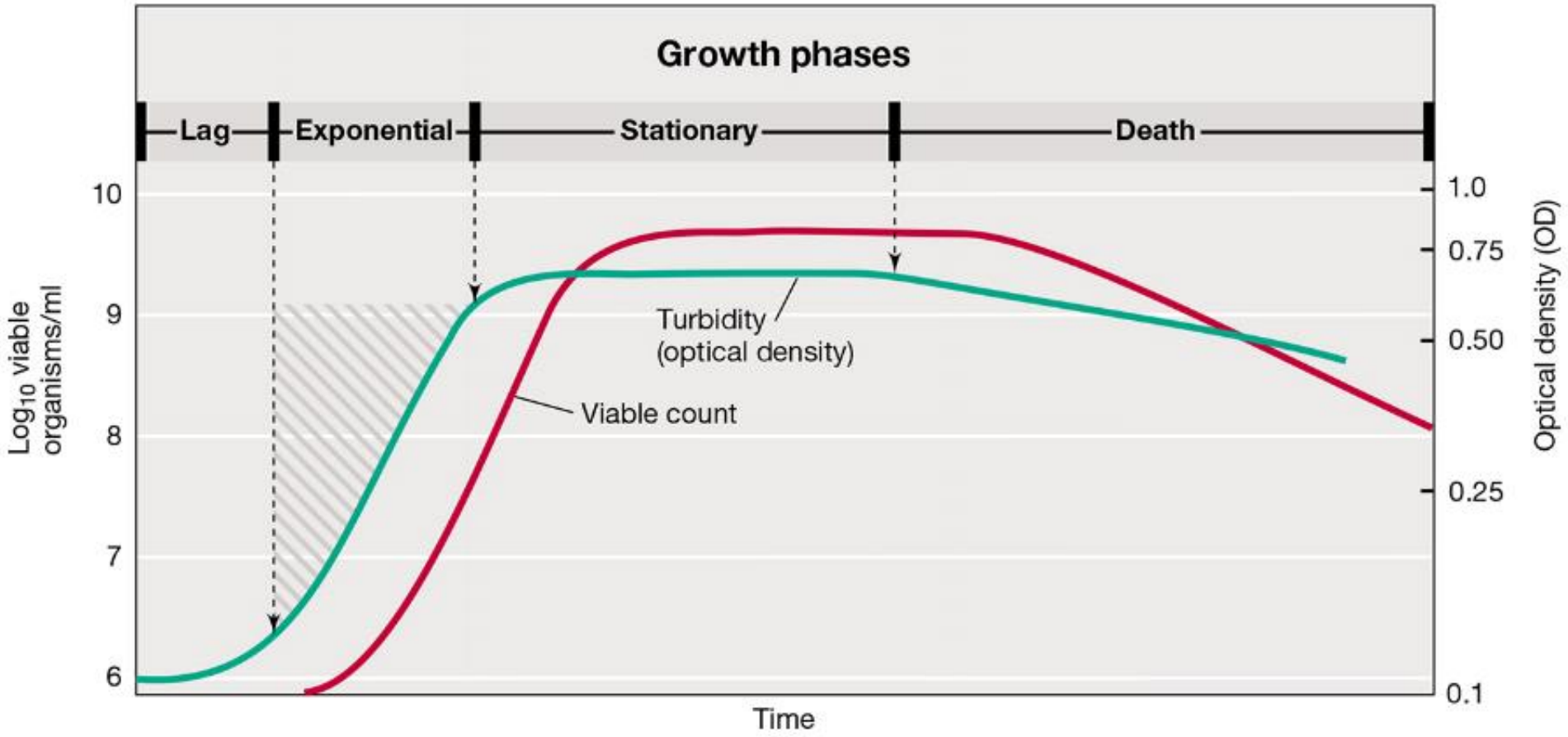
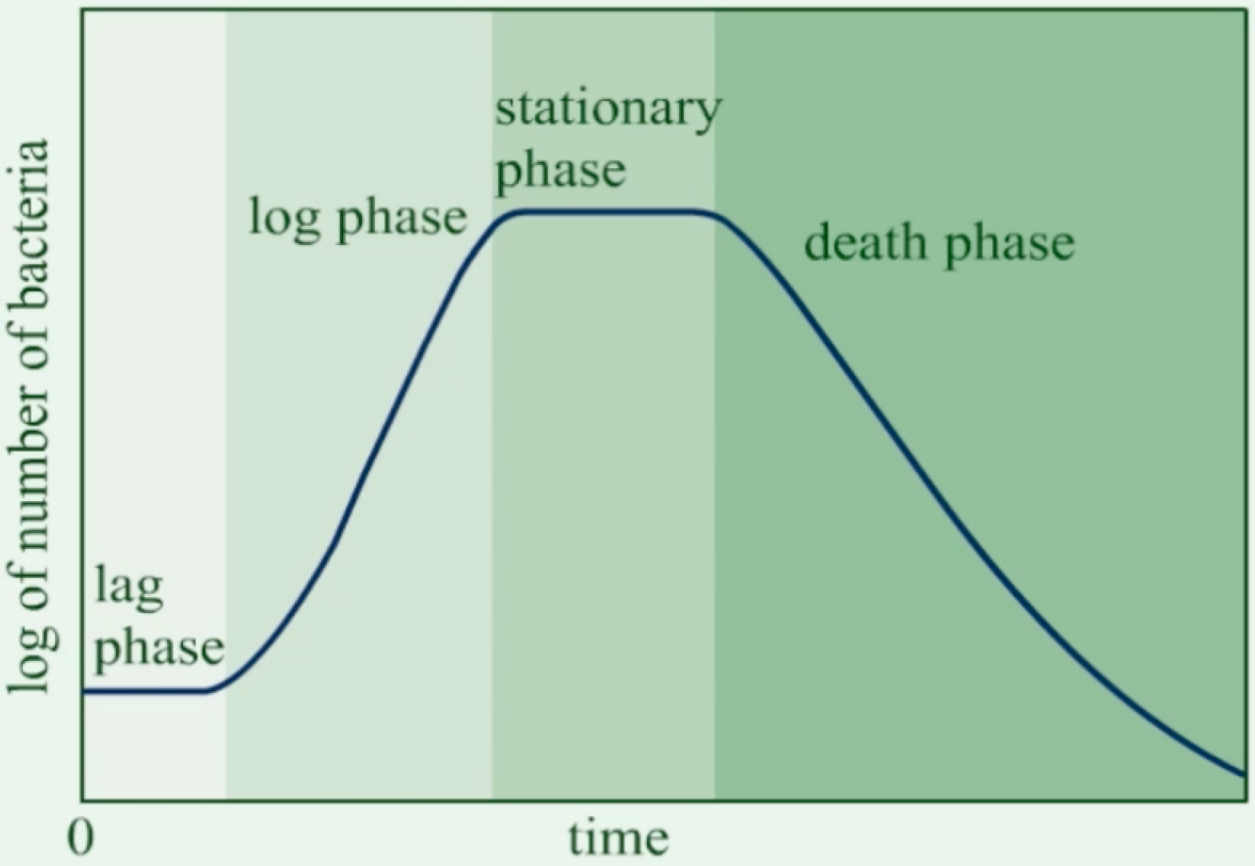


Figure 5.7

Bacterial Growth Curve



5.3 The Microbial Growth Cycle

- *Lag phase*
  - interval between inoculation of a culture and beginning of growth
  - time needed for biosynthesis of new enzymes and to produce required metabolites before growth can begin
- *Exponential phase*
  - Cells in this phase are typically in the healthiest state.

5.3 The Microbial Growth Cycle

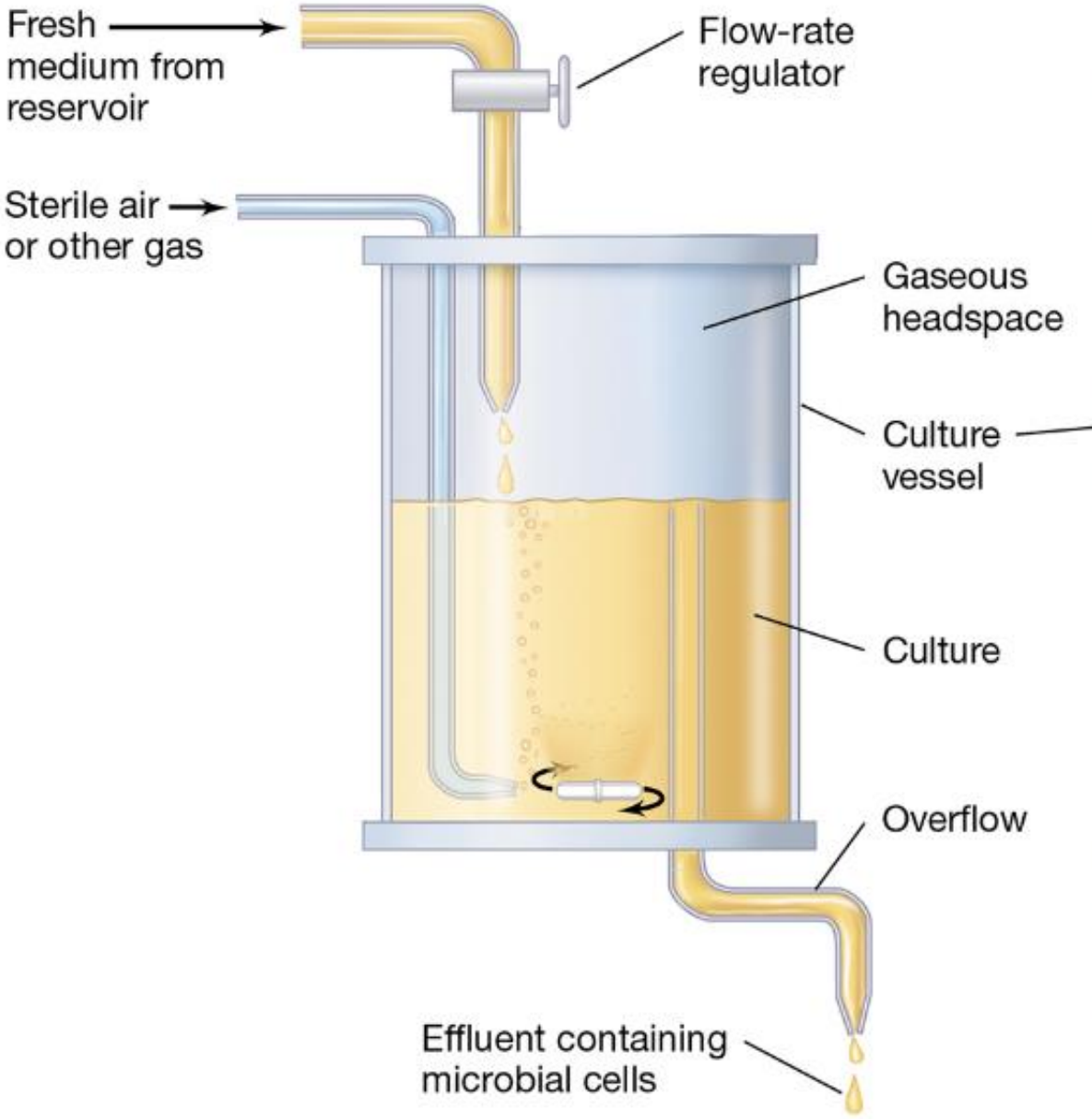
- *Stationary phase*
  - Growth rate of population is zero.
  - Either an essential nutrient is used up or waste products accumulate.
  - Metabolism continues at greatly reduced rate.
  - Some cells grow while others die, balancing each other.

