Lecture 18
BT 206
13/03/2023

Microbial Growth

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

The Growth Cycle

- Microorganisms show a characteristic growth pattern when inoculated into a fresh culture medium.

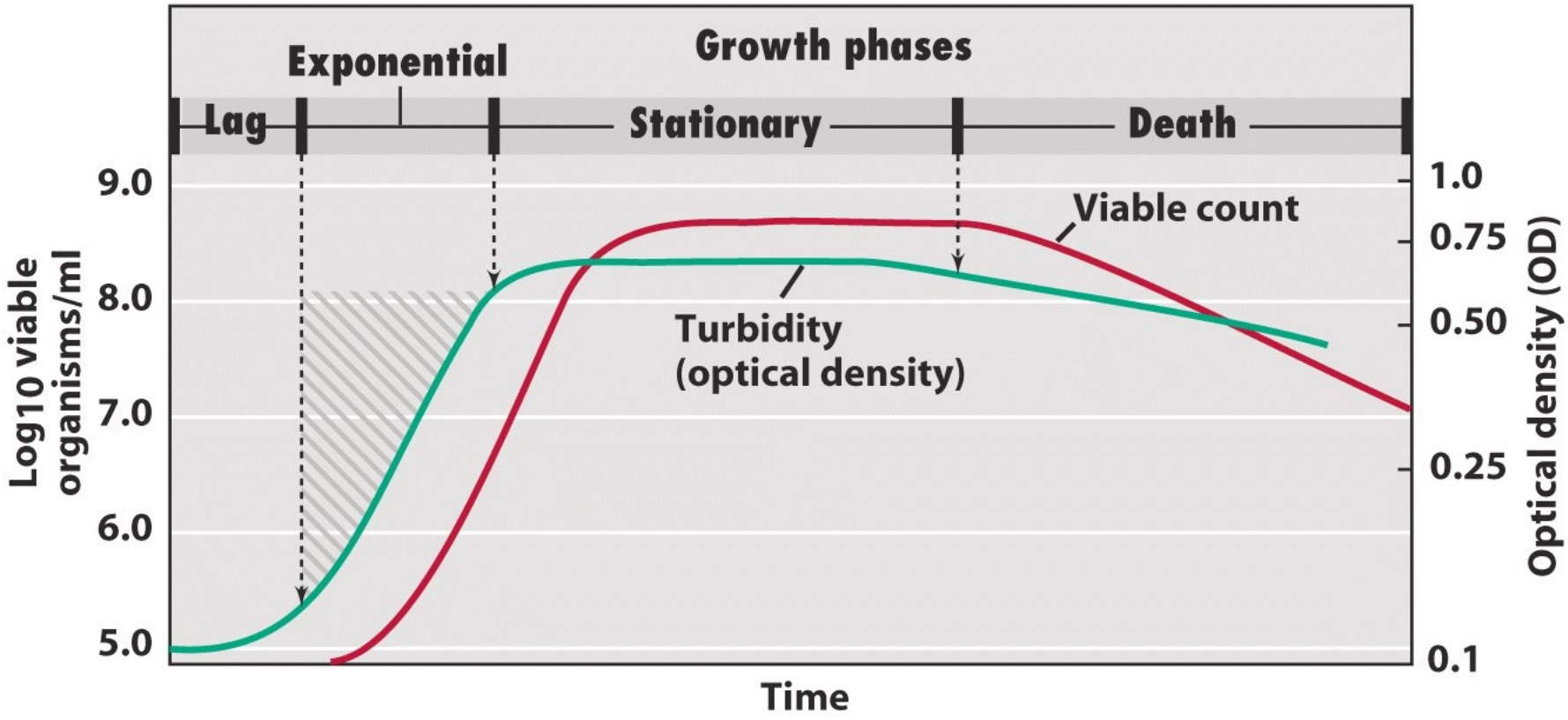


Figure 6-8 Brock Biology of Microorganisms 11/e

The Requirements for Growth: Physical Requirements

Physical and Chemical

- Physical: Temperature, pH, Osmotic Pressure
- Chemical: Carbon, Nitrogen, Sulfur, Phosphorous, Trace Elements, Oxygen, and Organic Growth Factors

On Temperature

- Classified based on Three: Psychrophiles, Mesophiles, Thermophiles
- Range
  - Minimum growth temperature: Psychrophiles
  - Optimum growth temperature: Mesophiles
  - Maximum growth temperature: Thermophiles

