Micro 260

Chapter 6A

Bacterial Nutrition and Growth

Types of Bacteria

	Energy	Electron	Carbon
Photo Autotrophs (photo lithotrophs)	Light	Inorganic Molecule	CO_2 (plants & cyanobacteria)
Chemo heterotrophs (Chemo organotrophs)	Organic molecule reduced carbon such as glucose	Organic molecule H+ ions stripped from carbon	Organic (animals, most bacteria)
Photo Heterotrops (Photo organotrophs)	Light	Organic molecule alcohol, fatty acids, & organic acid.	Organic (unique to some bacteria) Green non-sulfur bacteria
Chemo Autotrophs (chemo lithotrophs)	Inorganic molecule uses reduced inorganic compounds	Inorganic molecule (H_2S, S, NH_3, NO_2^-, Fe^{+2}, CO.)	CO_2 (unique to bacteria) Some bacteria are know to require organic carbon

The Requirements for Growth: Chemical Requirements

- Carbon
  - Structural organic molecules, energy source
  - Chemoheterotrophs use organic carbon sources
  - Autotrophs use CO_2
  - CHNOPS
    - Carbon
    - Hydrogen
    - Nitrogen
    - Oxygen
    - Phosphorous
    - Sulfur

The Requirements for Growth: Chemical Requirements

- Nitrogen
  - In amino acids, proteins
  - Most bacteria decompose proteins
  - Some bacteria use NH_4^+ or NO_3^-
  - A few bacteria use N_2 in nitrogen fixation
- Sulfur
  - In amino acids, thiamine, 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

The Requirements for Growth: Chemical Requirements

- Trace Elements
  - Inorganic elements required in small amounts
    - Mg, Mn, Mo, Cu, Fe, Na, K, Ca, Zn, Cl
  - Usually as enzyme cofactors

The Requirements for Growth: Chemical Requirements

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

Environmental Effects on the Growth of Bacteria

Microbial Adaptations to various types of environments

1) Temperatures;
2) Solution pH ;
3) Salinity;
4) Oxygen requirements (Aerobic/Anaerobic)
5) Other factors

Microbial Growth

- Microbial growth = increase in number of cells, not cell size

The Requirements for Growth: Physical Requirements

- Temperature
  - Minimum growth temperature
  - Optimum growth temperature
  - Maximum growth temperature

