Microbial growth

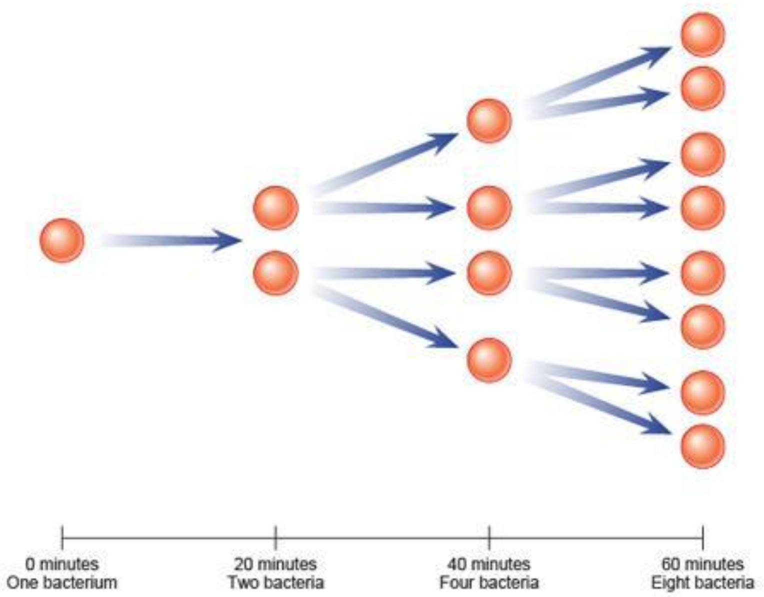
Microbiology - I

Growth:

- Microbial growth refers to the growth of a population or an increase in the number of cells.

- Not to an increase in the size of the individual cell.

- Cell division leads to the growth of cells in the population.



Factors influencing microbial growth:

Physical and chemical nature of the surrounding environment

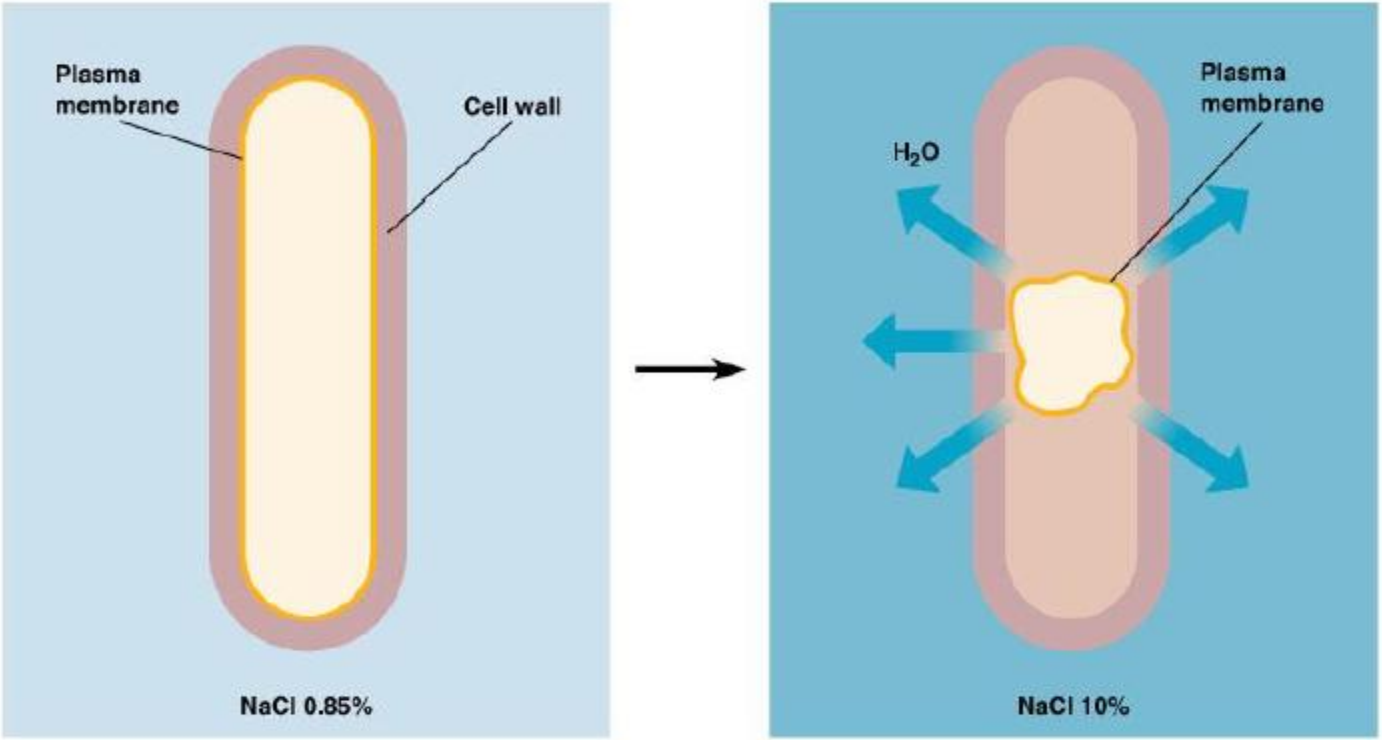
- Moisture
- Osmotic pressure
- Temperature
- pH
- Oxygen requirement
- CO_2
- Light

Moisture and osmotic pressure:
(Solutes and Water Activity)

Osmotic pressure: it expresses the concentration of the solution.