Temperature

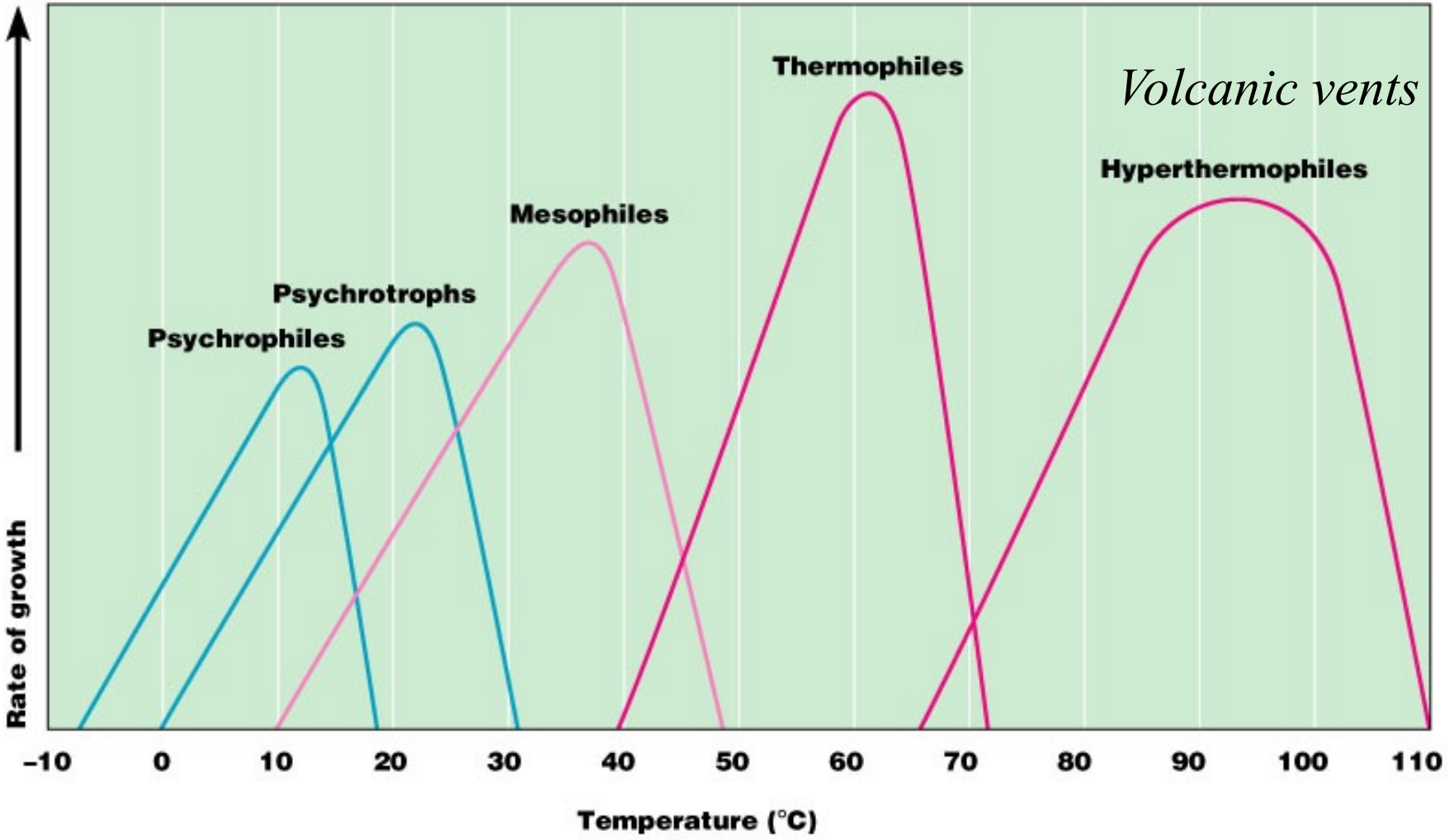
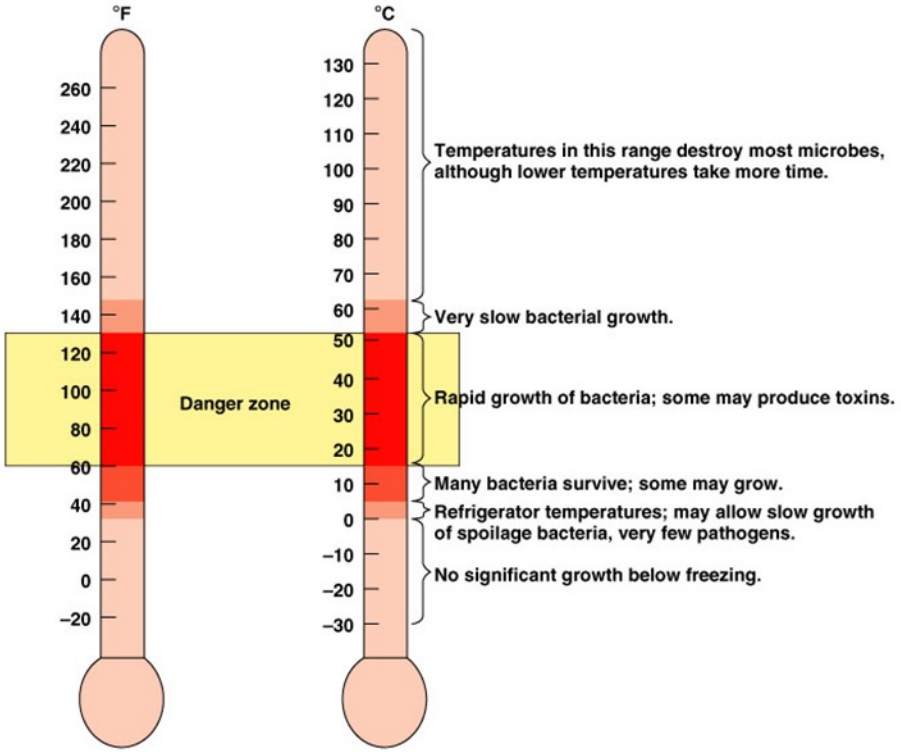