Temperature
For optimal growth and metabolism

- Psychrophile 0 to 15 C

- Psychrotrophs 0 to 32 C

- Mesophile- 20 to 40 C

- Thermophile- 45 to 80 C

- Hyperthermophiles 68 to 110 C

Temperature

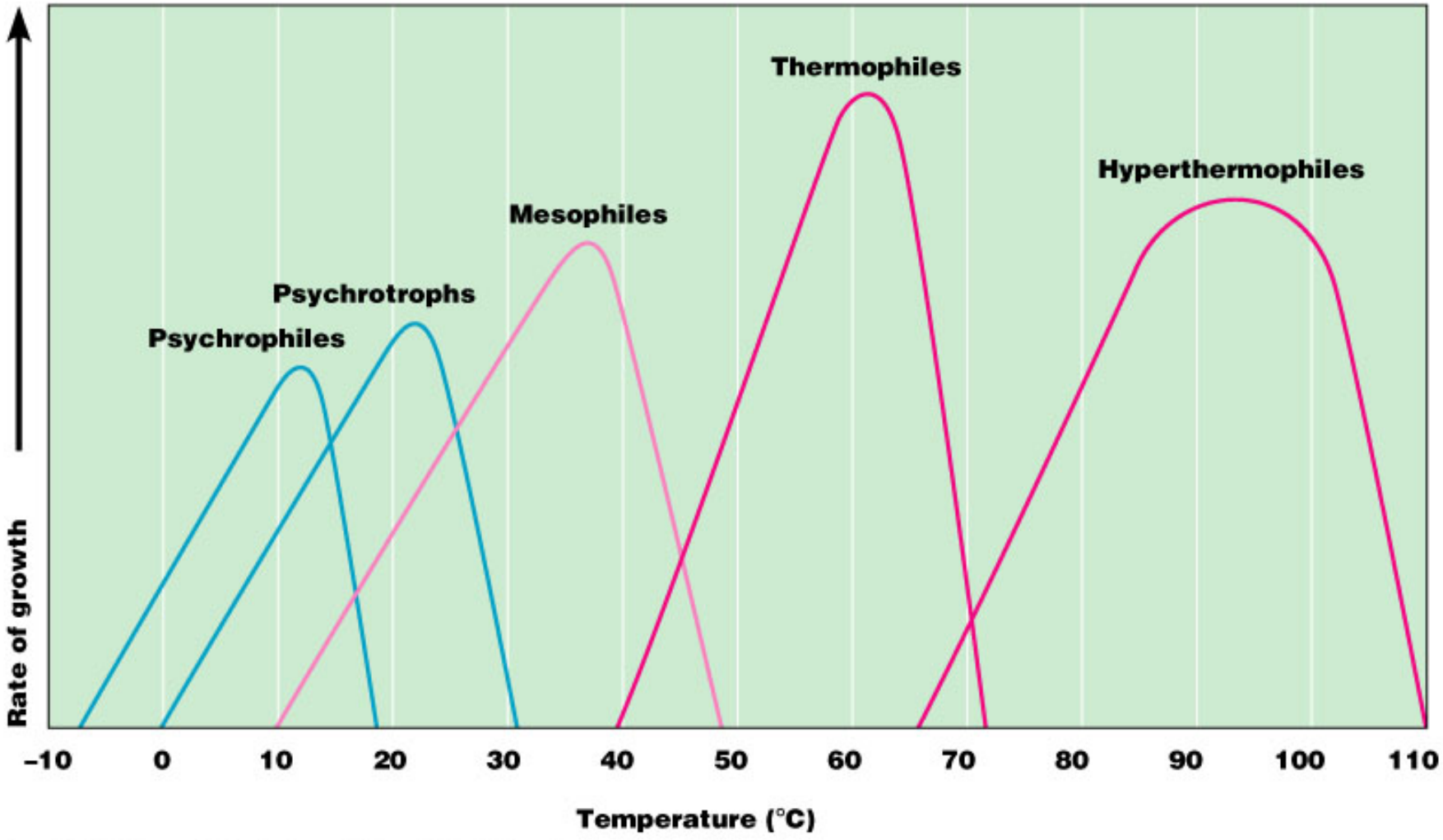


Figure 6.1

Psychrotrophs

- Grow between 0°C and 20-30°C
- Cause food spoilage

Psychrotrophs

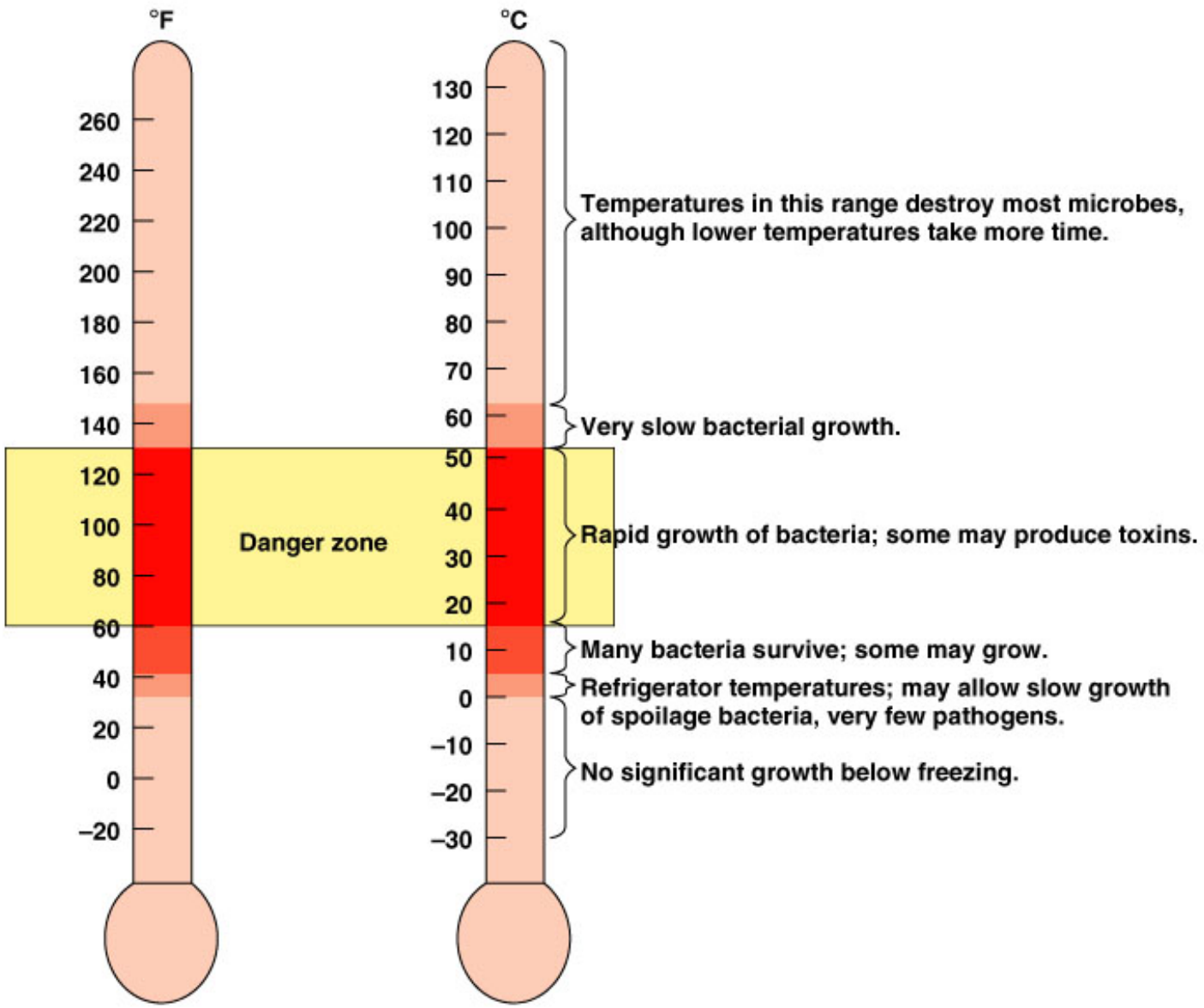


Figure 6.2

The Requirements for Growth: Physical Requirements

- pH
  - Most bacteria grow between pH 6.5 and 7.5
  - Molds and yeasts grow between pH 5 and 6
  - Acidophiles grow in acidic environments