5.3 The Microbial Growth Cycle

- *Death phase*
  - If incubation continues after cells reach stationary phase, the cells will eventually die.
  - exponential rate
  - typically much slower than exponential growth
  - Viable cells remain for months or years.

5.4 Continuous Culture

- *Continuous culture:* an open system microbial culture of fixed volume
- Chemostat: most common type of continuous culture device (Figure 5.8)
- Both growth rate and population density of culture can be controlled independently and simultaneously, depending on:
  - Dilution rate: F/V (F is flow rate of adding fresh medium and removing spent medium, V is culture volume).
  - concentration of a limiting nutrient.
- *Steady state:* cell density and substrate concentration do not change over time.



(a)

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Figure 5.8

5.4 Continuous Culture

- Experimental Uses
  - can maintain exponential growth phase for weeks/months
  - used to study physiology, microbial ecology and evolution, enrichment and isolation of bacteria from nature
  - growth rate controlled by dilution rate

II. Culturing Microbes and Measuring Their Growth

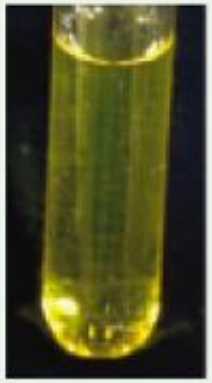
- 5.5 Growth Media and Laboratory Culture
- 5.6 Microscopic Counts of Microbial Cell Numbers
- 5.7 Viable Counting of Microbial Cell Numbers
- 5.8 Turbidimetric Measures of Microbial Cell Numbers

5.5 Growth Media and Laboratory Culture

- Culture media (Table 5.1)
  - nutrient solutions used to grow microbes in the laboratory
  - typically sterilized in an *autoclave*

- Two broad classes:
  - *defined media*: exact chemical composition known.
  - *complex media*: composed of digests of microbial, animal, or plant products (*e.g.,* yeast and meat extracts)

TABLE 5.1 Examples of culture media for microorganisms with simple and demanding nutritional requirements[a]

Defined culture medium for Escherichia coli	*Defined culture medium for* Leuconostoc mesenteroides	*Complex culture medium for either* E. coli *or* L. mesenteroides	*Defined culture medium for* Thiobacillus thioparus
K_2HPO_4 7 g	K_2HPO_4 0.6 g	Glucose 15 g	KH_2PO_4 0.5 g
KH_2PO_4 2 g	KH_2PO_4 0.6 g	Yeast extract 5 g	NH_4Cl 0.5 g
$(NH_4)_2SO_4$ 1 g	NH_4Cl 3 g	Peptone 5 g	$MgSO_4$ 0.1 g
$MgSO_4$ 0.1 g	$MgSO_4$ 0.1 g	KH_2PO_4 2 g	$CaCl_2$ 0.05 g
$CaCl_2$ 0.02 g	Glucose 25 g	Distilled water 1000 ml	KCl 0.5 g
Glucose 4–10 g	Sodium acetate 25 g	pH 7	$Na_2S_2O_3$ 2 g
Trace elements (Fe, Co, Mn, Zn, Cu, Ni, Mo) 2–10 µg each	Amino acids (alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine) 100–200 µg of each		Trace elements (as in first column) 2–10 µg each
Distilled water 1000 ml	Purines and pyrimidines (adenine, guanine, uracil, xanthine) 10 mg of each		Distilled water 1000 ml
pH 7	Vitamins (biotin, folate, nicotinic acid, pyridoxal, pyridoxamine, pyridoxine, riboflavin, thiamine, pantothenate, *p*-aminobenzoic acid) 0.01–1 mg of each		pH 7
	Trace elements (as in first column) 2–10 µg each		Carbon source: CO_2 from air
(a)	Distilled water 1000 ml	*(b)*	
	pH 7		

[a]The photos are tubes of (a) the defined medium described, and (b) the complex medium described. Note how the complex medium is colored from the various organic extracts and digests that it contains. Photo credits: Cheryl L. Broadie and John Vercillo, Southern Illinois University at Carbondale.

Table 5.1

5.5 Growth Media and Laboratory Culture

- *Enriched media*
  - contain complex media plus highly nutritious materials (*e.g.*, serum or blood)
  - used to culture *fastidious* (nutritionally demanding) microbes
- *Selective media*
  - contain compounds that selectively inhibit growth of some microbes but not others
- *Differential media*
  - contain an indicator, usually a dye, that detects particular metabolic reactions during growth

5.5 Growth Media and Laboratory Culture

- For successful cultivation of a microbe, it is important to know the nutritional requirements and supply them in proper form and proportions in a culture medium.
- Cells can be grown in liquid or solid culture media.
  - Solid media are prepared by addition of the gelling agent *agar* (Figure 5.11) to liquid media.
  - When grown on solid media, cells form isolated masses (*colonies*).