- **Hypotonic solution**: low osmotic concentration (solutes)
  - water will enter the cell and cause it to burst.
  - bacteria, algae, and fungi have **rigid cell walls** that maintain the shape and integrity of the cell
  - protozoa do not have a cell wall, they use contractile vacuoles to eliminate excess water
- **Hypertonic solution**: increase in salt or sugar concentration, cause plasmolysis
- Extreme or obligate halophiles require high osmotic pressure
- Facultative halophiles tolerate high osmotic pressure

Plasmolysis (shrinkage)



Isotonic solution

Hypertonic solution

Moisture: water activity

- The amount of water available to microorganisms affected by:
  - solute molecules (the osmotic effect)
  - adsorption to the surfaces of solids (the matric effect)
- **Water activity (aw):** $a_w = \frac{P_{soln}}{P_{water}}$
- The water activity of a solution is 1/100 the relative humidity of the solution (%).
- It is inversely related to osmotic pressure
- if a solution has high osmotic pressure, its **aw** is low.

Table 6.4 Approximate Lower a_w Limits for Microbial Growth

Water Activity	Environment	Bacteria	Fungi	Algae
1.00—Pure water	Blood, Plant wilt { Vegetables, meat, fruit } Seawater	Most gram-negative nonhalophiles		
0.95	Bread	Most gram-positive rods	*Basidiomycetes*	Most algae
0.90	Ham	Most cocci, *Bacillus*	*Fusarium* *Mucor, Rhizopus* Ascomycetous yeasts	
0.85	Salami	*Staphylococcus*	*Saccharomyces rouxii* (in salt)	
0.80	Preserves		*Penicillium*	
0.75	Salt lakes Salted fish	*Halobacterium* *Actinospora*	*Aspergillus*	*Dunaliella*
0.70			*Aspergillus*	
	Cereals, candy, dried fruit			
0.60			*Saccharomyces rouxii* (in sugars)	
	Chocolate			
	Honey		*Xeromyces bisporus*	
	Dried milk			
0.55—DNA disordered				

Adapted from A. D. Brown, "Microbial Water Stress," in *Bacteriological Reviews,* 40(4):803–846 1976.

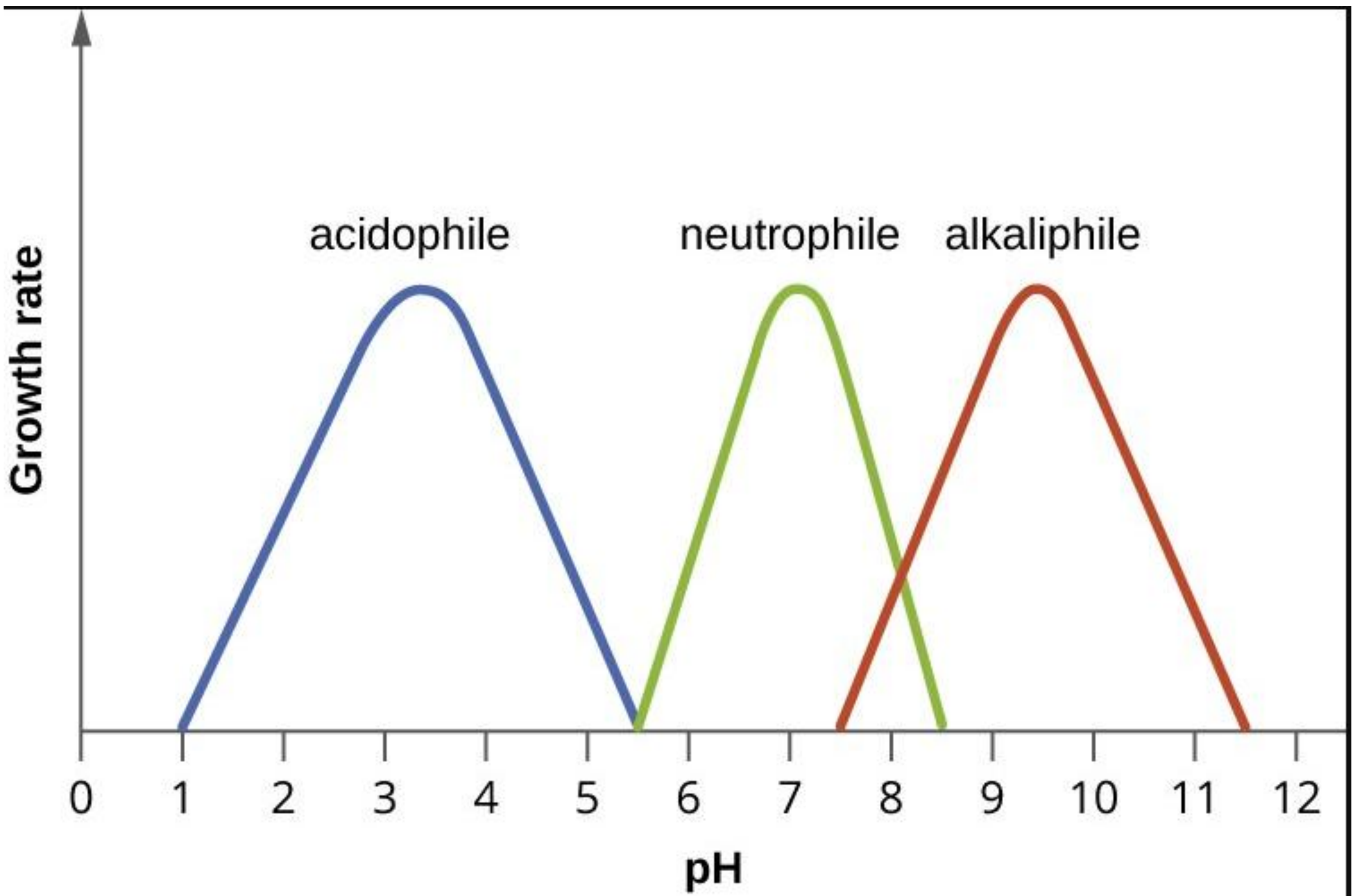
- **pH**: $$pH = -\log [H^+] = \log(1/[H^+])$$
- pH is a measure of the hydrogen ion activity of a solution
- Defined as the negative logarithm of the hydrogen ion concentration (molarity).

