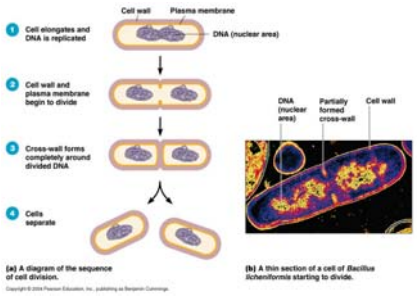
CHAPTER 6
MICROBIAL GROWTH

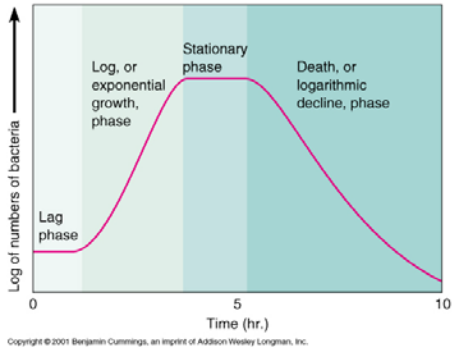
Microbial Growth

- **Growth=** an increase in the number of cells, not an increase in size
- **Generation**=growth by binary fission
- **Generation time**=time it takes for a cell to divide and the population to double; most are 1-3 hours (E.coli: every 20 min.)

Bacteria divide by binary fission



Bacterial growth curve



- Chemostat: keeps microbial growth at log stage by draining off used media and replacing with new nutrients.

Requirements for bacterial growth

- Physical requirements
  - Temperature, pH, osmotic pressure, oxygen
- Chemical requirements
  - Carbon, nitrogen, sulfur, and phosphorous
  - Trace elements

Optimum Growth Temperatures

- Psychrophiles-optimum 15°C
- Psychrotrophs- range from 20-30°C
- Mesophiles- range from 25-40°C
- Thermophiles-range from 50-60°C

Temperature

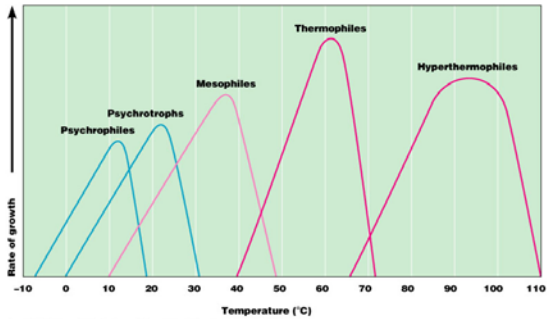


Figure 6.1

PSYCHROTROPHS

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

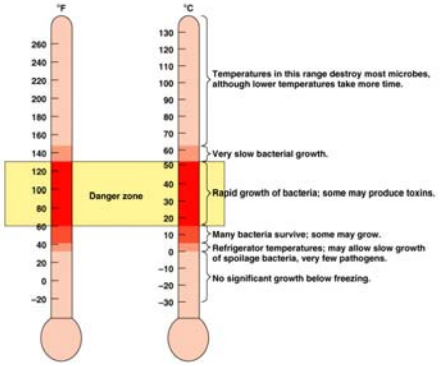


Figure 6.2

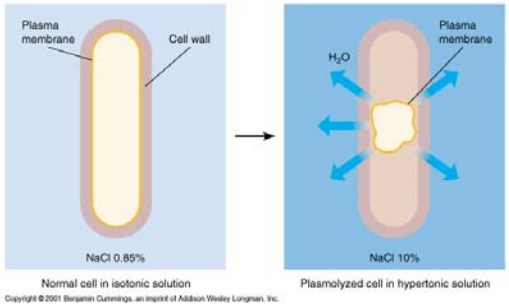
- pH
  - Most bacteria grow between pH 6.5 and 7.5
  - Molds and yeasts grow between pH 5 and 6
  - Acidophiles grow in acidic environments