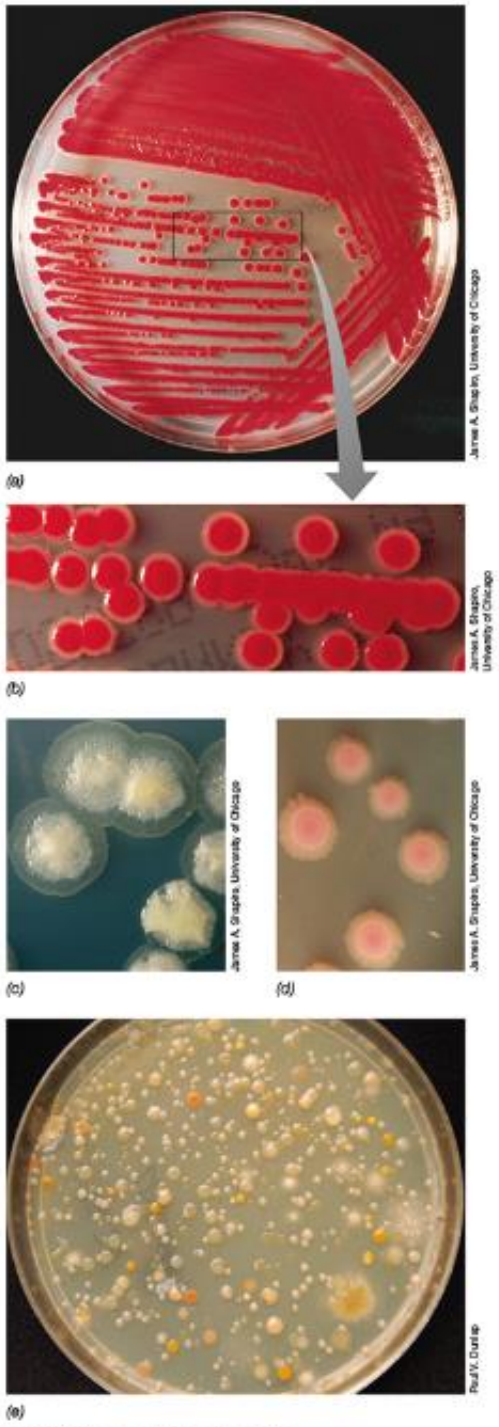


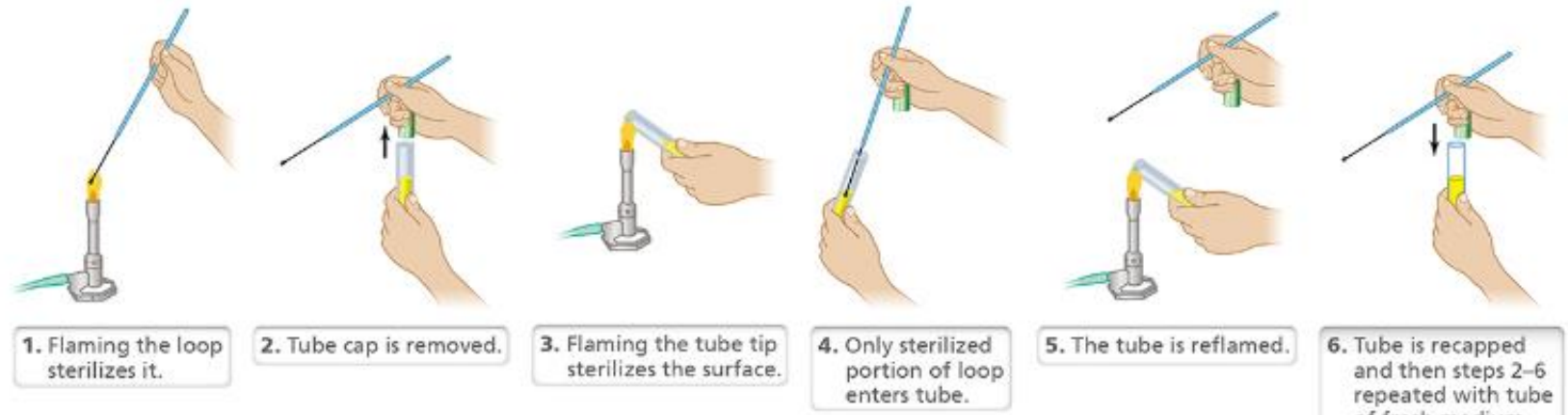
Figure 5.11

General Microbiology II

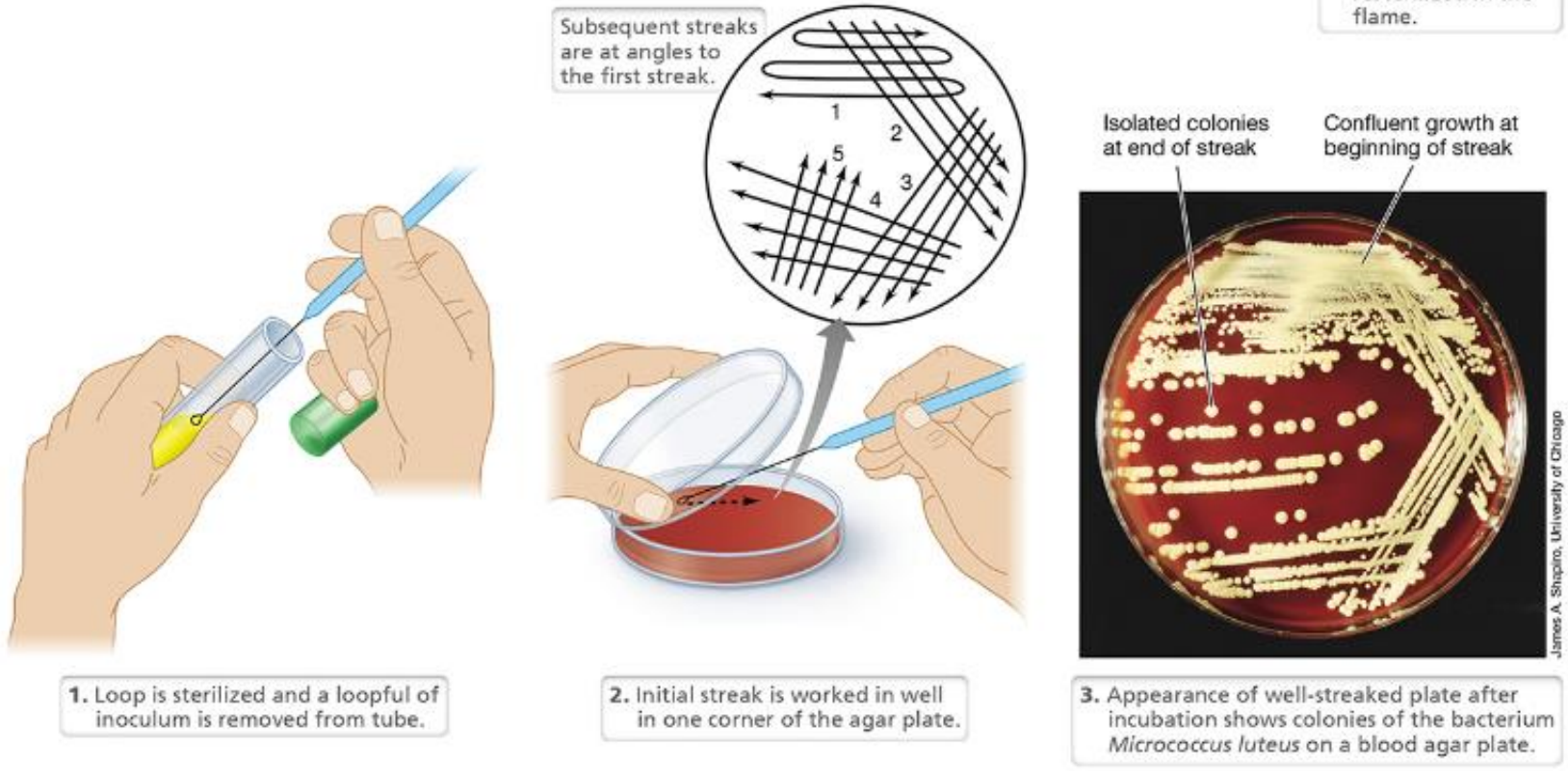
Lecture- 2

5.5 Growth Media and Laboratory Culture

- Microbes are everywhere.
  - Sterilization of media is critical.
  - To prevent contamination, aseptic technique should be followed. (Figure 5.12)



(a) **Transfering a liquid culture**



(b) **Streaking a Petri plate**

Figure 5.12

5.6 Microscopic Counts of Microbial Cell Numbers

- Total cell count
  - *microscopic cell count:* observing and enumerating cells present
  - dried on slides or on liquid samples
  - counting chambers with squares etched on a slide for liquid samples (Figure 5.13)

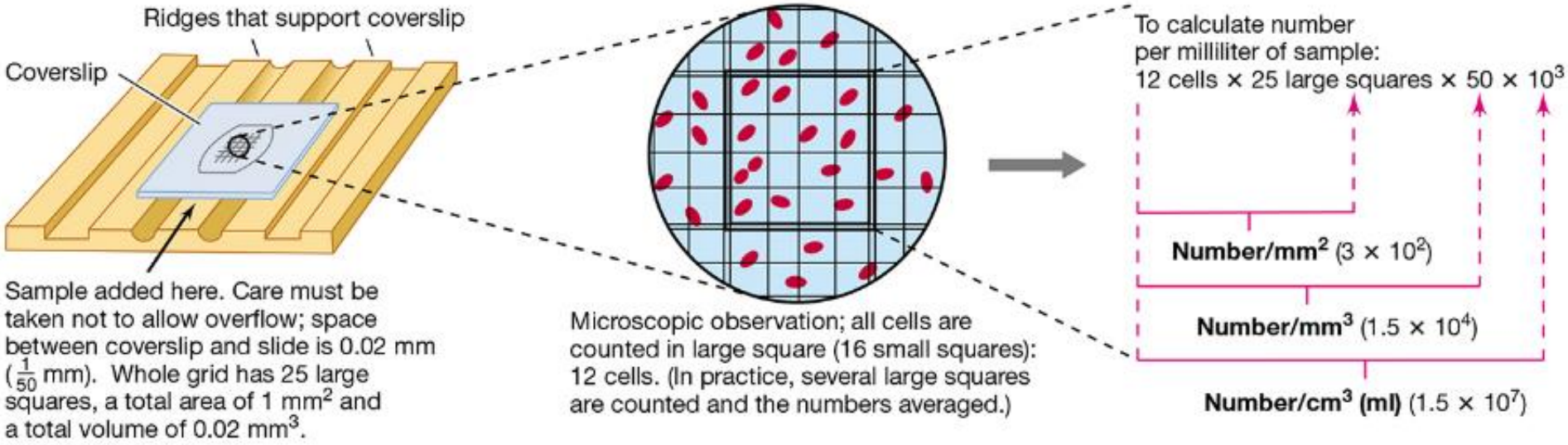


Figure 5.13

5.6 Microscopic Counts of Microbial Cell Numbers

- Limitations of microscopic cell counts
  - cannot distinguish between live and dead cells without special stains
  - Precision is difficult to achieve.
  - Small cells can be overlooked.
  - phase-contrast microscope required if a stain is not used
  - cell suspensions of low density (< 10^6 cells/ml) hard to count
  - Motile cells need to immobilized.
  - Debris in sample can be mistaken for cells.

5.6 Microscopic Counts of Microbial Cell Numbers

- Microscopic cell counts in microbial ecology
  - often used on natural samples
  - use stains to visualize and provide phylogenetic information or metabolic properties
  - DAPI reacts with DNA.
  - Other fluorescent stains differentiate live and dead cells.
  - *Phylogenetic stains* can determine proportion of *Bacteria* or *Archaea.*

5.7 Viable Counting of Microbial Cell Numbers

- Viable (plate) counts: measurement of living, reproducing population
  - two main ways to perform plate counts:
    - *spread-plate* method
    - *pour-plate* method
  - count colonies on plates with 30–300 colonies
  - To obtain the appropriate colony number, the sample to be counted should always be diluted. (Figure 5.15)

