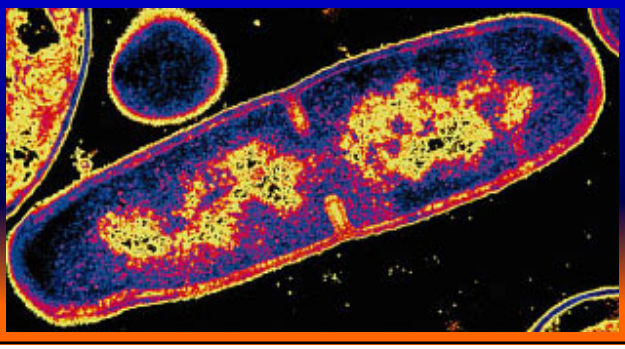
Chapter 6 *Microbial Growth*



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Chapter 6 Objectives:

Students should be able to: .....

1. **Ch. 6:** Describe several **physical and chemical requirements for microbial growth**, and explain what factors determine optimal conditions.
2. Define the **high, medium, or low levels of each physical factor** terms that describe organisms that prefer affecting growth.
3. Diagram and define the **four phases of a bacterial growth curve**. Compare several methods of **measuring microbial growth**.

❖ ***Objectives are your HOMEWORK between classes!!!***

- *Outline the concepts, define terms, DRAW structures and processes, and PRACTICE ALL!!!*

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Microbial Growth

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

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6.1) Requirements for Growth: Physical Requirements

A. Temperature: (Psychro-, meso-, thermo-philes)
   - *Minimum growth temperature*
   - *Optimum growth temperature*
   - *Maximum growth temperature*

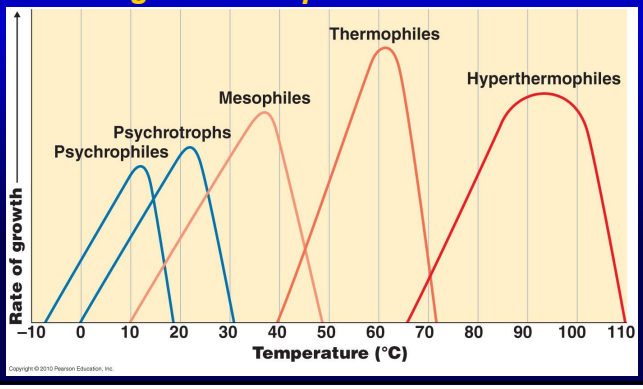

Figure 6.1

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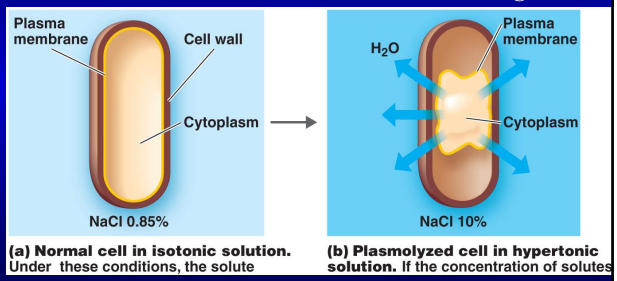
Physical Requirements for Growth

B. pH: (acido-, neutero-, alkalo-philes)
- Most bacteria grow between pH 6.5 & 7.5.
- Molds and yeasts grow between pH 5 & 6.
- Acidophiles grow in acidic environments.

Figure 6.4



(a) Normal cell in isotonic solution. Under these conditions, the solute

(b) Plasmolyzed cell in hypertonic solution. If the concentration of solutes

C. Osmotic Pressure:
- Hypertonic environments -- increase salt or sugar → cause plasmolysis.
- **Extreme or obligate *halophiles*** require high osmotic pressure.
- **Facultative halophiles** tolerate high osmotic pressure .

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6.2) Requirements for Growth: A. Chemical Requirements

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

2. 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

3. Sulfur
- In amino acids, thiamine, biotin
- Most bacteria decompose proteins
- Some bacteria use SO_4^{2-} or H_2S

4. Phosphorus
- In DNA, RNA, ATP, and membranes
- PO_4^{3-} is a source of phosphorus

5. Trace Elements
- Inorganic elements -- in small amounts
- Usually as enzyme cofactors

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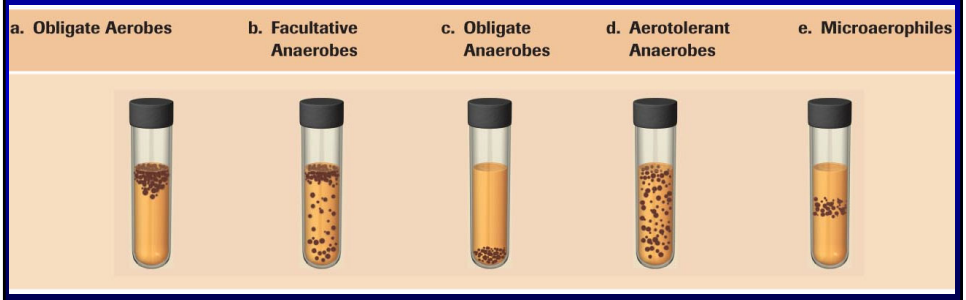
Chemical Requirements for Growth

6.) Oxygen (O_2)



(Eg: BHIA deep agar tubes)