Figure 6.1

Psychrotrophs

- Grow between 0°C and 20-30°C
- Cause food spoilage
- Also known as moderate psychrophiles or facultative psychrophiles



The Requirements for Growth: Physical Requirements

- pH
  - Most bacteria grow between pH 6.5 and 7.5
    - Food preservation using pH: pickles, cheeses
    - Acidophiles (e.g. survives pH=1 in the drainage waters of coal mines –oxidizes sulfur to form sulfuric acid)
  - Molds and yeasts grow between pH 5 and 6 (greater pH range compared to bacteria)
  - Media buffers: Peptones, amino acids, phosphate salts

The Requirements for Growth: Physical Requirements

- Osmotic Pressure
  - Hypertonic environments, increase salt or sugar, cause plasmolysis
    - Examples: salty fish, honey,
  - Extreme or obligate halophiles require high osmotic pressure 30% (Dead Sea)
  - Facultative halophiles tolerate high osmotic pressure (2 to 15%)
  - Usual concentration is 1.5%
  - Some bacteria are lysed in distilled water (hypotonic)

The Requirements for Growth: Physical Requirements

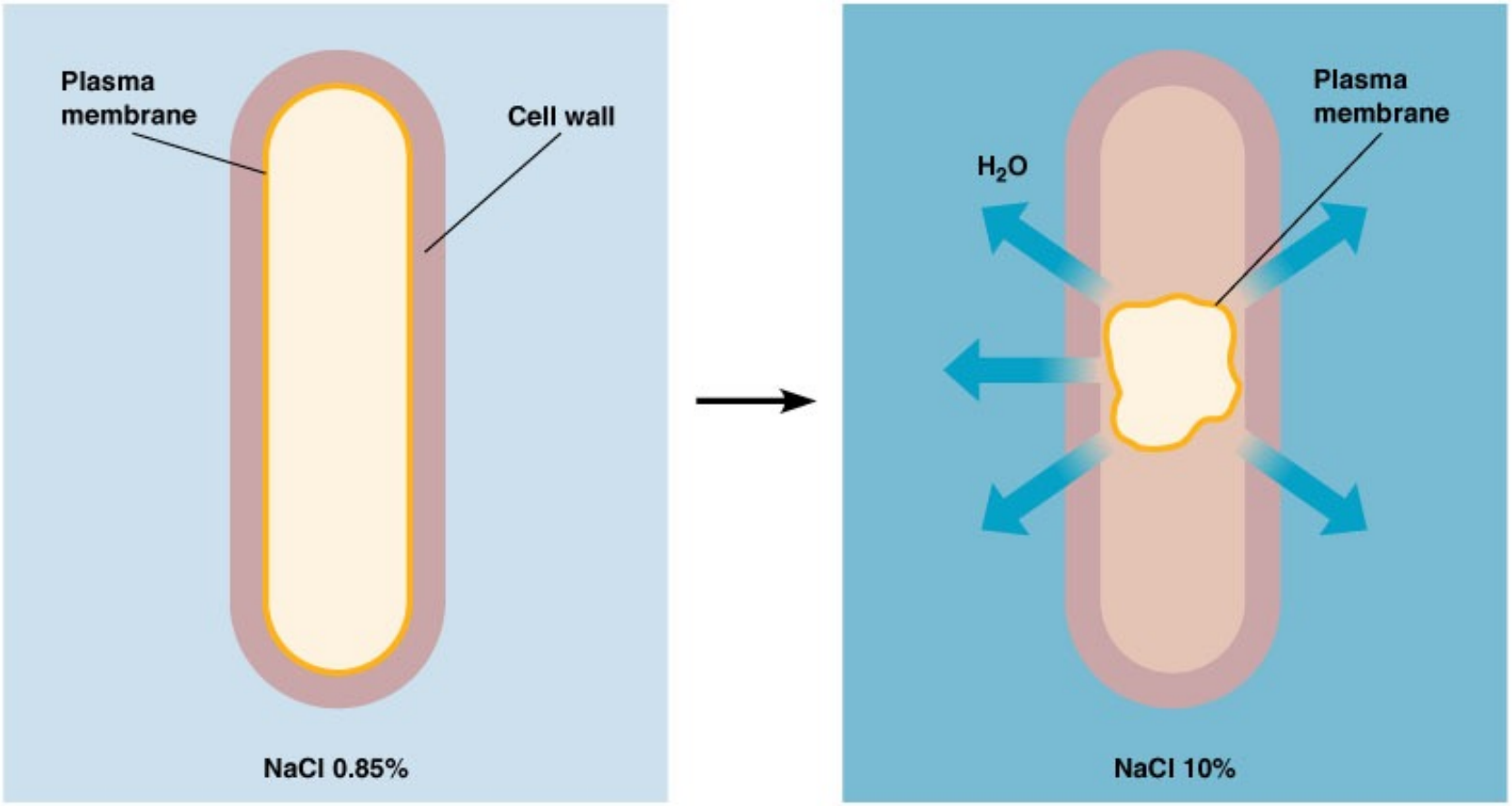


Figure 6.4

The Requirements for Growth: Chemical Requirements

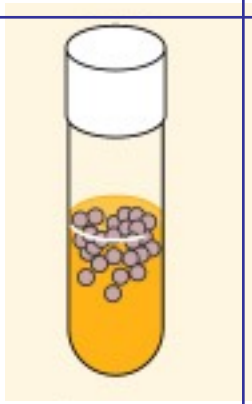
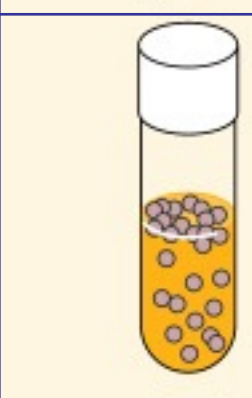
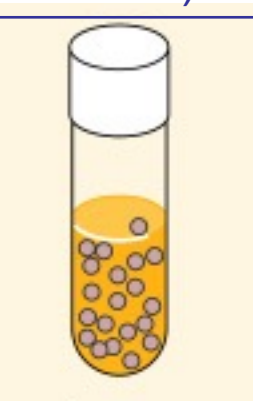
- Carbon
  - Structural organic molecules, energy source
  - ½ the dry wt is carbon
  - Chemoheterotrophs use organic carbon sources
  - Autotrophs use CO_2
- Nitrogen (14% of dry wt)
  - In amino acids, proteins
  - Most bacteria decompose proteins
  - Some bacteria use NH_4^+ or NO_3^-
  - A few bacteria use N_2 in nitrogen fixation-can form symbiotic relationship with the roots of legumes