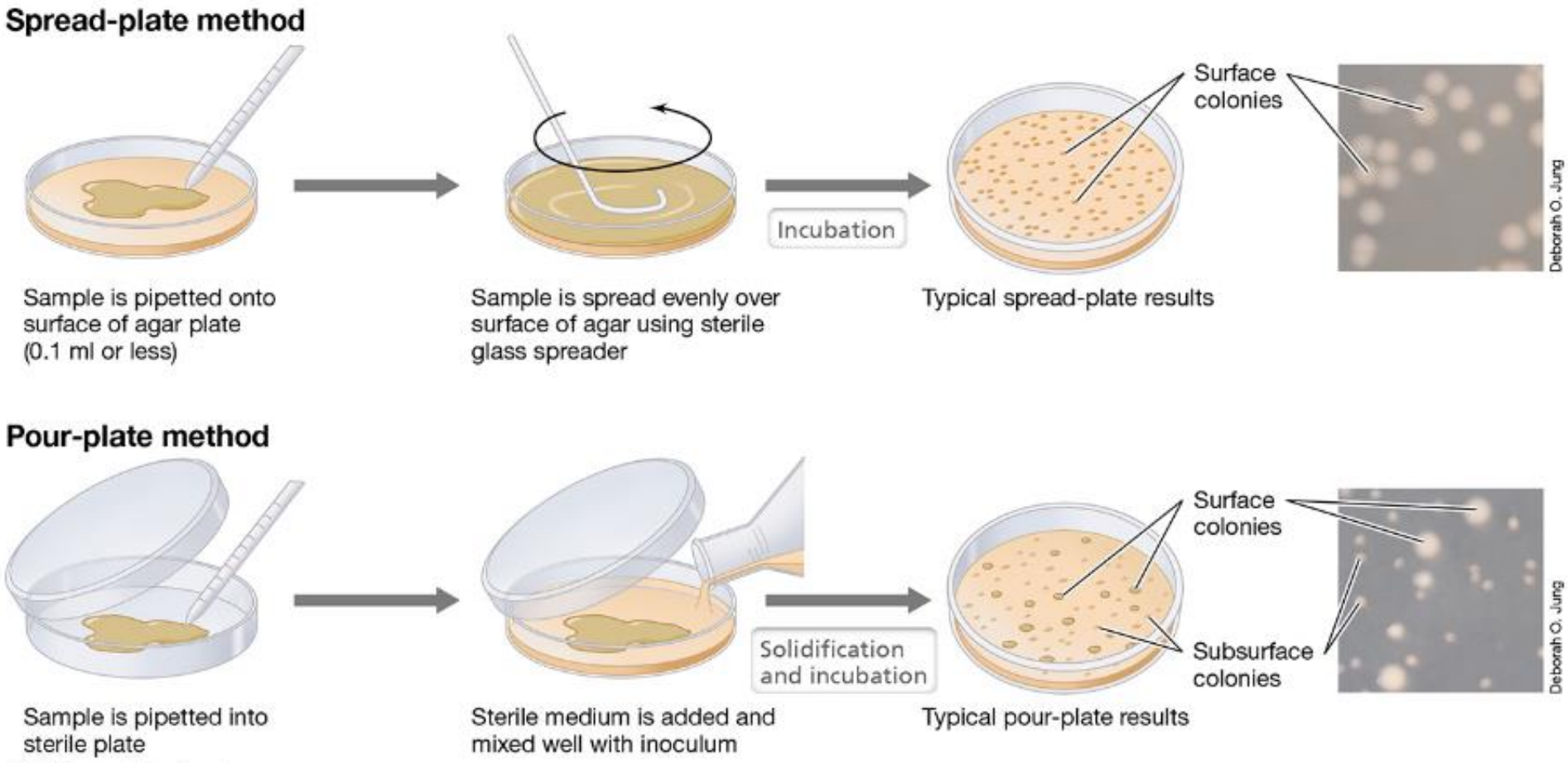


Figure 5.14

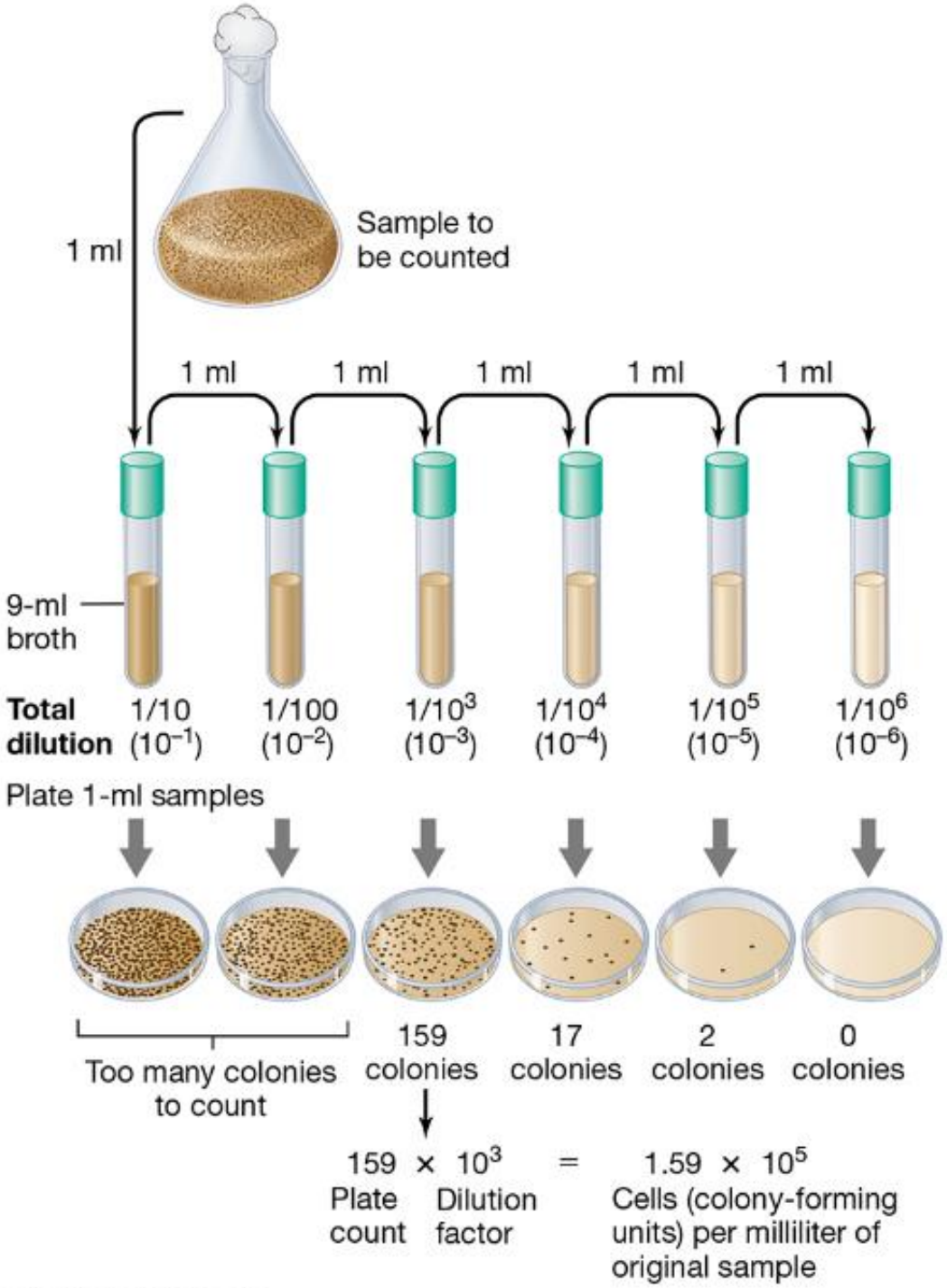


Figure 5.15

5.7 Viable Counting of Microbial Cell Numbers

- Sources of error in plate counting
  - depends on inoculum size, viability, culture medium, incubation conditions
  - Mixed cultures grow at different rates.
  - plating inconsistencies
  - Reporting in *colony-forming units* instead of number of viable cells accounts for clumps.

5.7 Viable Counting of Microbial Cell Numbers

- Applications
  - quick and easy
  - used in food, dairy, medical, and aquatic microbiology, and water analyses
  - high sensitivity
  - can target particular species in mixed samples

5.7 Viable Counting of Microbial Cell Numbers

- "The great plate count anomaly": Direct microscopic counts of natural samples reveal far more organisms than those recoverable on plates.
- Why is this?
  - Microscopic methods count dead cells, whereas viable methods do not.
  - Different organisms may have vastly different requirements for growth.

5.8 Turbidimetric Measures of Microbial Cell Numbers

- Cell suspensions are *turbid* (cloudy) because cells scatter light.
- Most often turbidity is measured with a spectrophotometer (Figure 5.16), and measurement is referred to as *optical density* (*OD*) at specified wavelength (*e.g.*, OD_{540} for measurements at 540 nm [green light]).
- For unicellular organisms, OD is proportional to cell number within limits.
- To relate a direct cell count to a turbidity value, a standard curve must first be established.

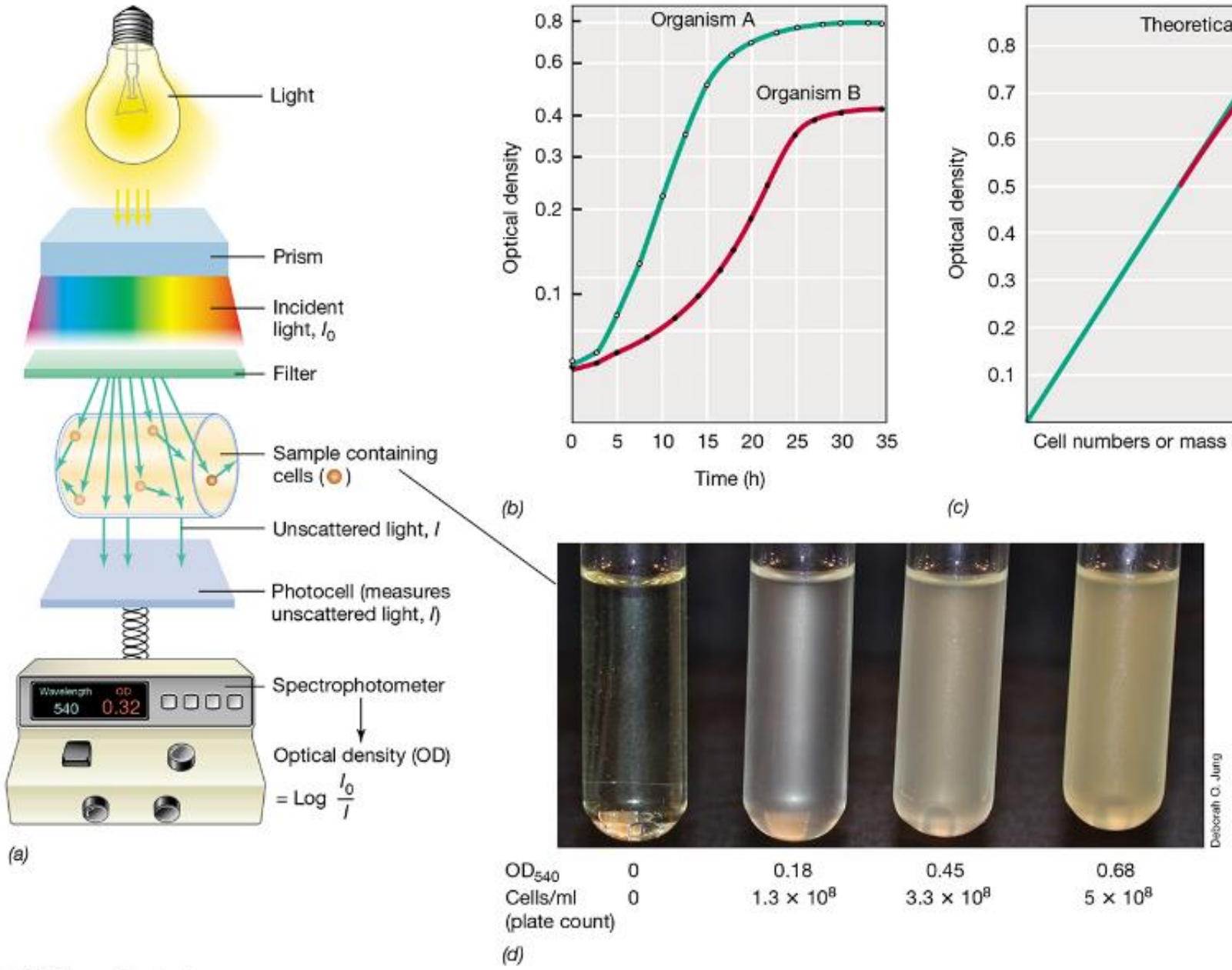


Figure 5.16

5.8 Turbidimetric Measures of Microbial Cell Numbers

- Advantages
  - quick and easy to perform
  - typically do not require destruction or significant disturbance of sample
  - Same sample can be checked repeatedly.
- Disadvantages
  - sometimes problematic (*e.g.,* microbes that form clumps or biofilms in liquid medium)

III. Environmental Effects on Growth: Temperature

- 5.9 Temperature Classes of Microorganisms
- 5.10 Microbial Life in the Cold
- 5.11 Microbial Life at High Temperatures

5.9 Temperature Classes of Microorganisms

- Temperature is a major environmental factor controlling microbial growth.
- Cardinal temperatures: the minimum, optimum, and maximum temperatures at which an organism grows (Figure 5.17)
- Range is typically < 40°C