- **Acidophiles** :pH 0 and 5.5
- Molds and yeasts (fungi) - pH 4 and 6

- **Neutrophiles**:pH 5.5 and 8.0
- bacteria and protozoa -pH 6.5 and 7.5

- **Alkalophiles:** pH range of 8.5 to 11.5
- Extreme alkalophiles - pH 10

pH range of microbes



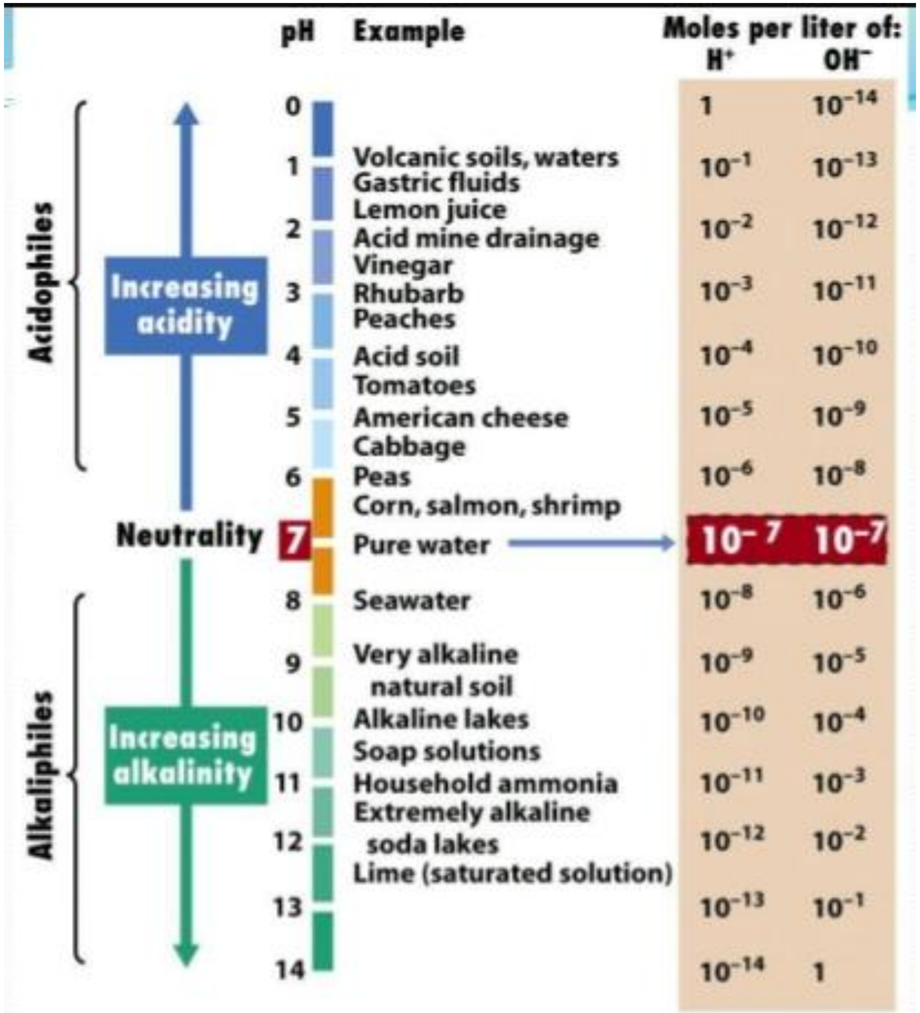


Figure 6-22 Brock Biology of Microorganisms 11/e

Affect of pH variations on microorganisms:

- Drastic variations in **cytoplasmic pH** harm microorganisms by:
  - Disrupting the plasma membrane
  - Inhibiting the activity of enzymes and membrane transport proteins.