Osmotic Pressure

- Hypotonic
- Isotonic (0.85% NaCl)
- Hypertonic

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

Osmotic environment influences growth



The Requirements for Growth: Chemical Requirements

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

The Requirements for Growth: Chemical

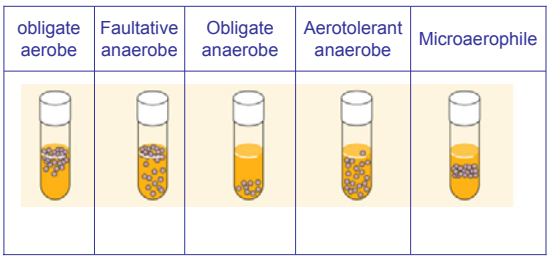
- Nitrogen
  - In amino acids, proteins
  - Most bacteria decompose proteins
  - Some bacteria use NH_4^+ or NO_3^-
  - A few bacteria use N_2 in nitrogen fixation
- Sulfur
  - In amino acids, thiamine, biotin
  - Most bacteria decompose proteins
  - Some bacteria use SO_4^{2-} or H_2S
- Phosphorus
  - In DNA, RNA, ATP, and membranes
  - PO_4^{3-} is a source of phosphorus

The Requirements for Growth: Chemical Requirements

- Trace Elements (Iron, Copper, Zinc, etc.)
  - Inorganic elements required in small amounts
  - Usually as enzyme cofactors

The Requirements for Growth: Chemical Requirements

- Oxygen (O_2)



Why can some organisms grow in the presence of oxygen?

- Toxic forms of oxygen need to be neutralized by enzymes
  - Superoxide dismutase
  - Catalase
  - Peroxidase

  If microbe does not produce these enzymes, must have anaerobic conditions.

Toxic forms of oxygen

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

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

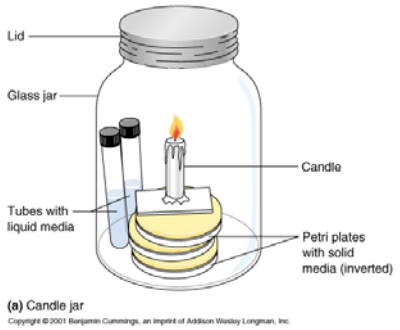
- Peroxide anion: O_2^{2-}

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

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

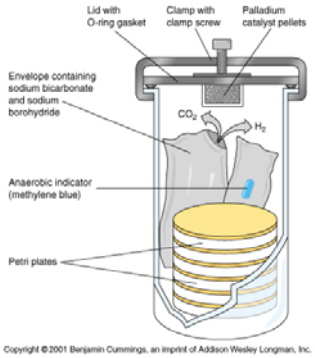
- Hydroxyl radical (OH•)

Candle jars increase CO_2 levels for growing capnophiles



(a) Candle jar

Anaerobic jars eliminate the oxygen for anaerobes to grow.



The Requirements for Growth: Chemical Requirements

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

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

TABLE 6.5	Culture Media
Type	**Purpose**
Chemically defined	Growth of chemoautotrophs and photoautotrophs, and microbiological assays.
Complex	Growth of most chemoheterotrophic organisms.
Reducing	Growth of obligate anaerobes.
Selective	Suppression of unwanted microbes; encouraging desired microbes.
Differential	Differentiation of colonies of desired microbes from others.
Enrichment	Similar to selective media but designed to increase numbers of desired microbes to detectable levels.

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
- Complex Media: Extracts and digests of yeasts, meat, or plants
  - Nutrient broth
  - Nutrient agar

Culture Media

- Chemically defined
  - GSA

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

Culture Media

- Complex
  - Nutrient Agar
  - TSA
  - BHI

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

- Selective media
- Suppress unwanted microbes and encourage desired microbes.

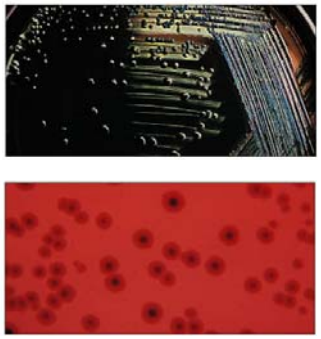


Figure 6.9b, c

Differential Media

- Make it easy to distinguish colonies of different microbes.