The Requirements for Growth: Chemical Requirements

- Sulfur (4% of dry wt)
  - In amino acids, thiamine, biotin
  - Most bacteria decompose proteins
  - Some bacteria use SO_4^{2-} or H_2S (eg. extremophiles)
- Phosphorus
  - In DNA, RNA, ATP, and membranes
  - PO_4^{3-} is a source of phosphorus
- Trace Elements (Zn, Cu, Mo)
  - Inorganic elements required in small amounts
  - Usually as enzyme cofactors
  - Usually present in tap water; even distilled

The Requirements for Growth: Chemical Requirements

- Oxygen (table 6.1) KNOW THIS!

obligate aerobes	Faultative anaerobes (use oxygen or ferment)	Obligate anaerobes (cannot use oxygen-cannot live in oxygen)	Aerotolerant anaerobes (only anaerobic growth-eg. Produce lactic acid-inhibit aerobes)	Microaerophiles (only aerobic growth, but low conc. of oxygen)
		 DO NOT make SOD or catalase	 *Possess SOD*	

(The presence of the enzymes SOD (superoxide dismutase and catalase allow for growth in the presence of oxygen)

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Toxic Forms of Oxygen

- Singlet oxygen: O_2 boosted to a higher-energy state
- Superoxide free radicals: O_2^-
  (formed in small amounts during normal respiration)

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

- Peroxide anion: O_2^{2-} (active ingredient in benzoyl peroxide)

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

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

- Hydroxyl radical (OH•) (formed in cellular respiration by ionizing radiation)
- All of these ions are used by WBCs to protect the body!