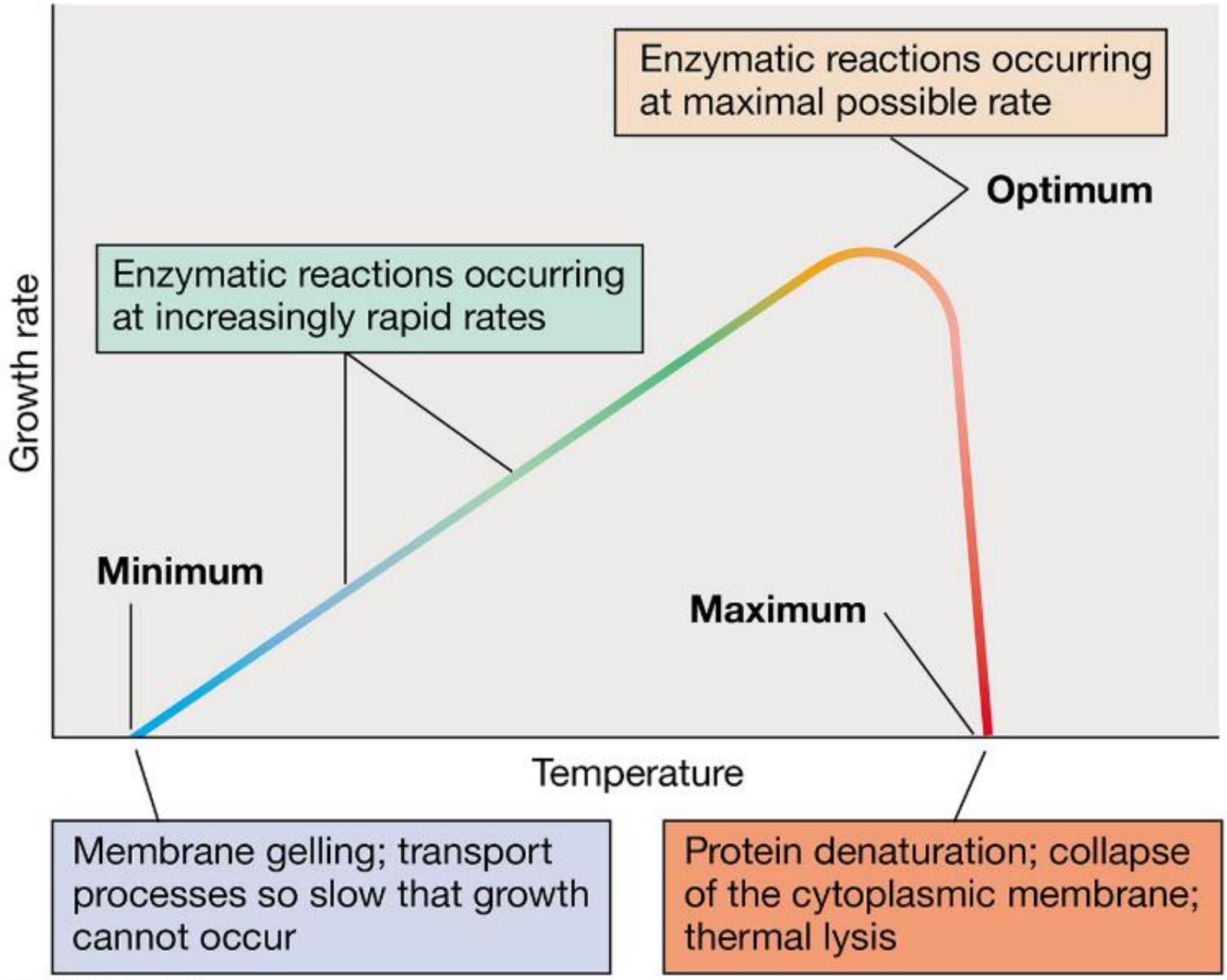


Figure 5.17

5.9 Temperature Classes of Microorganisms

- Microorganisms can be classified into groups by their growth temperature optima. (Figure 5.18)
  - psychrophile: low, found in cold environments
  - mesophile: midrange, most commonly studied
  - thermophile: high, found in hot environments
  - hyperthermophile: very high, found in extremely hot habitats such as hot springs and deep-sea hydrothermal vents

5.10 Microbial Life in the Cold

- *Extremophiles*
  - organisms that grow under very hot or very cold conditions
- Psychrophiles (Figures 5.19 and 5.20)
  - organisms with optimal growth temperature ≤ 15°C, maximum ≤ 20°C, minimum ≤ 0°C
  - inhabit constantly cold environments

5.10 Microbial Life in the Cold

- Psychrotolerant
  - organisms that can grow at 0°C but have optima of 20°C to 40°C
  - More widely distributed in nature than psychrophiles
  - isolated from soils and water in temperate climates and food at 4°C

5.10 Microbial Life in the Cold

- Molecular adaptations to life in the cold
  - production of enzymes that function optimally in the cold
    - more α-helices than β-sheets → greater flexibility for catalysis at cold temperatures
    - more polar and fewer hydrophobic amino acids
    - fewer weak bonds (*e.g.*, hydrogen and ionic bonds)

5.10 Microbial Life in the Cold

- Molecular adaptations to life in the cold
  - Cytoplasmic membranes function at low temperatures.
    - high unsaturated and shorter-chain fatty acid content
    - some polyunsaturated fatty acids, which remain flexible at very low temperatures
  - cold shock proteins (chaperones)
  - Cryoprotectants (*e.g.,* antifreeze proteins, certain solutes) prevent formation of ice crystals.
  - exopolysaccharide cell surface slime

5.11 Microbial Life at High Temperatures

- *Thermophiles:* organisms with growth temperature optima between 45°C and 80°C
- *Hyperthermophiles:* organisms with optima greater than 80°C
  - inhabit hot environments, including boiling hot springs and seafloor hydrothermal vents, that can experience temperatures in excess of 100°C
- Above 65°C, only prokaryotic life forms thrive, but extensive diversity present (Table 5.2)

5.11 Microbial Life at High Temperatures

- Hyperthermophiles in hot springs (Figure 5.21)
  - chemoorganotrophic and chemolithotrophic species present
  - generation times (*g*) as low as one hour common
  - high prokaryotic diversity (both *Archaea* and *Bacteria* represented)

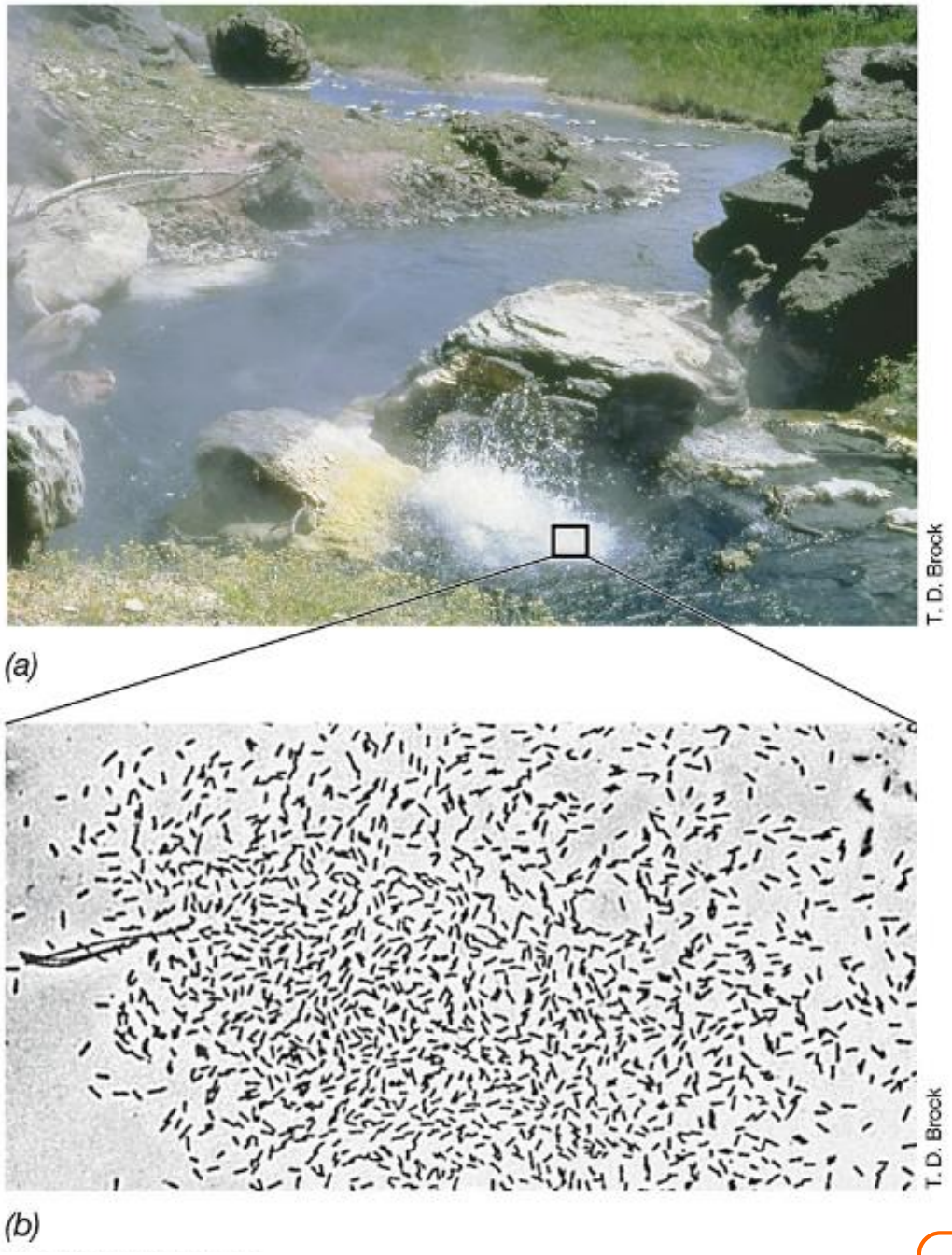


Figure 5.21

5.11 Microbial Life at High Temperatures

- Thermophiles inhabit moderately hot or intermittently hot environments.
- Thermal gradients form along edges of hot environments.
- Distribution of microbial species along the gradient is dictated by organism's biology. (Figure 5.22)

5.11 Microbial Life at High Temperatures

- Protein and membrane stability at high temperatures
  - Enzymes and proteins function optimally at high temperatures, features that provide thermal stability.
    - Critical amino acid substitutions in a few locations provide more heat-tolerant folds.
    - Increased number of ionic bonds between basic and acidic amino acids resists unfolding in the aqueous cytoplasm.
    - highly hydrophobic interiors
    - Production of solutes (*e.g.,* di-inositol phosphate, diglycerol phosphate) helps stabilize proteins.
- enzymes commercially useful
    - prolong shelf life, (*e.g., Taq polymerase* for polymerase chain reaction [PCR])

5.11 Microbial Life at High Temperatures

- Protein and membrane stability at high temperatures
  - modifications in cytoplasmic membranes to ensure heat stability
    - *Bacteria* have lipids rich in long-chain and saturated fatty acids, fewer unsaturated fatty acids.
    - *Archaea* have C_{40} hydrocarbons made of repeating isoprene units bonded to glycerol phosphate, and membrane forms lipid monolayer rather than bilayer.