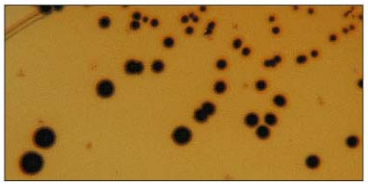
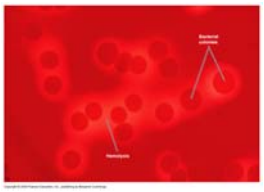


Figure 6.9a

Culture Media

- Selective
- Differential
  - Blood
- Selective and Differential
  - EMB



- 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

Transport Media

- Maintains bacterial growth when transporting to laboratory or mailing to state public health lab
- Thayer Martin (Martin-Lewis) Jembec packs for Neisseria gonorhoaeae

- 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

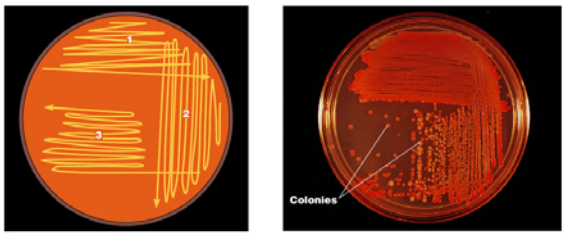
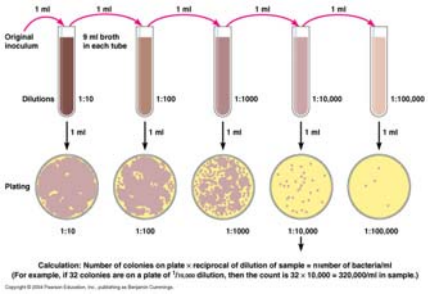


Figure 6.10a, b

Ways to measure bacterial growth

- Plate Count/Viable Cell Count
- Filtration (very small numbers)
- Most Probable Number
- Direct Microscopic Counts
  - Breed count method: Petroff-Hausser counter
- Measure Turbidity; Metabolic activity; Dry weight

Plate counts require dilutions to obtain colonies



Urine Cultures

- Urine cultures: calibrated loop for .01 or .001 ml of urine
- Cross hatch
- Count colonies:
  - $\geq 10^5$ colonies/ml indicate urinary tract infection

- Filtration

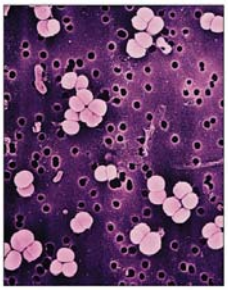


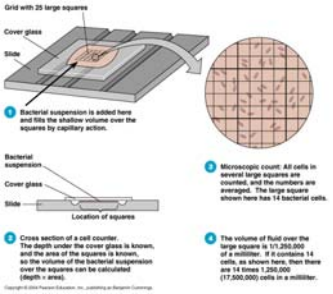
Figure 6.17a, b

- Multiple tube MPN test
- Count positive tubes and compare to statistical MPN table.

Combination of Positives	MPN Index/ 100 ml	95% Confidence Limits	
		Lower	Upper
4-2-0	22	9	56
4-2-1	26	12	65
4-3-0	27	12	67
4-3-1	33	15	77
4-4-0	34	16	80
5-0-0	23	9	86
5-0-1	30	10	110
5-0-2	40	20	140
5-1-0	30	10	120
5-1-1	50	20	150
5-1-2	60	30	180
5-2-0	50	20	170
5-2-1	70	30	210
5-2-2	90	40	250
5-3-0	80	30	250
5-3-1	110	40	300
5-3-2	140	60	360

Figure 6.18b

Direct Counts



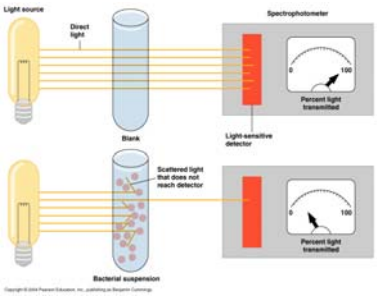
Direct Measurements of Microbial Growth

- Direct Microscopic Count

$$\text{Number of bacteria/ml} = \frac{\text{number of cells counted}}{\text{volume of area counted}}$$

$$\frac{14}{8 \times 10^{-7}} = 17{,}500{,}000$$

Turbidity gives a rough estimate



Estimating Bacterial Numbers by Indirect methods

- Metabolic activity
- Dry weight