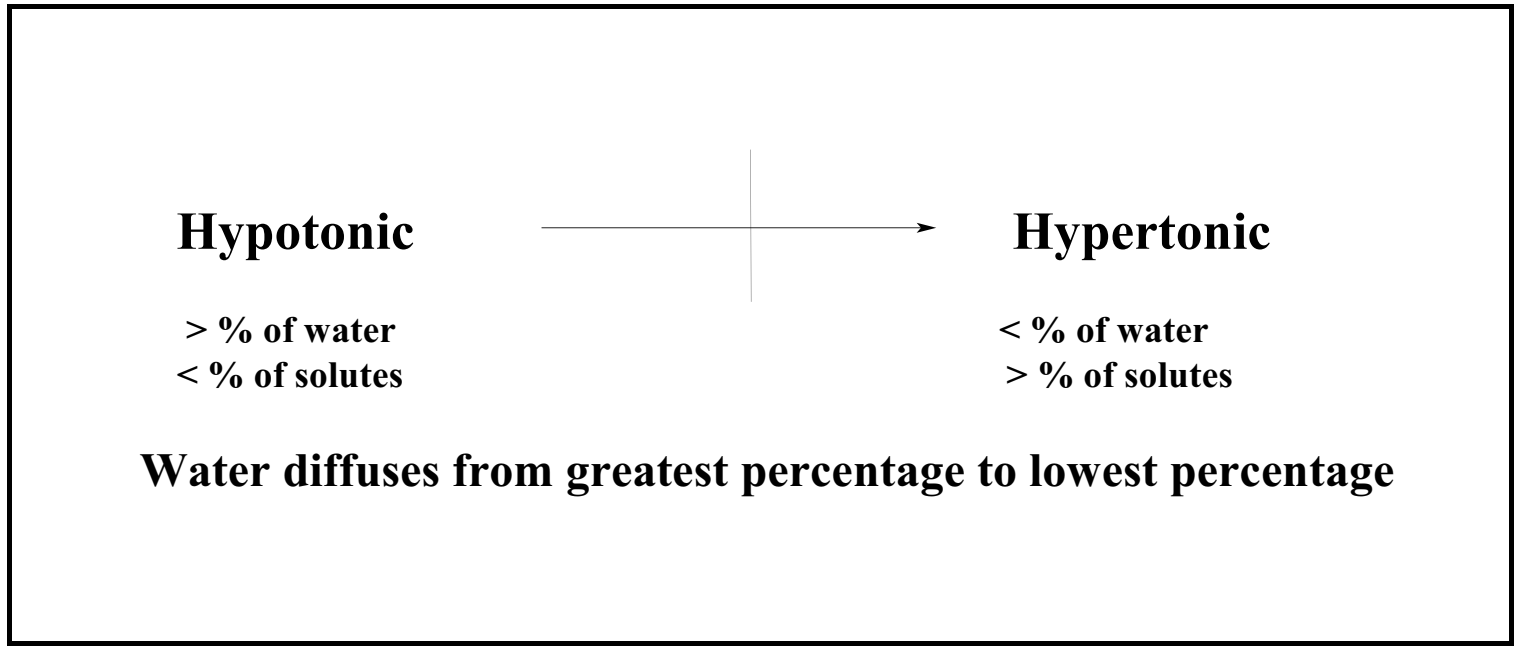
Diffusion

- Net movement of molecules from a high concentrated area to a low concentrated area
- No energy is expended (passive)
- Concentration gradient and permeability affect movement

The Requirements for Growth: Physical Requirements

- Osmotic Pressure
  - Hypertonic environments, increase salt or sugar, cause plasmolysis
  - Extreme or obligate halophiles require high osmotic pressure
  - Facultative halophiles tolerate high osmotic pressure