The Requirements for Growth: Chemical Requirements

- Organic Growth Factors (essential organic compounds an organism is unable to synthesize)
  - Organic compounds obtained from the environment
  - Vitamins, amino acids, purines, pyrimidines

Culture Media

- What is required to grow microorganisms?
- Culture Medium: Nutrients prepared for microbial growth (pH, moisture, trace elements, organic co-factors, C, N, P, S source, oxygen? Etc)
- Sterile: No living microbes
- Definitions
  - 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, slants, and deeps
- Generally not metabolized by microbes
- Liquefies at 100°C
- Solidifies ~40°C

Culture Media

- Chemically Defined Media: Exact chemical composition is known (table 6.2 and 6.3) be able to recognize this type of media
  - Fastidious organisms require more growth factors
- Complex Media: Extracts and digests of yeasts, meat, or plants (be able to recognize this type of media)
  - Nutrient broth
  - Nutrient agar (broth + agar)

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

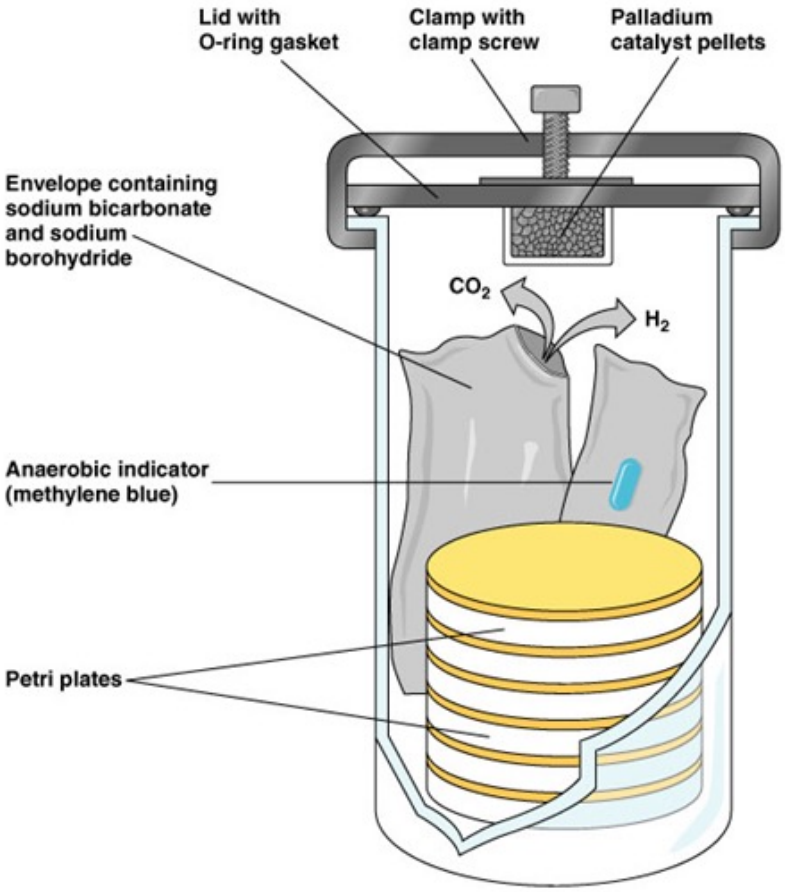
Constituent	Amount
Glucose	5.0 g
Ammonium phosphate, monobasic ($NH_4H_2PO_4$)	1.0 g
Sodium chloride (NaCl)	5.0 g
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	0.2 g
Potassium phosphate, dibasic (K_2HPO_4)	1.0 g
Water	1 liter