- Changes in the **external pH**:
  - Alters the ionization of nutrient molecules
  - Reduces their availability to the organism

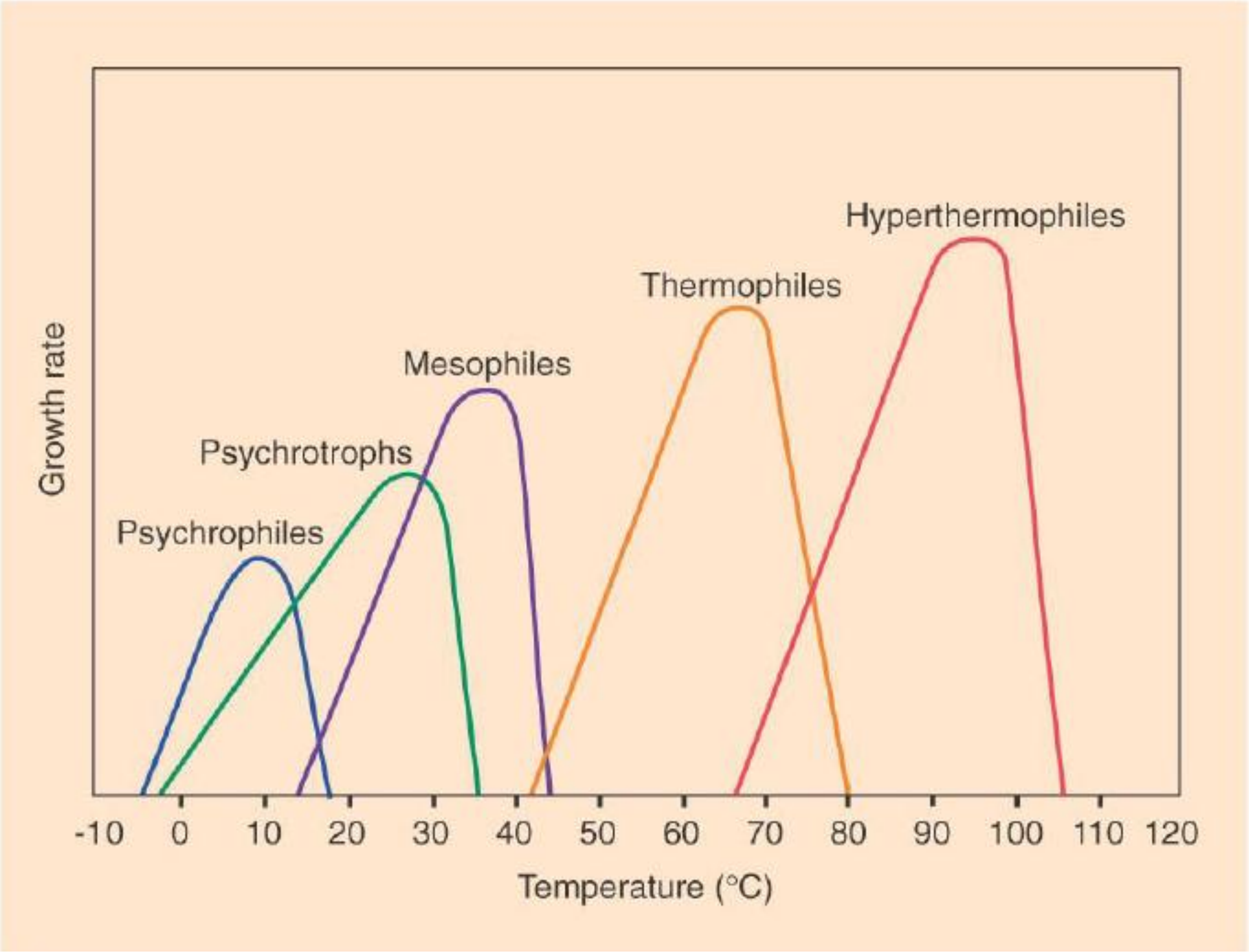
Temperature:

- A most important factor influencing the effect of temperature on **growth** is the temperature sensitivity of **enzyme** catalyzed reactions.
- It affects the **cellular functions** and structure.

- **High temperature** influence growth by:
  - Increasing **enzyme activity** that increases metabolism & leads to **rapid growth**.
  - The cell membrane exposed to high temp. **melts**, disintegrates.

- At **low temp**. the cell membrane **solidifies**.
- At low temp. the enzymes become inactive, slows down growth.

Temperature range of microbes



Classification of microbes on temperature ranges:

- **Psychrophiles:** Minimum: 0°C, optimum: 15°C, maximum: 20°C.
- **Psychrotrophs** or **facultative Psychrophiles:** grow at 0 to 7°C, optima between 20 and 30°C, and maxima at about 35°C.
- **Mesophiles:** growth optima - 20 to 45°C; minimum - 15 to 20°C, maximum - 45°C.
- **Thermophiles:** minimum: 45°C, optima: 55 and 65°C
- **Hyperthermophiles:** 90°C or above, some have maxima above 100°C.

Oxygen Concentration:

Microbes are classified based on O_2 requirements as:

Aerobe: require atmospheric O_2 for growth.

Obligate aerobes: completely dependent on atmospheric O_2 .

Anaerobe : grow in absence of O_2 .
Strict or **obligate anaerobes** do not tolerate O_2 at all and die in its presence.

Facultative anaerobes do not require O_2 for growth but do grow better in its presence.

Aerotolerant anaerobe: ignore O_2 and grow equally well whether it is present or not.

Microaerophiles: damaged by the normal atmospheric level of O_2 (20%) and require O_2 levels below the range of 2 to 10% for growth.