Tonicity and Diffusion of Water



The Requirements for Growth: Physical Requirements

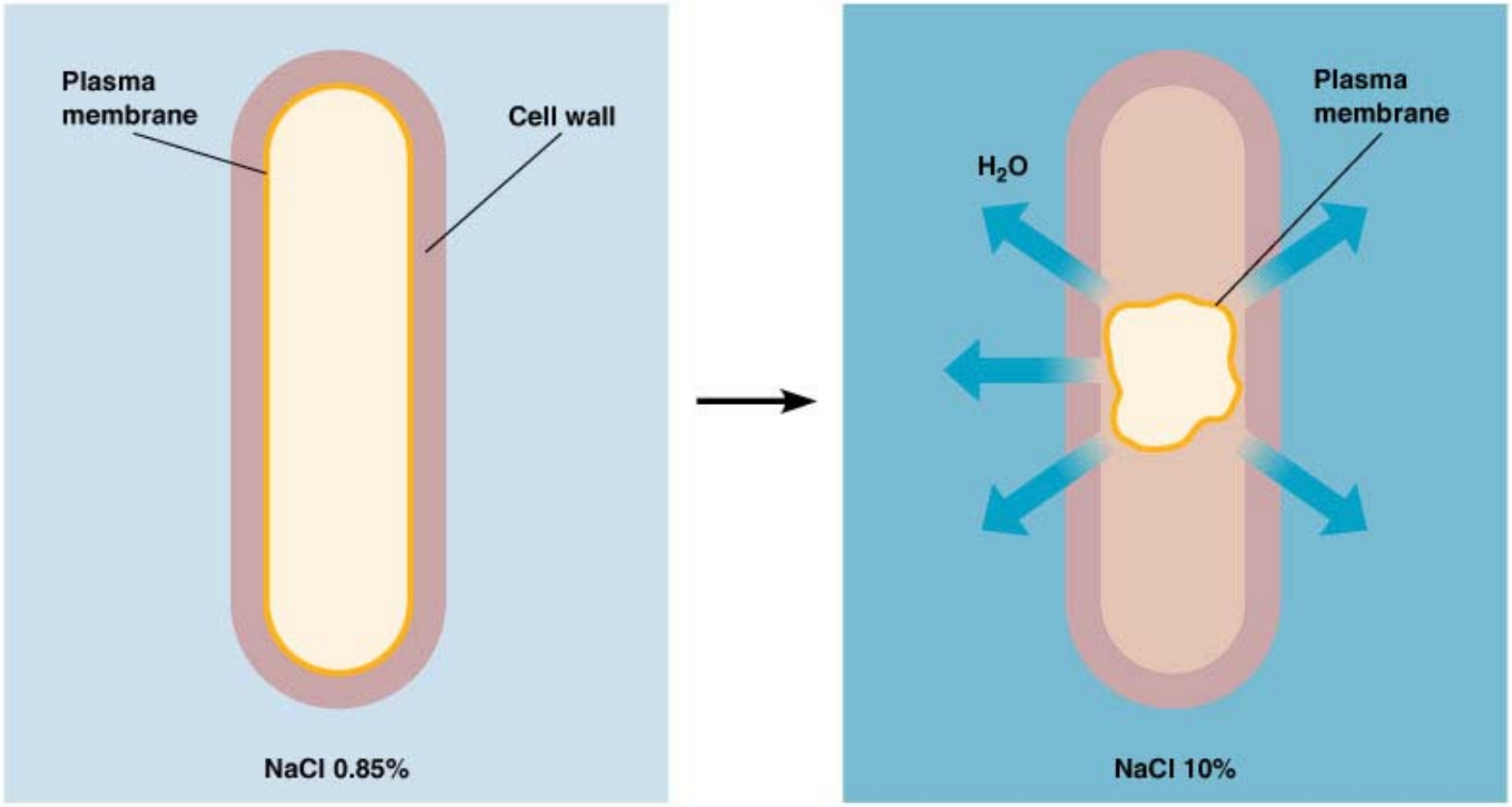
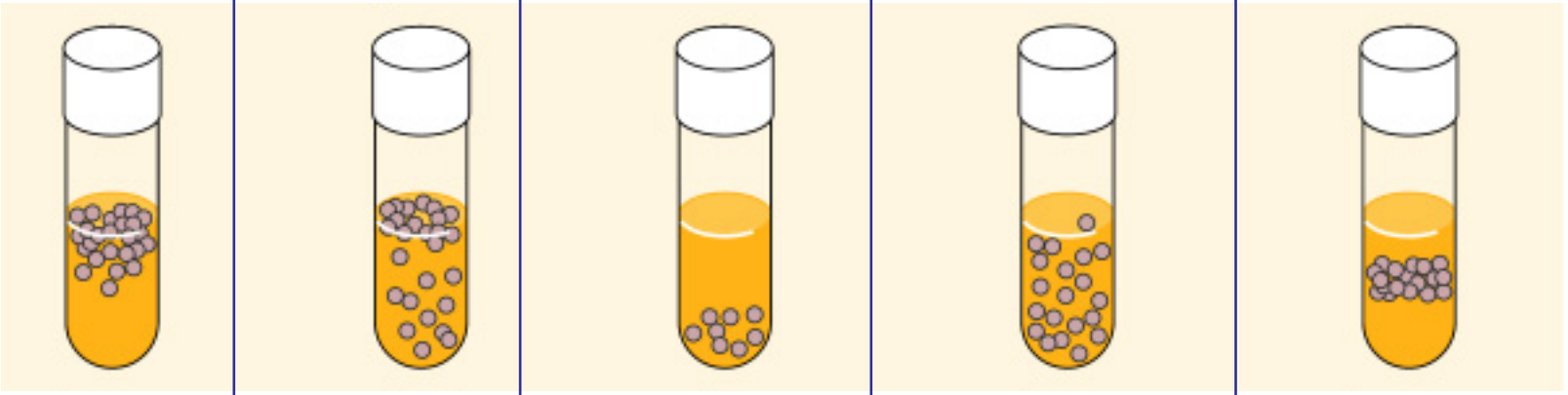


Figure 6.4

The Requirements for Growth: Chemical Requirements

- Oxygen (O_2)

obligate aerobes	Faultative anaerobes	Obligate anaerobes	Aerotolerant anaerobes	Microaerophiles
				