General Microbiology II

Lecture- 3

IV. Environmental Effects on Growth: pH, Osmolarity, and Oxygen

- 5.12 Effects of pH on Microbial Growth
- 5.13 Osmolarity and Microbial Growth
- 5.14 Oxygen and Microbial Growth

5.12 Effects of pH on Microbial Growth

- pH expresses acidity or alkalinity of a solution.
- pH 7 = neutral (Figure 5.23)
- *acidic* pH < 7, *alkaline* pH > 7
- Each microbe has a pH range ~2–3 pH units within which growth is possible.
- Most natural environments are pH 3–9. (Table 5.3)

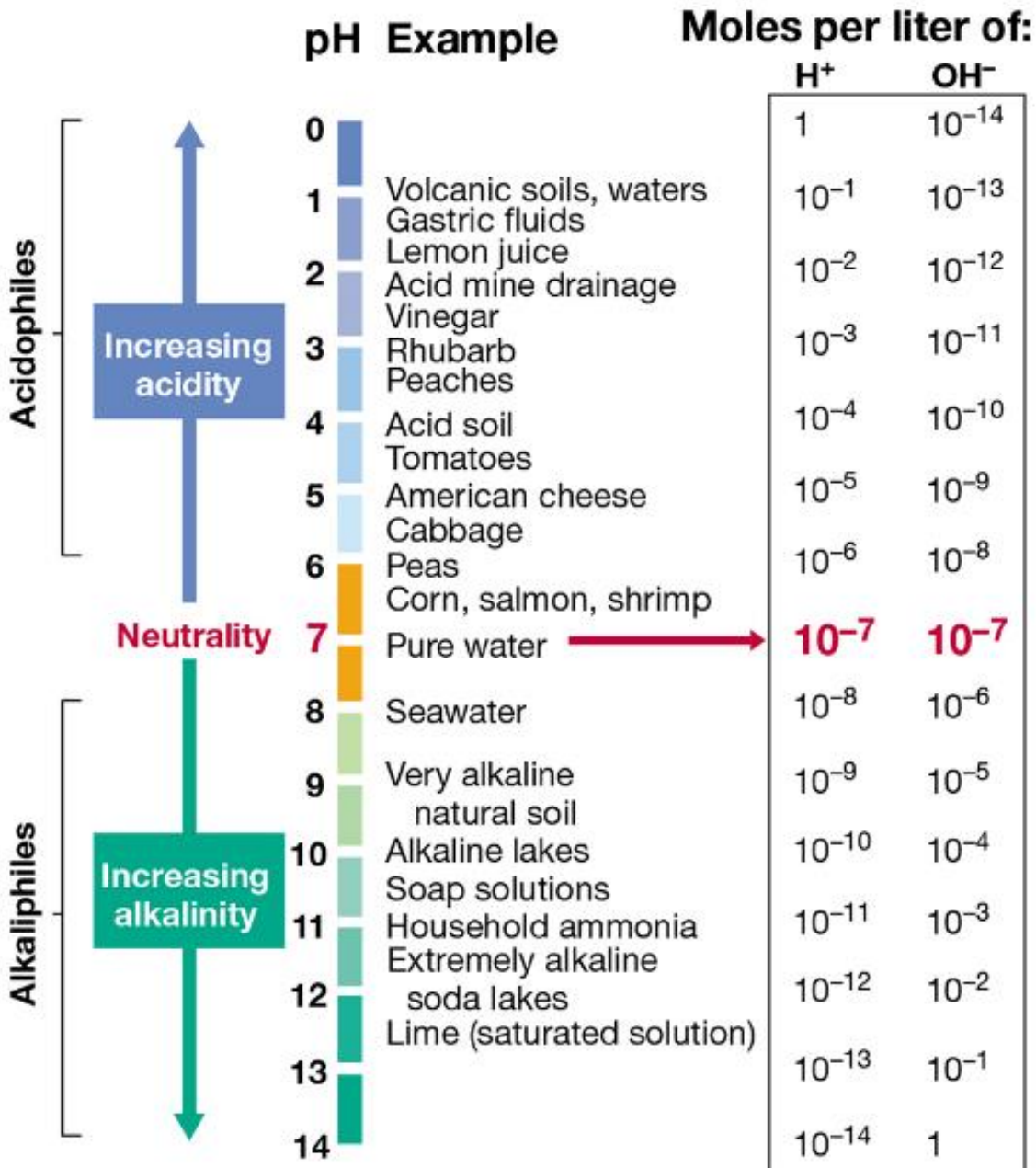


Figure 5.23

5.12 Effects of pH on Microbial Growth

- Neutrophiles: organisms that grown optimally at pH 5.5–7.9
- Acidophiles: organisms that grow best at low pH (< 5.5)
  - stability of cytoplasmic membrane critical
  - Some are obligate acidophiles—membranes destroyed at neutral pH.

5.12 Effects of pH on Microbial Growth

- Alkaliphiles: organisms that grow best at high pH (≥ 8)
  - found in soda lakes and high-carbonate soils
  - used commercially (*e.g.,* secreted proteases and lipases that are added to laundry detergents)
  - Some have sodium (Na^+) motive force rather than proton motive force.

5.12 Effects of pH on Microbial Growth

- The *intracellular* pH must stay relatively close to neutral (pH 5–9) even if the external pH is highly acidic or basic.
- Microbial culture media typically contain *buffers* to maintain constant pH.

5.13 Osmolarity and Microbial Growth

- Water activity (a_w): water availability (Table 5.4)
  - defined as ratio of vapor pressure of air in equilibrium with a substance or solution to the vapor pressure of pure water
  - varies from zero (no free water) to one (pure water)
- *Osmosis:* Water diffuses from high to low concentrations.

5.13 Osmolarity and Microbial Growth

- Typically, the cytoplasm has a higher solute concentration than the surrounding environment; thus, the tendency is for water to move into the cell (*positive water balance*).
- When a cell is in an environment with a higher external solute concentration, water will flow out unless the cell has a mechanism to prevent this.

5.13 Osmolarity and Microbial Growth

- *Halophiles:* organisms that grow best at a_w = 0.98 (seawater); have a specific requirement for NaCl (Figure 5.24)
- *Halotolerant:* organisms that can tolerate some additional dissolved solutes but generally grow best in the absence of the added solute
- *Extreme halophiles:* organisms that require very high levels (15 percent to 30 percent) of NaCl; often unable to grow at lower concentrations

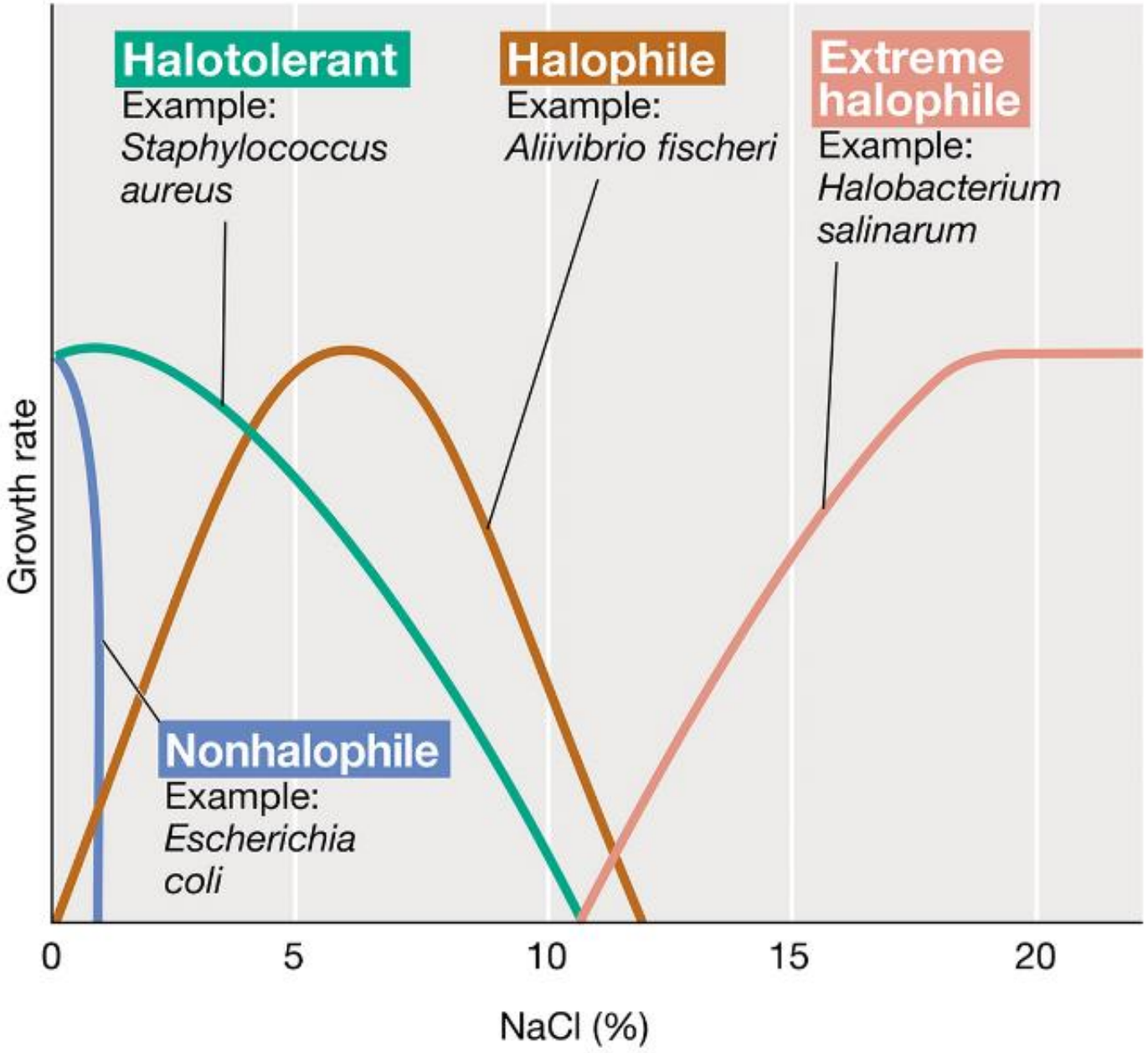


Figure 5.24

5.13 Osmolarity and Microbial Growth

- Osmophiles: organisms that live in environments high in sugar as solute
- Xerophiles: organisms able to grow in very dry environments
- See Table 5.5 for examples.
- Lowest a_w = 0.61; physiochemical constraints on obtaining water at lower a_w

5.13 Osmolarity and Microbial Growth

- Compatible solutes: used by cell to maintain positive water balance
  - pumping solutes from environment into cell
  - synthesizing cytoplasmic solutes
  - highly water-soluble, (*e.g.,* sugars, alcohols, glycine betaine, KCl) (Table 5.5)

5.14 Oxygen and Microbial Growth

- Table 5.6
- Aerobes: require oxygen (respiration) and grow at full oxygen tension (~21 percent)
- Microaerophiles: can use oxygen only when it is present at levels reduced from that in air due to limited respiration or oxygen sensitivity
- Facultative organisms: can live with or without oxygen

TABLE 5.6 Oxygen relationships of microorganisms

Group	Relationship to O_2	Type of metabolism	Example[a]	Habitat[b]
Aerobes				
Obligate	Required	Aerobic respiration	*Micrococcus luteus* (B)	Skin, dust
Facultative	Not required, but growth better with O_2	Aerobic respiration, anaerobic respiration, fermentation	*Escherichia coli* (B)	Mammalian large intestine
Microaerophilic	Required but at levels lower than atmospheric	Aerobic respiration	*Spirillum volutans* (B)	Lake water
Anaerobes				
Aerotolerant	Not required, and growth no better when O_2 present	Fermentation	*Streptococcus pyogenes* (B)	Upper respiratory tract
Obligate	Harmful or lethal	Fermentation or anaerobic respiration	*Methanobacterium formicicum* (A)	Sewage sludge, anoxic lake sediments

[a]Letters in parentheses indicate phylogenetic status (B, *Bacteria*; A, *Archaea*). Representatives of either domain of prokaryotic cells are known in each category. Most eukaryotes are obligate aerobes, but facultative aerobes (for example, yeast) and obligate anaerobes (for example, certain protozoa and fungi) are known.
[b]Listed are typical habitats of the example organism; many others could be listed.