Table 6.1 The Effect of Oxygen on the Growth of Various Types of Bacteria

	a. Obligate Aerobes	b. Facultative Anaerobes	c. Obligate Anaerobes	d. Aerotolerant Anaerobes	e. Microaerophiles
Effect of Oxygen on Growth	Only aerobic growth; oxygen required	Both aerobic and anaerobic growth; greater growth in presence of oxygen	Only anaerobic growth; ceases in presence of oxygen	Only anaerobic growth; but continues in presence of oxygen	Only aerobic growth; oxygen required in low concentration
Bacterial Growth in Tube of Solid Growth Medium					
Explanation of Growth Patterns	Growth occurs only where high concentrations of oxygen have diffused into the medium	Growth is best where most oxygen is present, but occurs throughout tube	Growth occurs only where there is no oxygen	Growth occurs evenly; oxygen has no effect	Growth occurs only where a low concentration of oxygen has diffused into medium
Explanation of Oxygen's Effects	Presence of enzymes catalase and superoxide dismutase (SOD) allows toxic forms of oxygen to be neutralized; can use oxygen	Presence of enzymes catalase and SOD allows toxic forms of oxygen to be neutralized; can use oxygen	Lacks enzymes to neutralize harmful forms of oxygen; cannot tolerate oxygen	Presence of one enzyme, SOD, allows harmful forms of oxygen to be partially neutralized; tolerates oxygen	Produce lethal amounts of toxic forms of oxygen if exposed to normal atmospheric oxygen

Group	Spoilage organisms	Pathogens
Aerobes	*Micrococcus* sp. Moulds, e.g. *Botrytis cinerea* *Pseudomonas sp*	*Bacillus cereus* *Yersinia enterocolitica* *Vibrio parahaemolyticus* *Camplobacter jejuni*
Microaeophiles	*Lactobacillus* sp. *Bacillus* spp. *Enterobacteriaceae*	*Listeria monocytogenes* *Aeromonas hydrophilia* *Escherichia coli*
Facultative anaerobes	*Brocothrix thermosphacta* *Shewanella putrefaciens* Yeasts	*Salmonella* spp. *Staphylococcus* spp. *Vibrio* sp.
Anaerobes	*Clostridium sporogenes* *Clostridium tyrobutyricum*	*Clostridium perfringens* *Clostridium botulinum*

10.A2.2.1 *Effect of oxygen*

Bacteria, yeasts and moulds have different respiratory and metabolic needs and can be grouped according to their O_2 needs (Table 10.1).

Toxic forms of Oxygen:

- Singlet oxygen: O_2 boosted to a higher-energy state
- Superoxide free radicals: O_2^-

$$2\ O_2^- + 2\ H^+ \xrightarrow{\text{superoxide dismutase}} H_2O_2 + O_2$$

- Peroxide anion: O_2^{2-}

$$2\ H_2O_2 \xrightarrow{\text{catalase}} 2\ H_2O + O_2$$

$$H_2O_2 + 2\ O^+ \xrightarrow{\text{peroxidase}} 2\ H_2O$$

- Hydroxyl radical (OH•)

Category	Relationship to oxygen	Type of metabolism	Enzymes used in detoxification of toxic forms of oxygen
Aerobes Obligate	Oxygen required	Aerobic respiration	SOD and catalase
Facultative	Oxygen not essential for growth, but growth is better with oxygen	Aerobic respiration (when oxygen is present); anaerobic respiration or fermentation (when oxygen is not present)	SOD and catalase
Micro-aerophilic	Oxygen required at low levels	Aerobic respiration (limited capacity)	SOD (catalase may be present at low levels)
Anaerobes Obligate	Oxygen inhibitory or lethal	Anaerobic respiration or fermentation	None
Aerotolerant	Oxygen not required (growth is the same in the presence or in the absence of oxygen)	Anaerobic respiration or fermentation	SOD

SOD = Superoxide dismutase

CARBON DIOXIDE

- Approximately half of dry weight
- CO_2 is provided by cellular metabolism and from environment.
- Autotrophic organisms are able to use carbon dioxide as source of carbon.
- Heterotrophic bacteria require some amount of carbon dioxide from exogenous sources.
- 5-10 % CO_2 is supplied for them in culture.
- Capnophilic = requiring excess amount of CO_2 eg Brucella abortus (10% CO_2).

- The carbon available in the carbohydrate sugar molecules is cycled further by microorganisms in a series of reactions that tricarboxylic acid (or TCA) cycle.

- The breakdown of the carbohydrate serves to supply energy to the microorganism.
- This process is also known as respiration.

- In anaerobic environments, microorganisms can cycle the carbon compounds to yield energy in a process known as fermentation

LIGHT

- Most bacteria prefer darkness for growth.
- Cultures die if exposed to sunlight.
- However some bacteria require sunlight and are called phototropic.
- Exposure to light may influence pigment production.
- Photochromogenic mycobacteria form a pigment only on exposure to light and not when incubated in the dark

Pigments called photosensitizers: chlorophyll, bacteriochlorophyll, cytochromes, and flavins. Absorb light energy and get excited.
Many microorganisms that are airborne or live on exposed surfaces use carotenoid pigments for protection against photo-oxidation.