Toxic Forms of Oxygen

- Singlet oxygen: O_2 boosted to a higher-energy state
- Superoxide free radicals: O_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•)

Toxic Forms of Oxygen

Electron reduction of oxygen in a stepwise manner

Reaction	Reaction type
$O_2 + e^- \longrightarrow O_2^-$	**Superoxide**
$O_2^- + e^- + 2H^+ \longrightarrow H_2O_2$	**Hydrogen peroxide**
$H_2O_2 + e^- + H^+ \longrightarrow H_2O_2 + OH\bullet$	**Hydroxyl radical**
$OH\bullet + e^- + H^+ \longrightarrow H_2O$	**Water**

Enzyme required to carry out reaction:

Catalase

$H_2O_2 + H_2O_2 \longrightarrow 2\,H_2O + O_2$

Peroxidase

$H_2O_2 + NADH + H^+ \longrightarrow 2\,H_2O + NAD^+$

Superoxide dismutase

$O_2^- + O_2^- + H^+ \longrightarrow H_2O_2 + O_2$

Superoxide dismutase/catalase in combination

$4\,O_2^- + 4H^+ \longrightarrow 2H_2O + 3\,O_2$

Superoxide reductase

$O_2^- + 2H^+ + \text{cyt } c_{\text{reduced}} \longrightarrow H_2O_2 + \text{Cyt c}_{\text{oxidized}}$

Other factors

Radiation- withstand UV, infrared

Barophiles – withstand high pressures

Spores and cysts- can survive dry habitats

Terms for Culture Media

- Culture Medium: Nutrients prepared for microbial growth
- Sterile: No living microbes
- Inoculum: Introduction of microbes into medium
- Culture: Microbes growing in/on culture medium

Agar

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

Culture Media

- Chemically Defined Media: Exact chemical composition is known
- Complex Media: Extracts and digests of yeasts, meat, or plants
  - Nutrient broth
  - Nutrient agar

Culture Media

TABLE 6.2 **A Chemically Defined Medium for Growing a Typical Chemoheterotroph, Such as *E. coli***

Constituent	Amount
Glucose	5.0 g
Ammonium phosphate, monobasic ($NH_4H_2PO_4$)	1.0 g
Sodium chloride (NaCl)	5.0 g
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	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**

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

Table 6.2 & 6.4

Medium	Selective Agent(s)	Organism Encouraged to Grow
Brilliant green agar	Brilliant green	Gram-negative rods*
Eosin-methylene blue agar	Eosin Y, methylene blue	Grams-negative rods
Hektoen enteric agar	Bile salts	Grams-negative rods
MacConkey agar	Bile salts, crystal violet	Grams-negative rods
Mannitol-salt agar	Sodium chloride	*Staphylococcus aureus*

* this medium is not used for the isolations of *Salmonella typhi.*

A selective medium is defined as one that permits the growth of certain organisms while preventing or retarding the growth of others. Selection, in general, can be carried out through (1) control of ingredients of the medium, (2) alteration of atomospheric components, or (3) adjustment of incubation temperature.

Selective media may contain selective agents the inhibit the growth of one or more unwanted organisms in a specimen without preventing the growth of the wanted organism... i.e...Different nutrient rich media or Anti-biotic media impregnated media.

Summary of Reactions Associated with Selected Differential (D) and Selective Differential (SD) Media Used for Isolation and/or Identification.

Medium	Substrates(s)	Type of medium	Reaction and descriptions
Blood agar	Hemoglobin	D	1. Alpha hemolysis (green zones around colonies) 2. Beat hemolysis (clear zones around colonies) 3. Gamma hemolysis (no zone around colonies)
brilliant green agar	Lactose, sucrose	SD	1. Lactose-fermenter (yellow-green colonies) 2. Non-lactose fermenter (pink to white colonies Surrounded by brilliant red zones)
Eosin-methylene blue agar	Lactose, sucrose	SD	1. Lactose-fermenter (dark purple colonies or colonies With dark centers and transparent colorless borders) 2. Non-lactose or non-sucrose fermenters (colorless colonies.
Hektoen enteric agar	Lactose, sucrose salicin, and amino acids containing sulfur	SD	1. Lactose-fermenter (salmon-pink colonies) 2. Non-lactose-fermenters (green, most colonies) 3. Salicin-fermenters (pink zones around colonies) 4. Non-salicin-fermenters (no change) 5. H_2S producers (colonies with black centers)
MacConkey agar	Lactose	SD	1. Lactose-fermenter (pink-red colony is surrounded by pink zones due to precipitated bile). 2. Non-lactose fermenter (colorless and translucent colonies)
Mannitol-salt agar	Mannitol	SD	1. Mannitol fermenter (colonies surrounded by yellow zone. 2. Non-mannitol fermenter (small colonies with no color yellow change)

Anaerobic Culture Methods

- Reducing media
  - Contain chemicals (thioglycollate or oxyrase) that combine O_2
  - Heated to drive off O_2