Table 5.6

5.14 Oxygen and Microbial Growth

- Anaerobes: cannot respire oxygen
- Aerotolerant anaerobes: tolerate oxygen and grow in its presence even though they cannot respire
- Obligate anaerobes: inhibited or killed by oxygen, (*e.g.,* some *Bacteria* and *Archaea*, few fungi, and few protozoa)

5.14 Oxygen and Microbial Growth

- Special techniques are needed to grow aerobic and anaerobic microorganisms.
- Aerobes need extensive aeration (*e.g.,* shaking, bubbling).
- Anaerobes need oxygen excluded.
  - *reducing agents:* chemicals that may be added to culture media to reduce oxygen (*e.g.,* thioglycolate broth) (Figure 5.25)
    - complex medium that separates microbes based on oxygen requirements
    - Oxygen can penetrate only the top of the tube.
    - Microbes grow at different heights based on oxygen exposure.
  - can flush or consume oxygen (*e.g.,* glove box) (Figure 5.26)

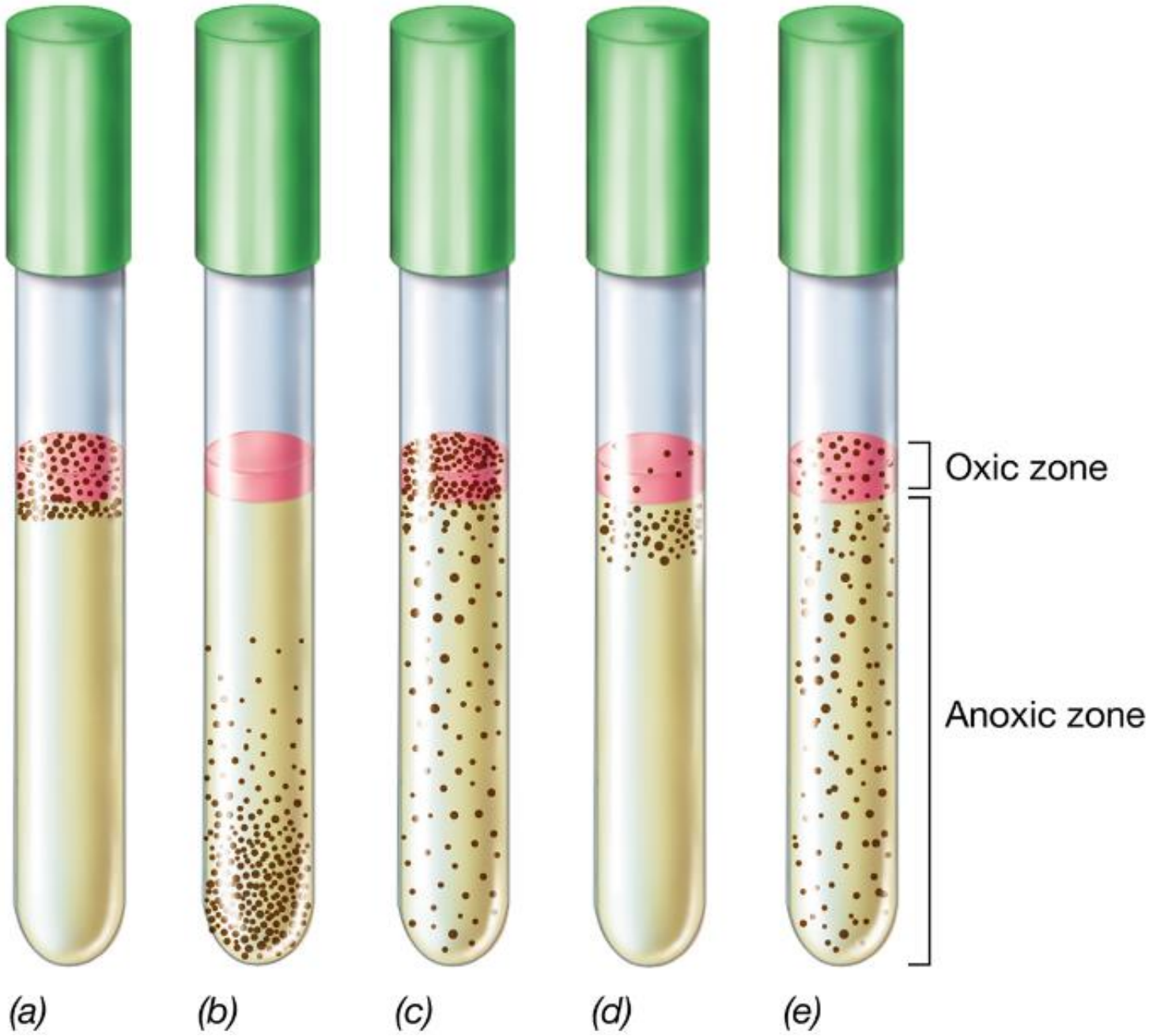


Figure 5.25

Deborah O. Jung and M. T. Madigan

(a)

Coy Laboratory Products

(b)

Figure 5.26

5.14 Oxygen and Microbial Growth

- Why is oxygen toxic?
  - Molecular oxygen (O_2) is not toxic.
  - Exposure to oxygen yields toxic byproducts. (Figure 5.27)
    - *superoxide anion* (O_2^-)
    - *hydrogen peroxide* (H_2O_2)
    - *hydroxyl radical* ($OH\cdot$)

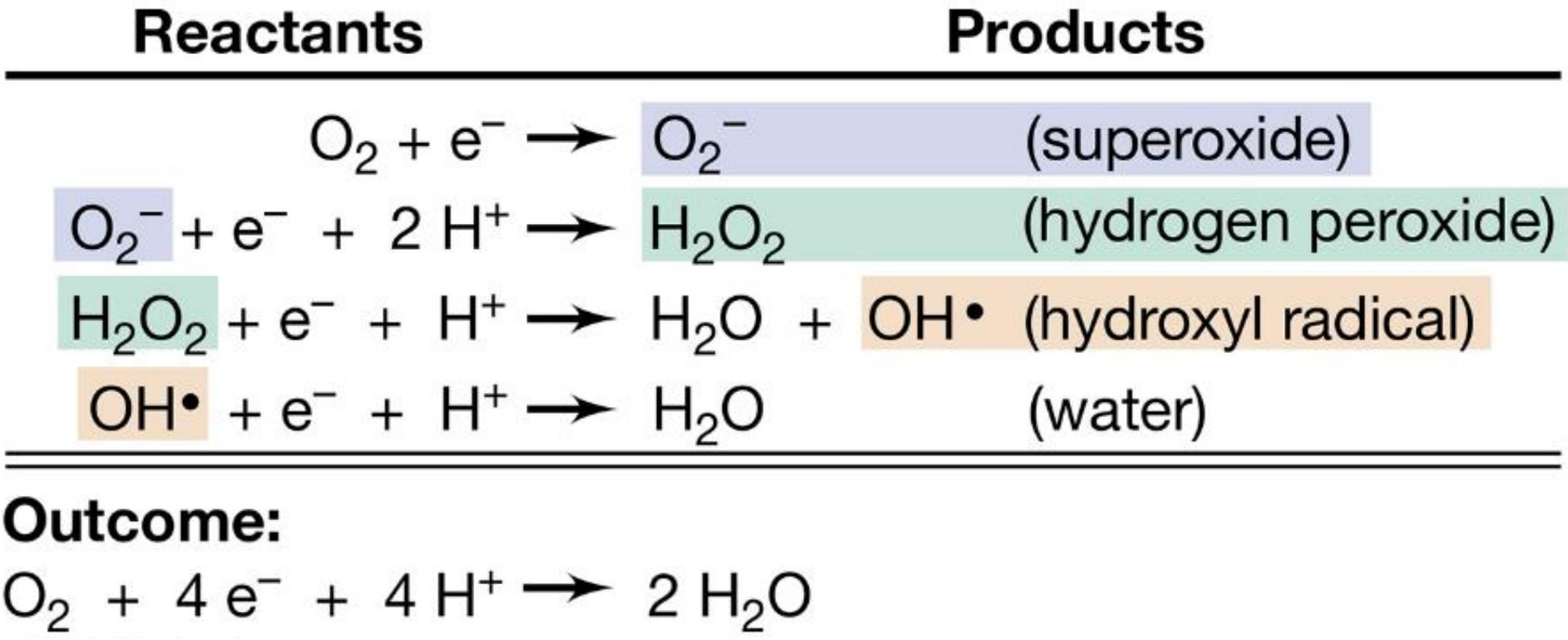


Figure 5.27

5.14 Oxygen and Microbial Growth

- Enzymes are present to neutralize most of these toxic oxygen species. (Figure 5.28)
  - Catalase and peroxidase convert H_2O_2 to O_2 and H_2O. (Figure 5.29)
  - *Superoxide dismutase* converts 2 O_2^- to H_2O_2 and O_2.
  - *Superoxide reductase* in some strict anaerobes converts O_2^- to H_2O_2 without producing O_2.