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

Anaerobic Culture Methods

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



Anaerobic Culture Methods

- Anaerobic chamber

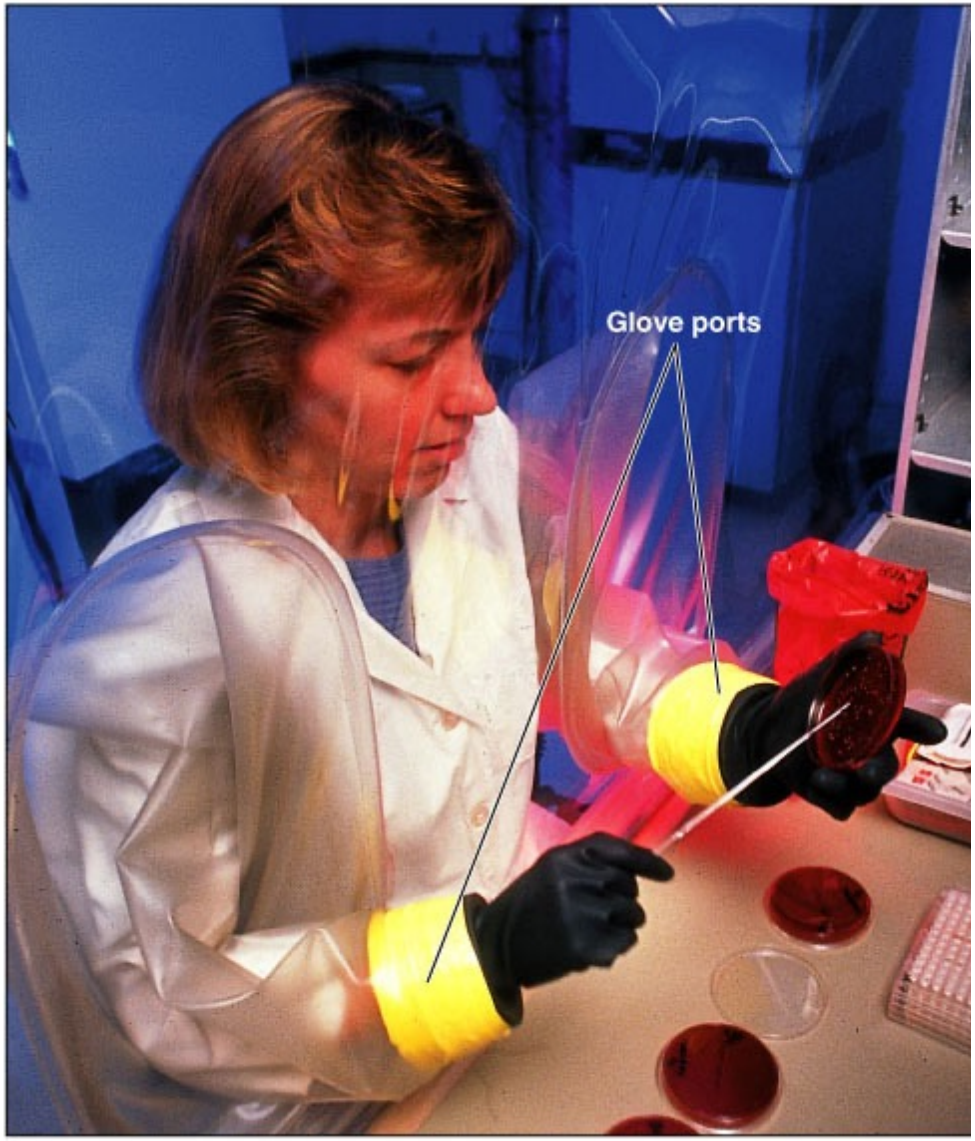


Figure 6.6

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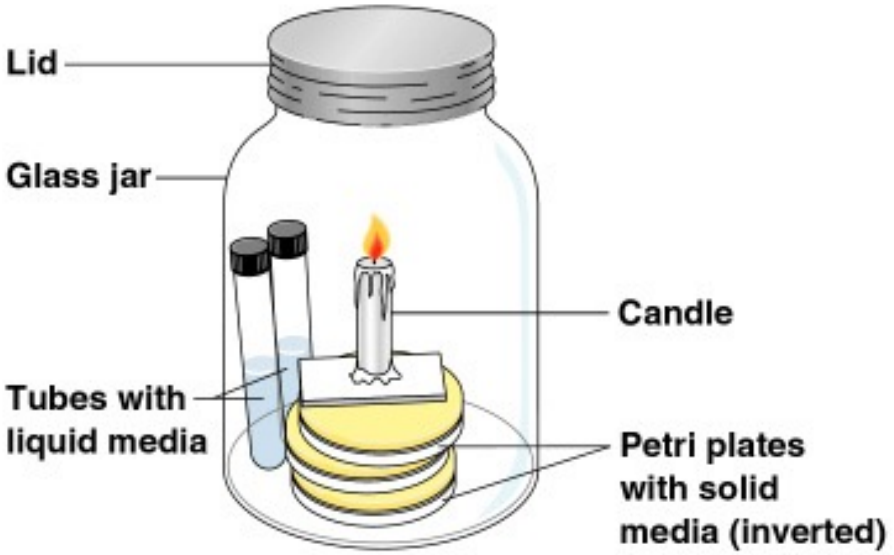
Capnophiles require high CO_2