Anaerobic Culture Methods

- Anaerobic jar

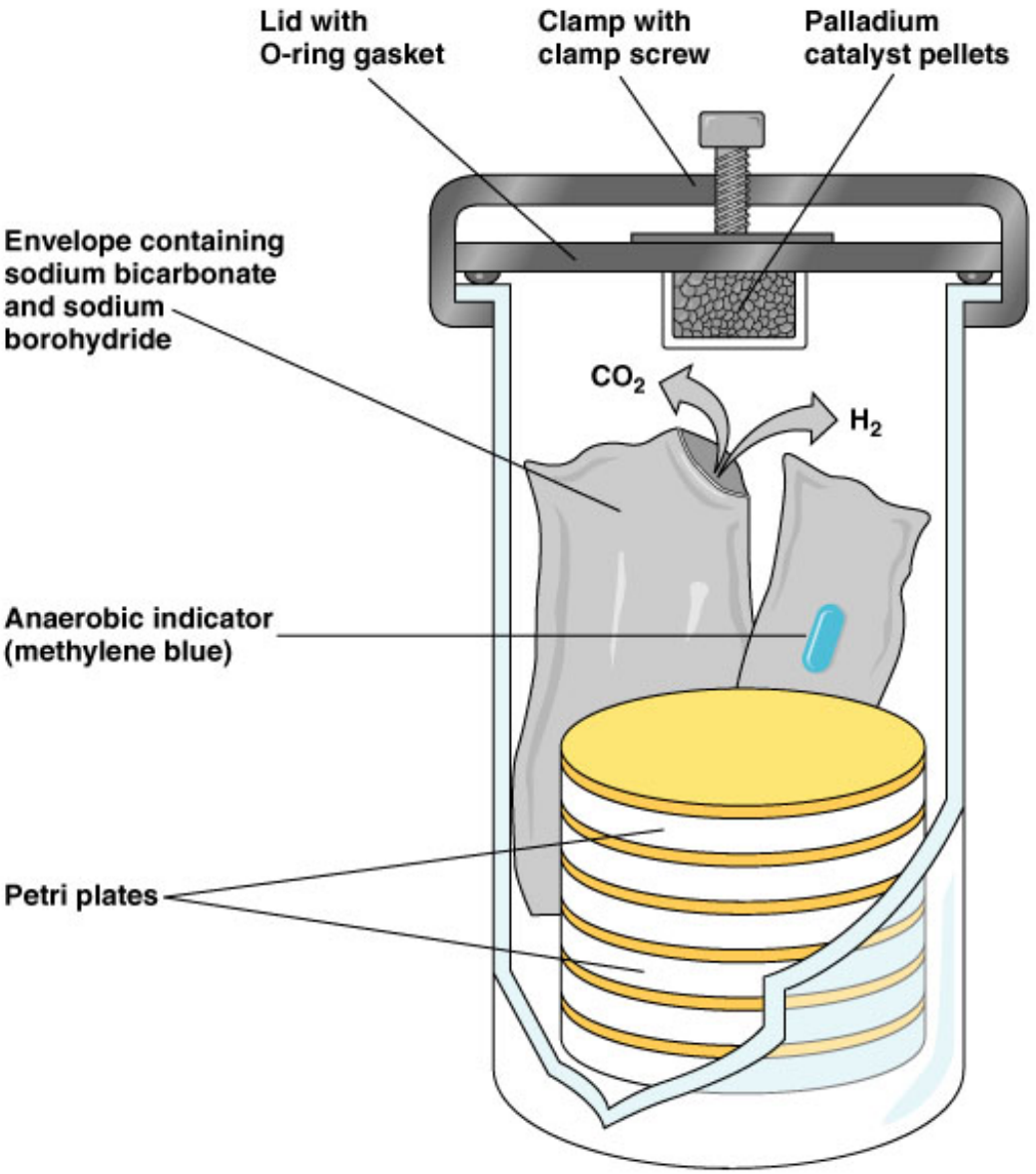
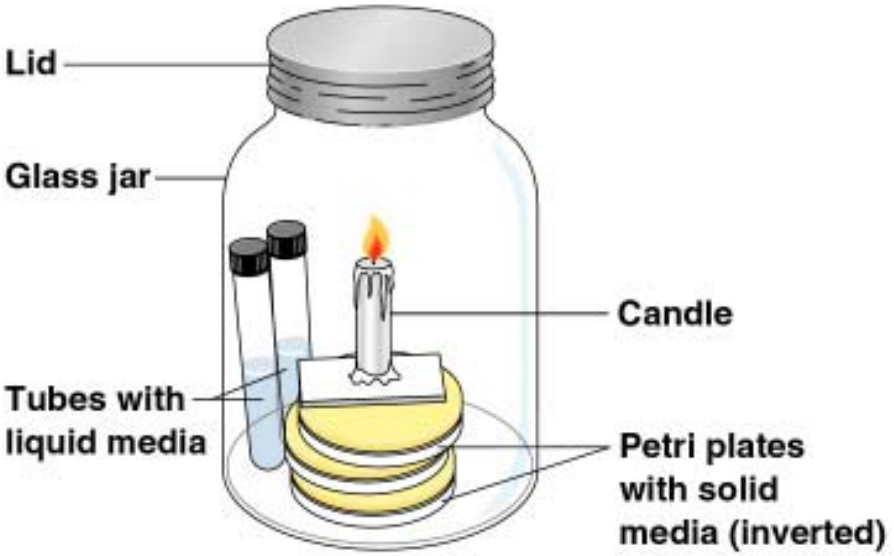