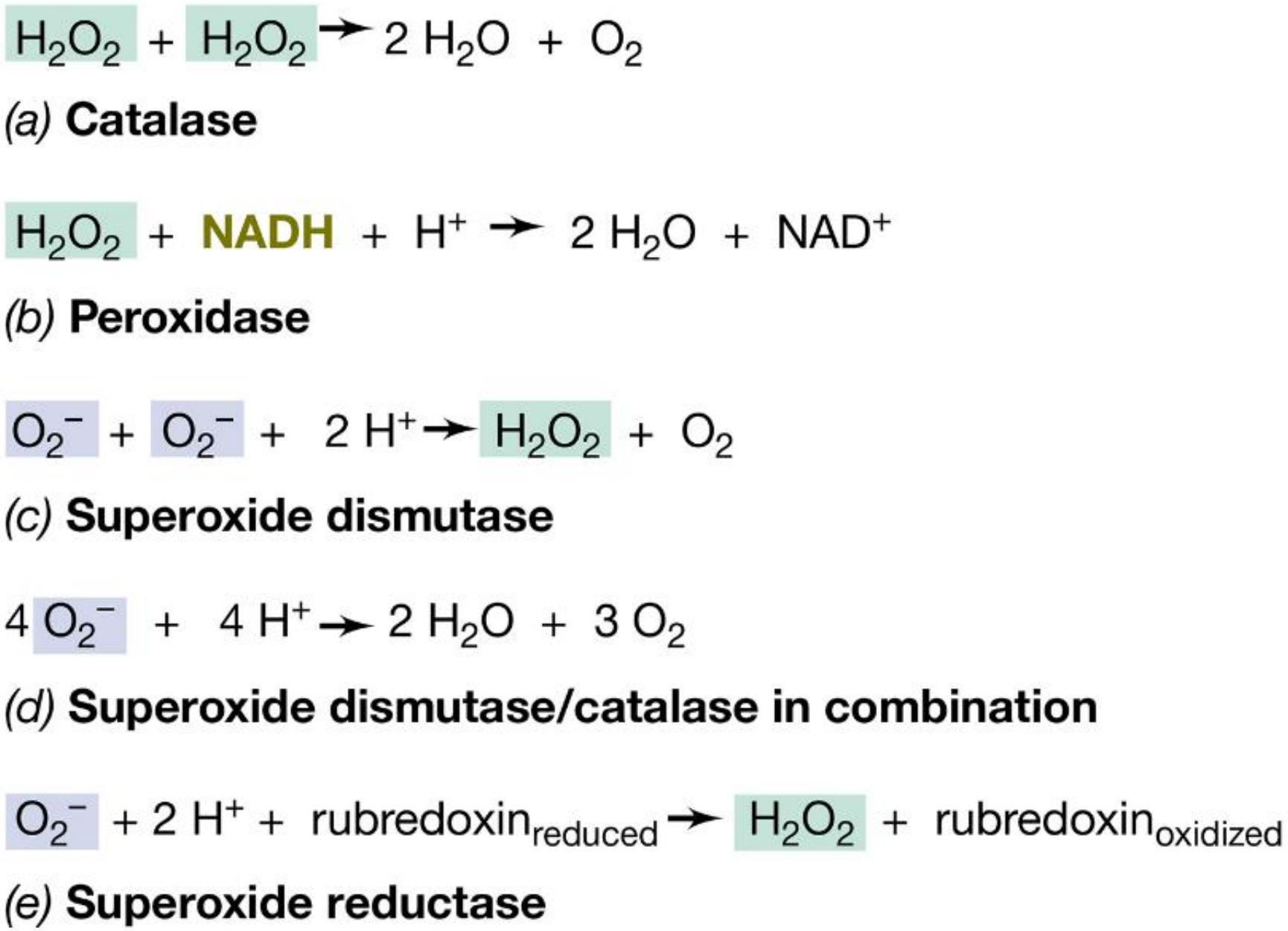
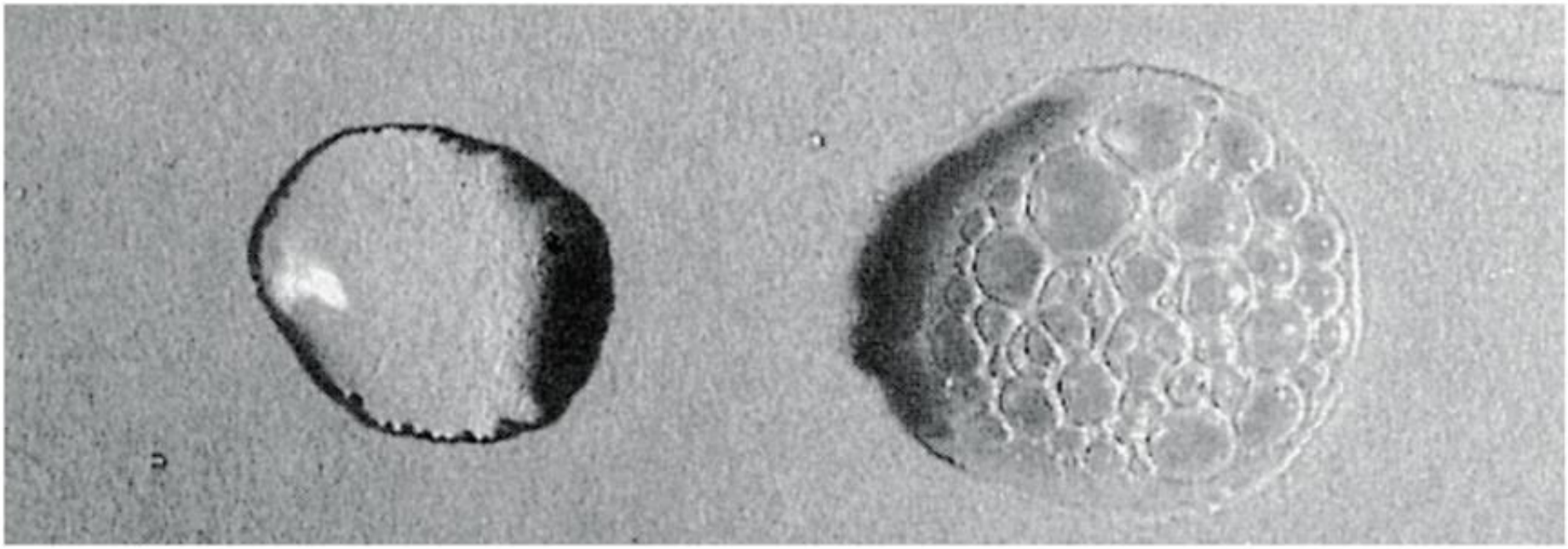


Figure 5.28



T. D. Brock

Figure 5.29

5.15 General Principles and Growth Control by Heat

- *Decontamination*
  - the treatment of an object to make it safe to handle
- Disinfection
  - directly targets the removal of all pathogens, not necessarily all microorganisms

5.15 General Principles and Growth Control by Heat

- Heat sterilization is the most widely used method of controlling microbial growth.
  - Amount of time required to reduce viability tenfold is called the *decimal reduction time* (*D*). (Figure 5.30)
  - exponential relationship
  - heat killing faster as temperature rises
  - Moist heat works better than dry heat.
  - *thermal death time:* time to kill all cells at a given temperature; affected by population size
  - Endospores can survive heat that would rapidly kill vegetative cells.

5.15 General Principles and Growth Control by Heat

- The autoclave is a sealed device that uses steam under pressure. (Figure 5.31)
  - allows temperature of water to get above 100°C
  - kills endospores
  - not the pressure but the high temperature that kills the microbes
- Pasteurization is the process of using precisely controlled heat to reduce the microbial load in heat-sensitive liquids.
  - does not kill all organisms, so it is different from sterilization

5.16 Other Physical Control Methods: Radiation and Filtration

- Ultraviolet (UV) radiation (between 220 and 300 nm) has sufficient energy to cause modifications and breaks in DNA.
  - UV useful for decontaminating surfaces (Figure 5.32)
  - cannot penetrate solid, opaque, or light-absorbing surfaces

5.16 Other Physical Control Methods: Radiation and Filtration

- Ionizing radiation
  - electromagnetic radiation that produces ions and other reactive molecules upon collision
  - amount of energy required to reduce viability tenfold (*D*10) is analogous to *D* value (Table 5.7, Figure 5.35)
  - some microorganisms more resistant to radiation than others (*e.g.,* endospores vs. vegetative cells, viruses vs. bacteria)
  - used for diverse items including surgical supplies, plastic labware, drugs, fresh produce, meat

5.16 Other Physical Control Methods: Radiation and Filtration

- Sources of radiation include cathode ray tubes, X-rays, and radioactive nuclides.
- Radiation is used for sterilization in the medical field and food industry.
  - Radiation is approved by the WHO and is used in the United States for decontaminating foods particularly susceptible to microbial contamination.
    - Hamburger, chicken, and spices may all be irradiated.

5.16 Other Physical Control Methods: Radiation and Filtration

- Filtration avoids the use of heat on sensitive liquids and gases.
  - pores of filter (0.45 and 0.2 μm) are too small for living organisms to pass through but do not trap most viruses
  - pores allow liquid or gas to pass through
- Depth filters made of overlapping paper or glass fibers
  - *HEPA filters* (Figure 5.34*a*)
- Membrane filters function more like a sieve. (Figure 5.34*b* and Figure 5.35)
- Nucleopore filters for scanning electron microscopy (Figure 5.34*c*)

5.17 Chemical Control of Microbial Growth

- Antimicrobial agents are chemicals that kill or inhibit growth.
- *-cidal* kills microorganisms (*e.g.,* bactericidal, *fungicidal*, *viricidal*)
- *-static* inhibits growth (*e.g.,* bacteriostatic, *fungistatic*, *viristatic*)

5.17 Chemical Control of Microbial Growth

- Antibacterial agents can be classified as bacteriostatic, bacteriocidal, and bacteriolytic. (Figure 5.36)
  - Bacteriostatic agents inhibit biochemical processes such as protein synthesis and bind weakly.
  - Bactericidal agents bind tightly and kill the cell.
  - Bacteriolytic agents kill by lysis (*e.g.,* detergents).

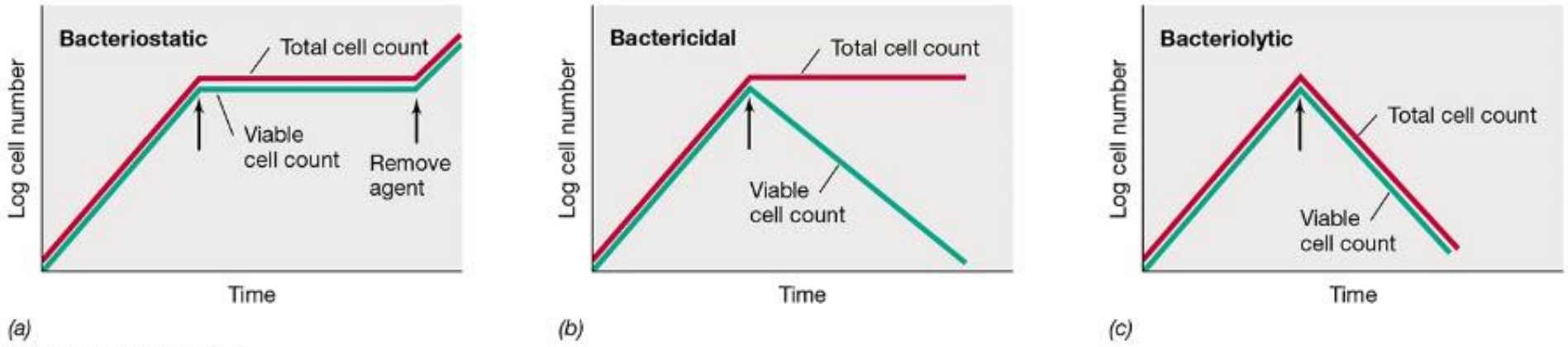


Figure 5.36

5.17 Chemical Control of Microbial Growth

- Minimum inhibitory concentration (MIC) is the smallest amount of an agent needed to inhibit growth of a microorganism. (Figure 5.37)
- Disc diffusion assay uses solid media. (Figure 5.38)
  - Antimicrobial agent added to filter paper disc, diffuses into agar.
  - MIC is reached at some distance.
    - *Zone of inhibition:* area of no growth around disc

5.17 Chemical Control of Microbial Growth

- Sterilants, disinfectants, sanitizers, and antiseptics (Table 5.8) are used to prevent growth on inanimate surfaces and external body surfaces.
  - Sterilants destroy all microorganisms, including endospores.
  - Disinfectants are used on surfaces to kill microorganisms but not necessarily endospores.
  - Sanitizers reduce microbial numbers but do not sterilize.
  - Antiseptics (germicides) kill or inhibit microbial growth but are nontoxic enough to be applied to living tissues.