- Candle jar



Campylobacter jejuni

- CO_2-packet

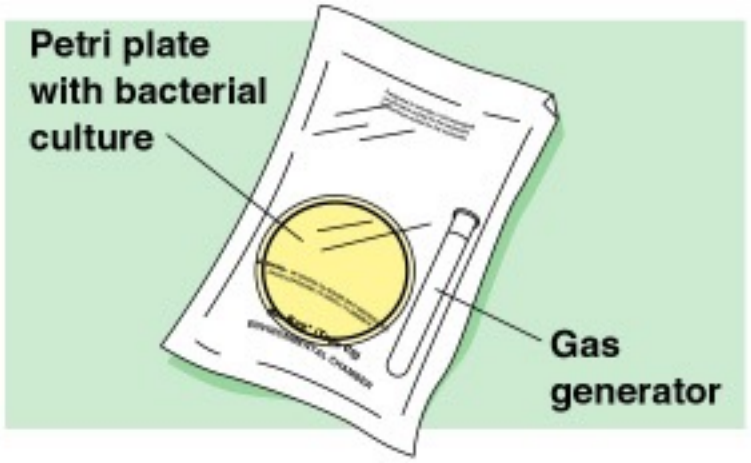


Figure 6.7

Selective Media

- Suppress unwanted microbes and encourage desired microbes.
- Bismuth sulfate suppresses gram + and normal gram – intestinal bacteria and is used to isolate *Salmonella*

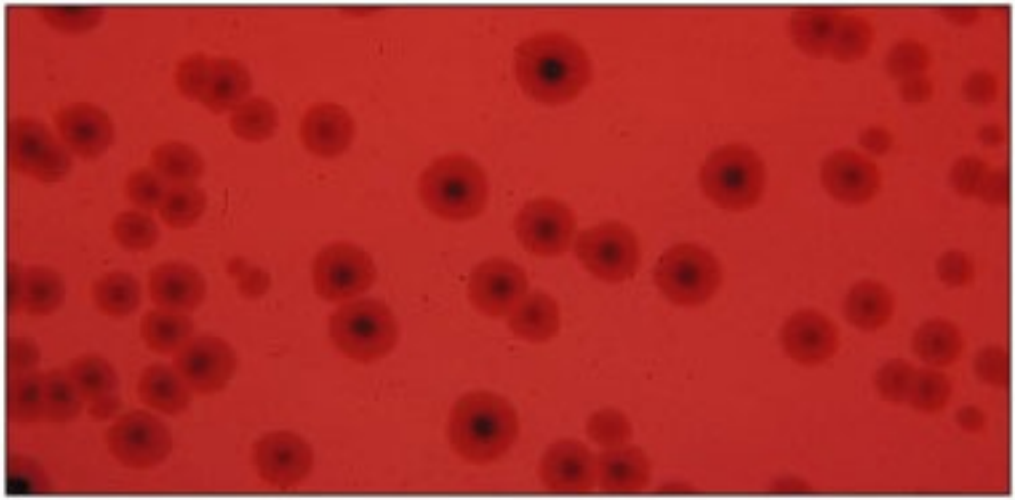


Figure 6.9b, c

Differential Media

- Make it easy to distinguish colonies of different microbes.
- Blood agar-select for organisms that produce hemolysins (Group A strep)
- Selective and Differential: High salt + fermentation of mannitol –isolate *S. aureus*