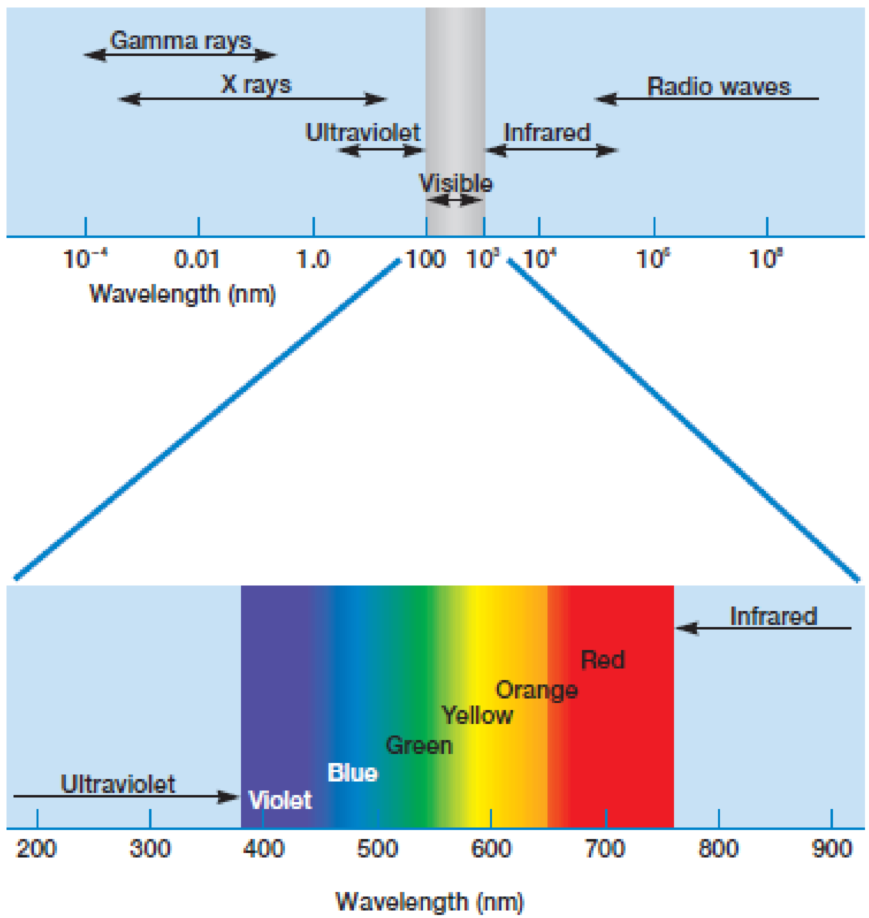
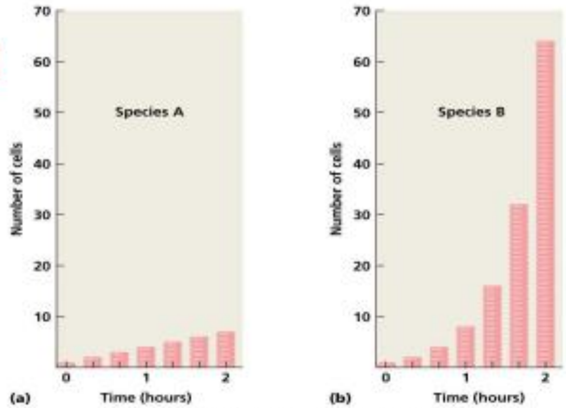
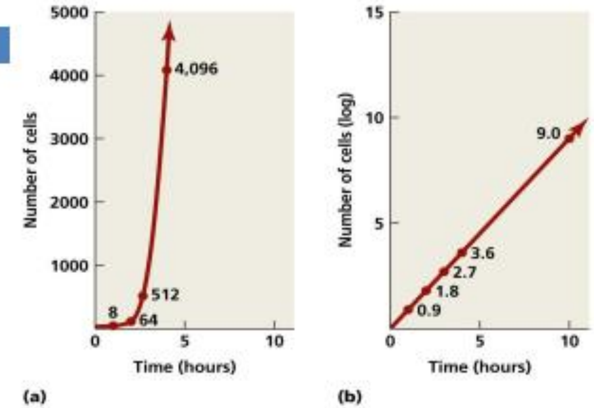


Figure 6.17 The Electromagnetic Spectrum. The visible portion of the spectrum is expanded at the bottom of the figure.

Microbial growth





<u>Bacterial Division</u>: Occurs mainly by binary fission.

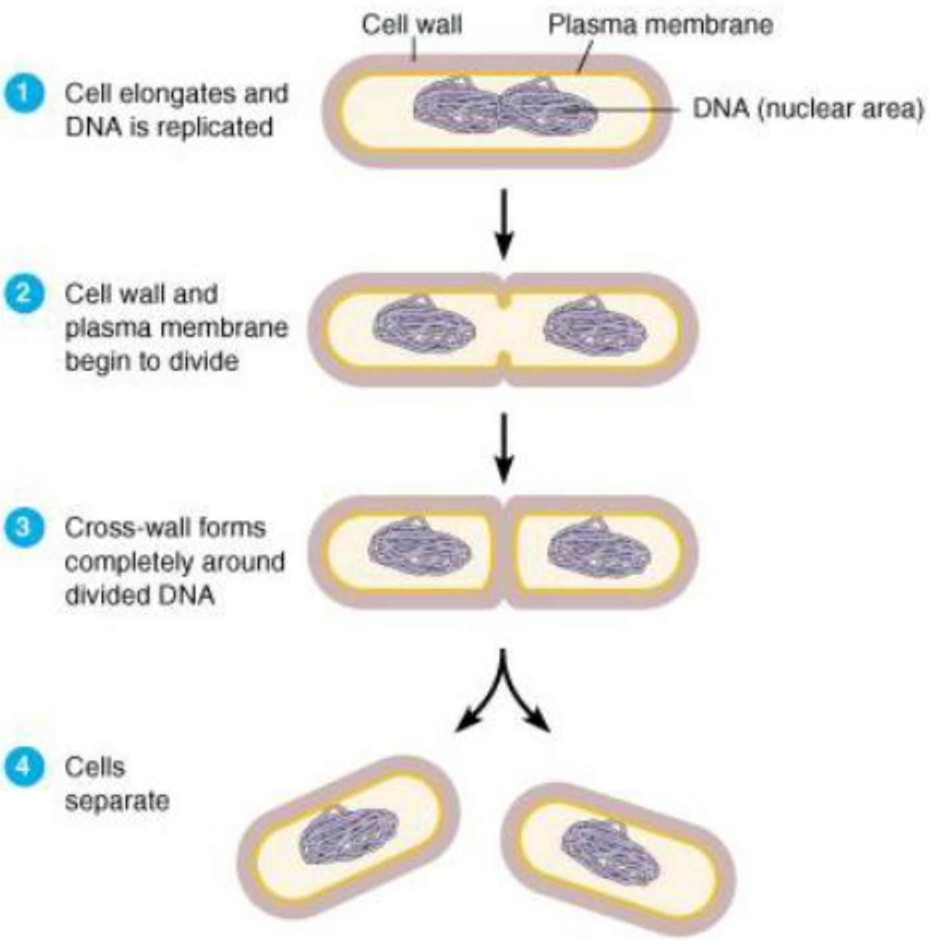
A few bacterial species reproduce by budding.