Figure 6.5

Capnophiles require high CO_2

- Candle jar



- CO_2-packet

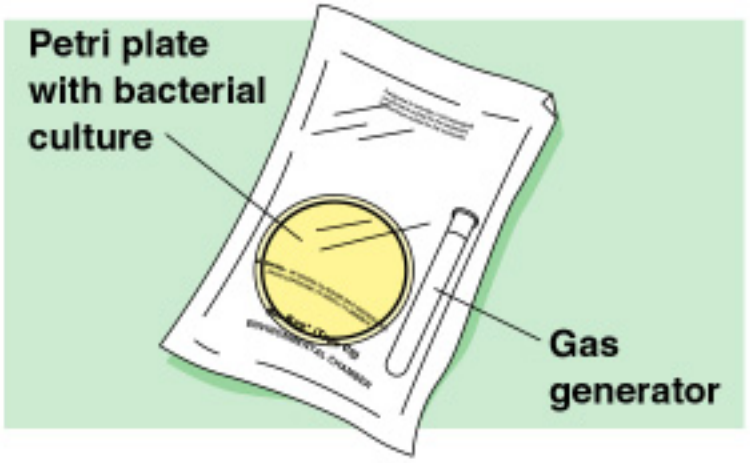


Figure 6.7

Selective Media

- Suppress unwanted microbes and encourage desired microbes.

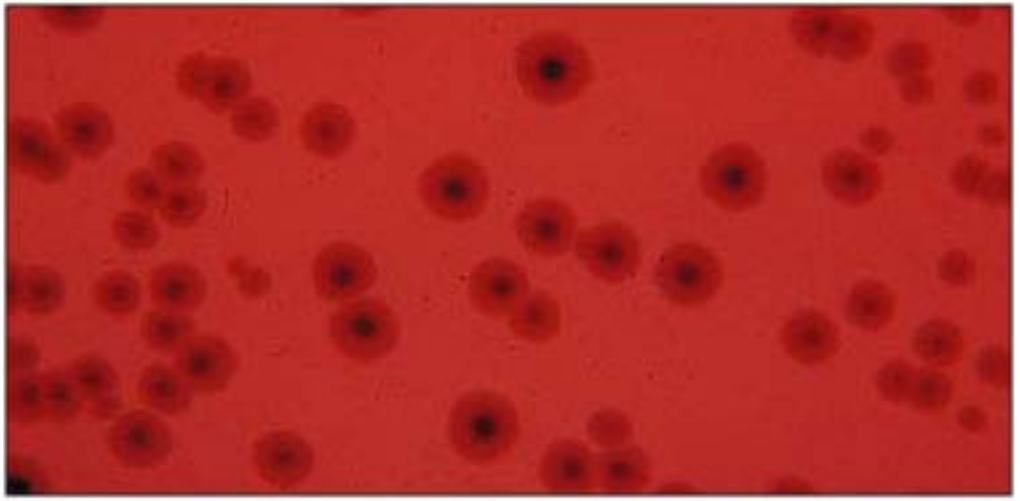


Figure 6.9b, c

Differential Media

- Make it easy to distinguish colonies of different microbes.