S. aureus on Tellurite-Glycine medium

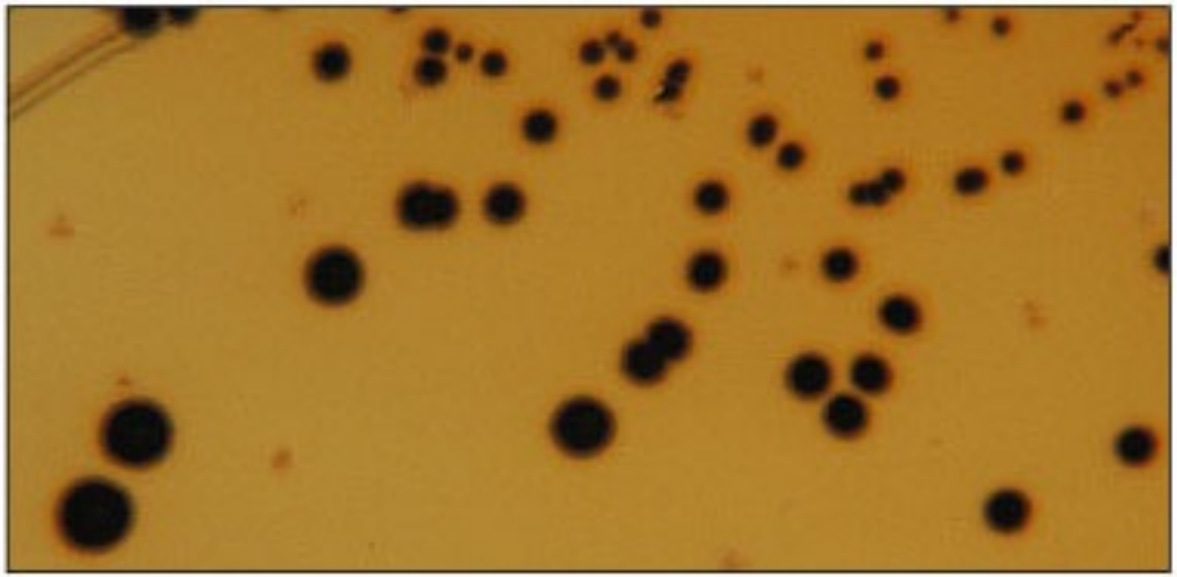


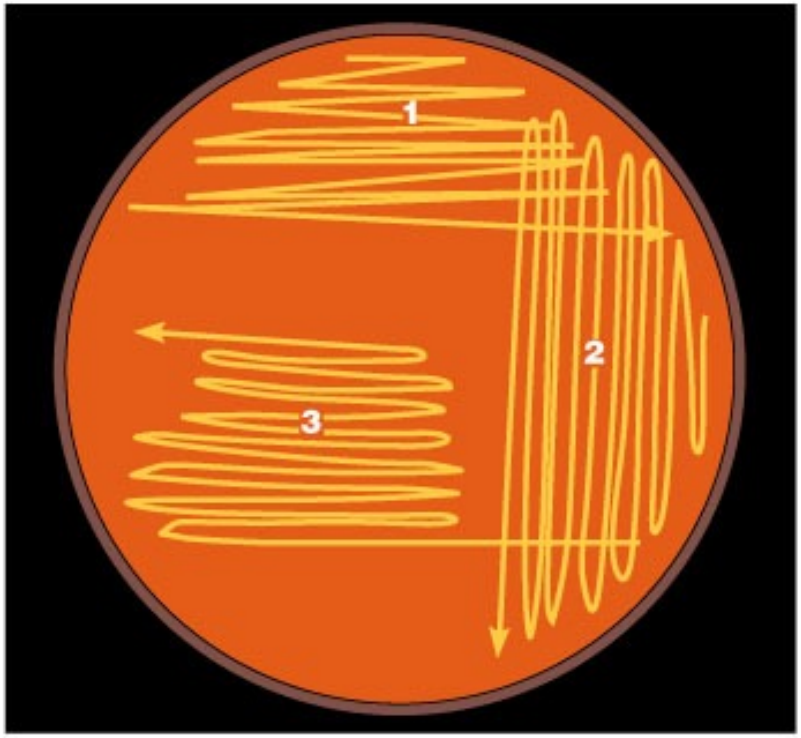
Figure 6.9a

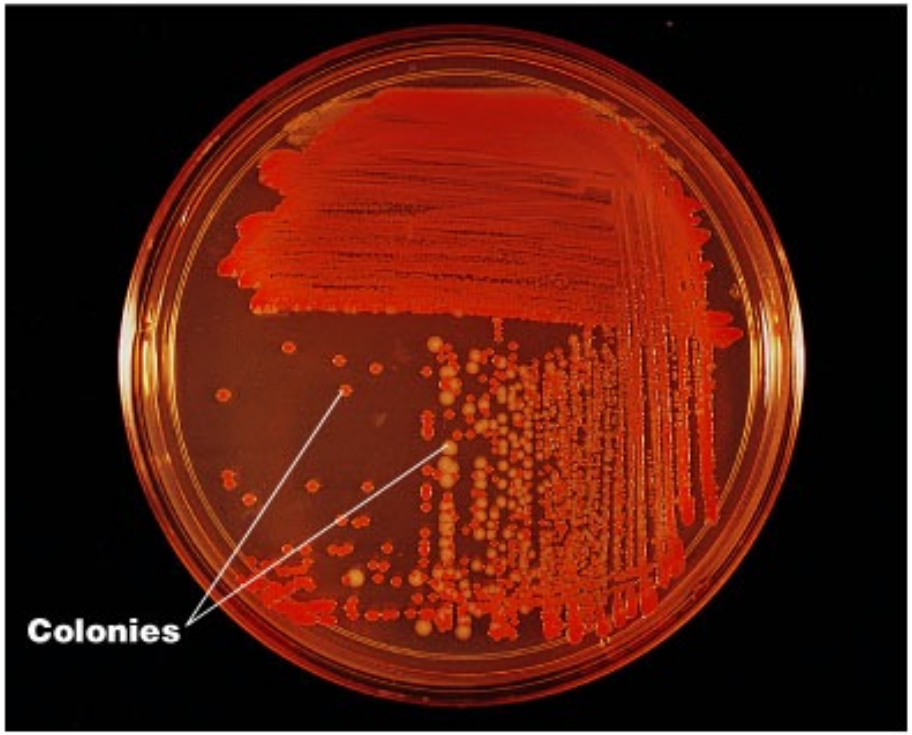
Enrichment Media

- 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





Works well when the bacteria is present in high numbers

Figure 6.10a, b