<u>Generation Time:</u> Time required for a cell to divide, ***and*** its population to double.

Generation time varies considerably:

- *E. coli* divides every 20 minutes.
- Most bacteria divide every 1 to 3 hours.
- Some bacteria require over 24 hours to divide.

Binary Fission

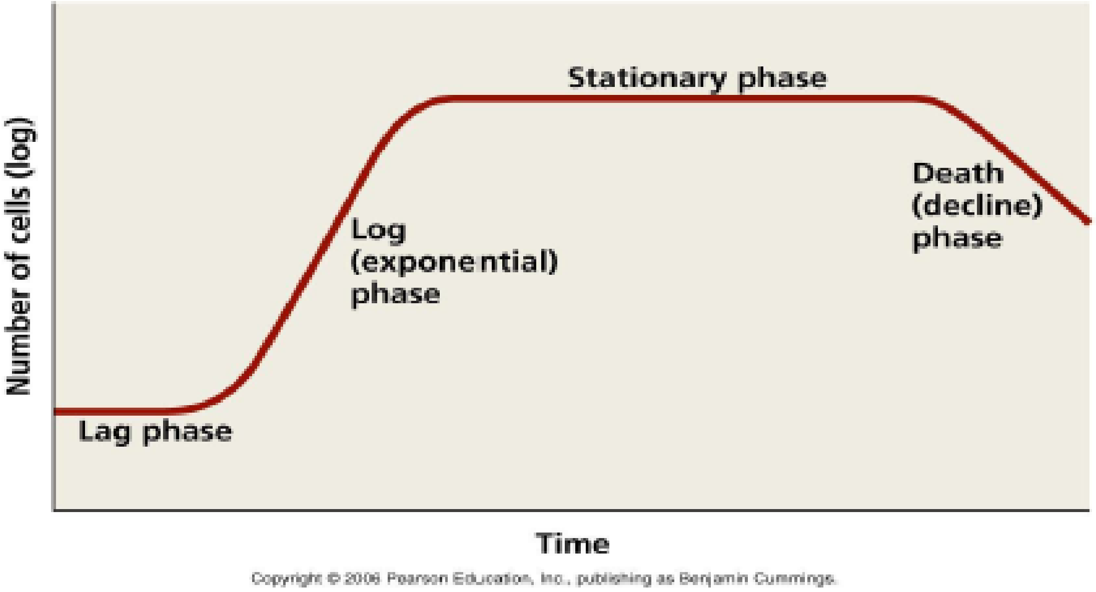


(a)

Rapid Growth of Bacterial Population

Arithmetic Numbers of Cells	Numbers Expressed as a Power of 2	Visual Representation of Numbers
1	2^0	●
2	2^1	●●
4	2^2	●●●●
8	2^3	●●●●●●●●
16	2^4	●●●●●●●●●●●●●●●●
32	2^5	●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●

Growth curve



Bacterial Growth Curve : When bacteria are inoculated into a liquid growth medium, we can plot of the number of cells in the population over time.

Four phases of Bacterial Growth:

1. Lag Phase:

- ✓ Period of adjustment to new conditions.
- ✓ Little or no cell division occurs, population size doesn't increase.
- ✓ Phase of intense metabolic activity, in which individual organisms grow in size.
- ✓ May last from one hour to several days.

2. Log Phase:

- ✓ Cells begin to divide and generation time reaches a constant minimum.
- ✓ Period of most rapid growth.

  Number of cells produced > Number of cells dying
- ✓ Cells are at highest metabolic activity.
- ✓ Cells are ***most susceptible*** to adverse environmental factors at this stage.
  - Radiation
  - Antibiotics

3. Stationary Phase:

- ✓ Population size begins to stabilize.

  Number of cells produced = Number of cells dying

- ✓ Overall cell number does not increase.
- ✓ Cell division begins to slow down.
- ✓ Factors that slow down microbial growth:
  - Accumulation of toxic waste materials
  - Acidic pH of media
  - Limited nutrients
  - Insufficient oxygen supply

4. Death or Decline Phase:

- ✓ Population size begins to decrease.