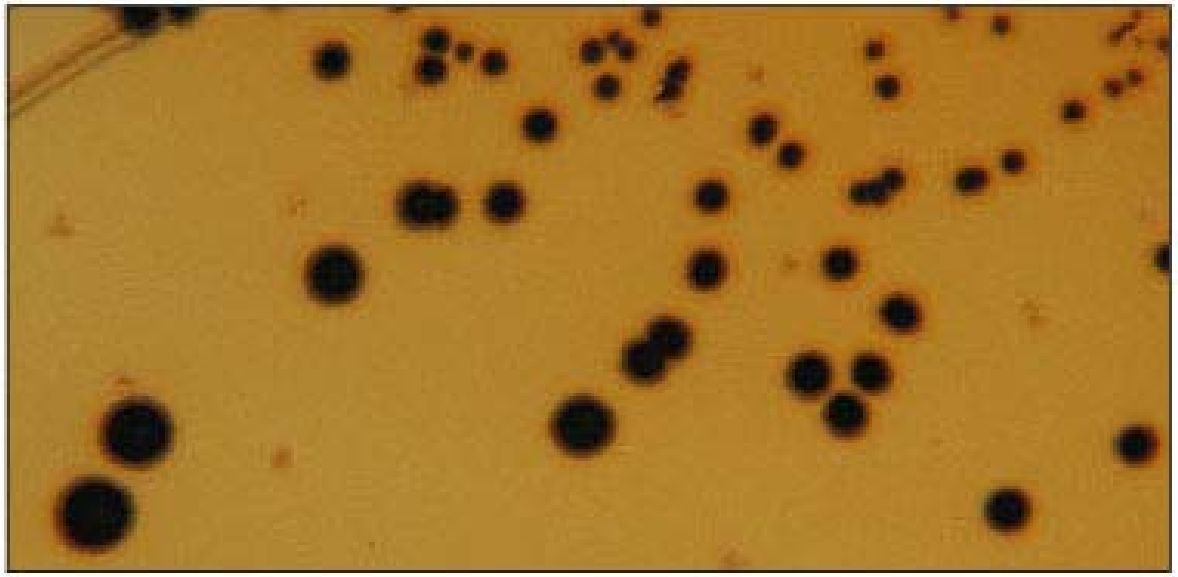


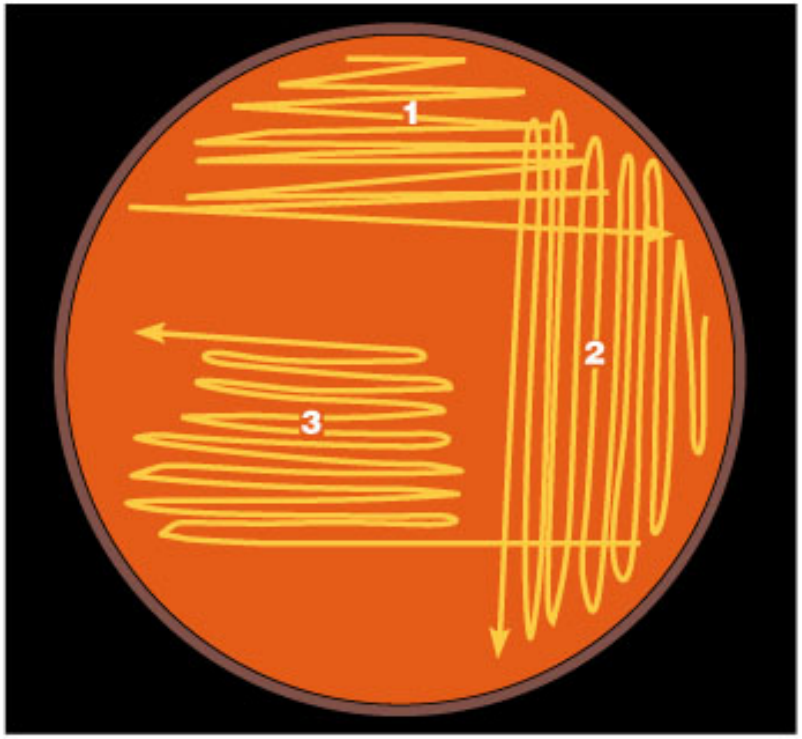
Figure 6.9a

Enrichment Media

- Encourages growth of desired microbe
- Assume a soil sample contains a few phenol-degrading bacteria and thousands of other bacteria
  - Inoculate phenol-containing culture medium with the soil and incubate
  - Transfer 1 ml to another flask of the phenol medium and incubate
  - Transfer 1 ml to another flask of the phenol medium and incubate
  - Only phenol-metabolizing bacteria will be growing

- A pure culture contains only one species or strain
- A colony is a population of cells arising from a single cell or spore or from a group of attached cells
- A colony is often called a colony-forming unit (CFU)

Streak Plate



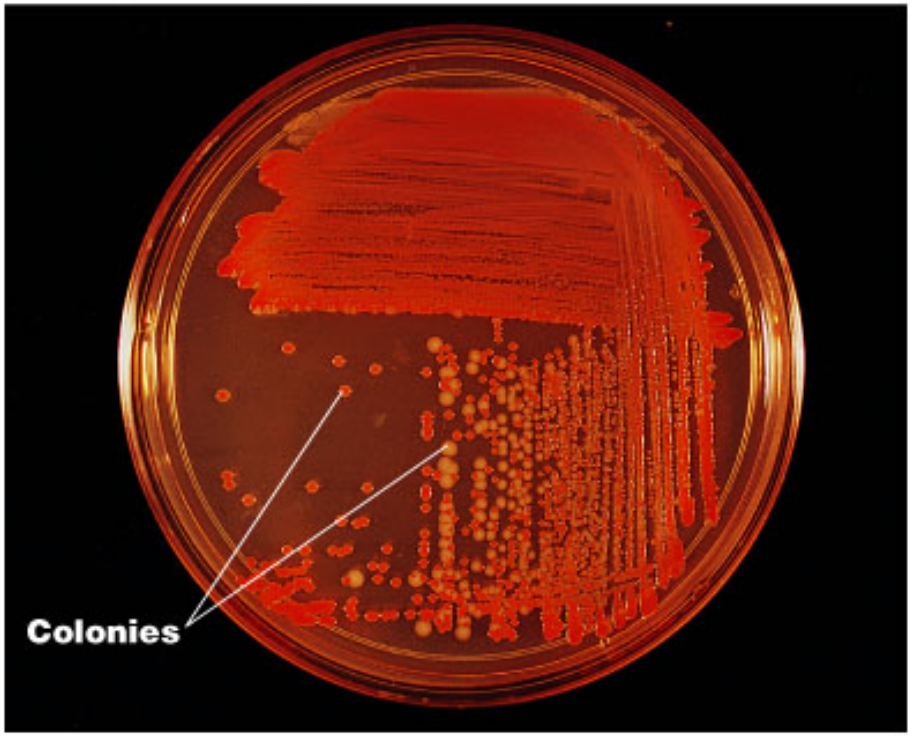


Figure 6.10a, b