Number of cells dying > Number of cells produced

- ✓ Cell number decreases at a logarithmic rate.
- ✓ Cells lose their ability to divide.
- ✓ A few cells may remain alive for a long period of time.

Relationship Involving Microorganisms

- Despite an apparent simplicity, bacteria can form complex associations with other organisms. This process is known as **symbiosis**.

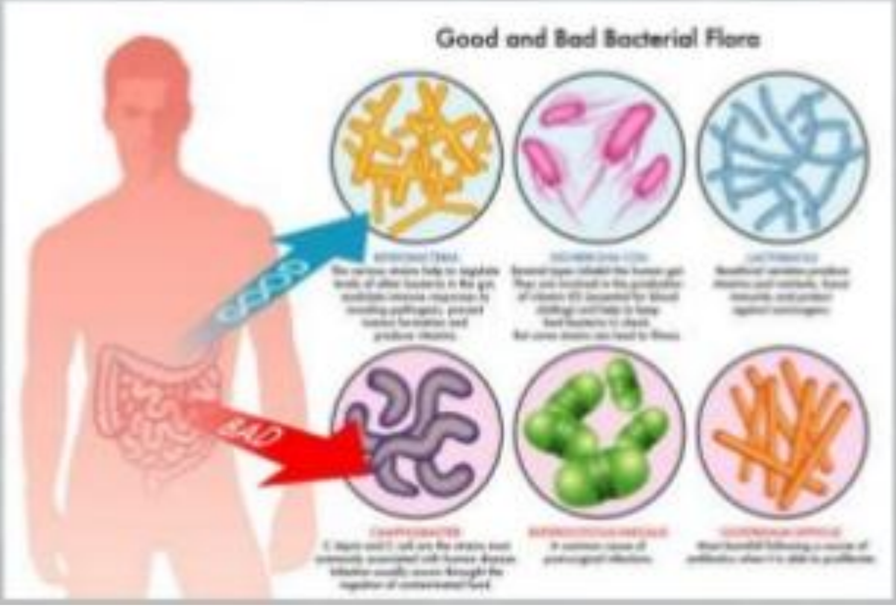
- **Symbiosis** - defined as the living together in more or less intimate association or close union of two dissimilar organisms

- The organisms that live together in such relationship are called **Symbionts.**

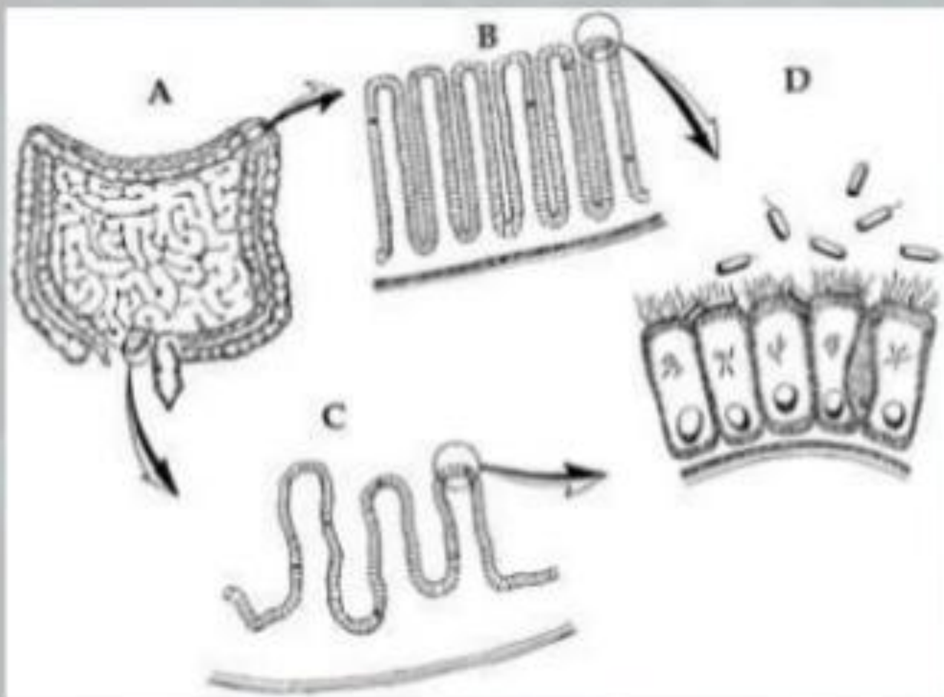
- 4 types :
  - **MUTALISM**
  - **COMMENSALISM**
  - **PARASITISM**
  - **SYNERISIM**

Mutalism

- A type of symbiotic interaction in which both organisms benefit from the relationship in some way.
- In humans, gut bacteria assist in breaking down additional carbohydrates, out-competing harmful bacteria, and producing hormones to direct fat storage.



- The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K2, and preventing colonization of the intestine with pathogenic bacteria



Commensalism

- one organism benefits while the other organism neither benefits nor suffers from the interaction.
- Humans are host to a variety of commensal bacteria in their bodies that do not harm them but rely on them for survival.
- e.g. staph epidermis on skin

Parasitism

- Parasitic relationships, in which one species benefits and the other suffers, are very common in nature.
- Most of the microorganisms studied in medical microbiology are parasitic and feed on human tissue.
- For e.g., cholera, leshmaniasis, and Giardia are all parasitic microbes.