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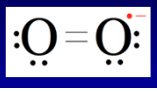
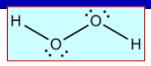
B. Toxic Forms of Oxygen

1. ***Singlet oxygen: O_2*** boosted to a higher-energy state
2. ***Superoxide free radicals: $O_2^{\bullet -}$***



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

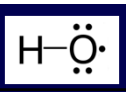
3. ***Peroxide anion: O_2^{2-}***



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

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

4. ***Hydroxyl radical ($^{\bullet}OH$)***



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6.3) Culture Media

- ***Chemically Defined Media***: Exact chemical composition is known
  - "minimal media"
  - many additives for ***"fastidious" species***
- ***Complex Media***: Extracts and digests of yeasts, meat, or plants
  - Nutrient broth
  - Nutrient agar

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A. Selective Media

- *Suppress unwanted microbes and encourage desired microbes.*
  - EMB
  - MacConkey
  - NaCl-Mannitol
  - Min. Glc/Nitrate

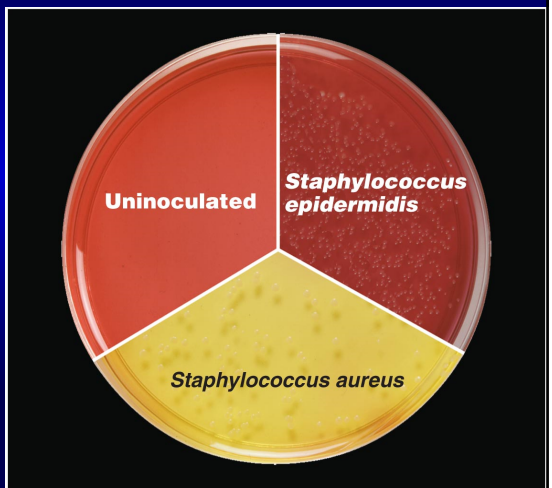

Figure 6.10

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B. Differential Media

- *Make it easy to distinguish colonies of different microbes.*
  - *[All the examples on previous slide]*
  - Fermentation tubes
  - Blood agar, YE-$CaCO_3$-sucrose, Snyder deep

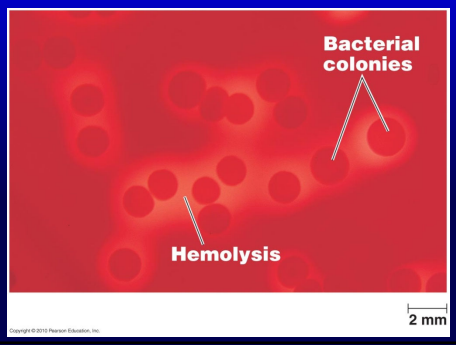

Figure 6.9

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6.4) Bact. Growth: Binary Fission

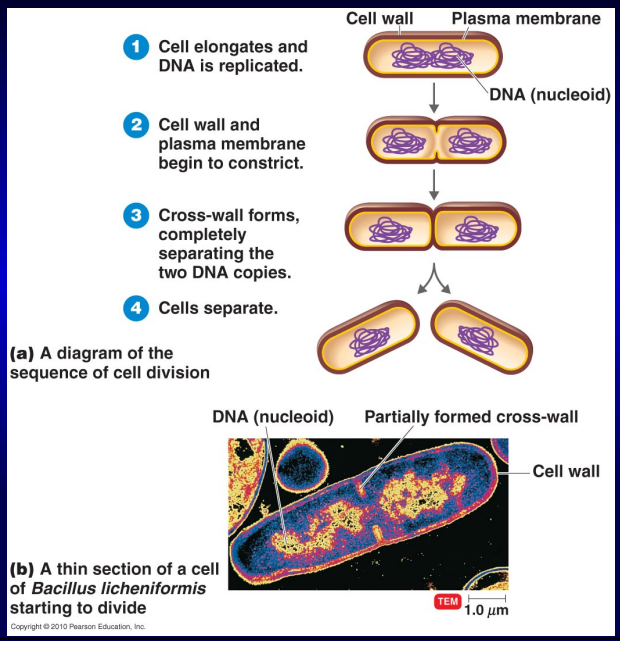

Figure 6.12

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A. Cell Division: Exponential Increases!!

Generation Number	Number of Cells	Log_{10} of Number of Cells
0	$2^0 = 1$	0
5	$2^5 = 32$	1.51
10	$2^{10} = 1{,}024$	3.01
15	$2^{15} = 32{,}768$	4.52
16	$2^{16} = 65{,}536$	4.82
17	$2^{17} = 131{,}072$	5.12
18	$2^{18} = 262{,}144$	5.42
19	$2^{19} = 524{,}288$	5.72
20	$2^{20} = 1{,}048{,}576$	6.02

(b) Conversion of the number of cells in a population into the logarithmic expression of this number. To arrive at the numbers in the center column, use the y^x key on your calculator. Enter 2 on the calculator; press y^x; enter 5; then press the = sign. The calculator will show the number 32. Thus, the fifth-generation population of bacteria will total 32 cells. To arrive at the numbers in the right-hand column, use the log key on your calculator. Enter the number 32; then press the log key. The calculator will show, rounded off, that the log_{10} of 32 is 1.51.

2^n, where n = # cell division cycles

Figure 6.13b

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B. Bacterial Growth Curve

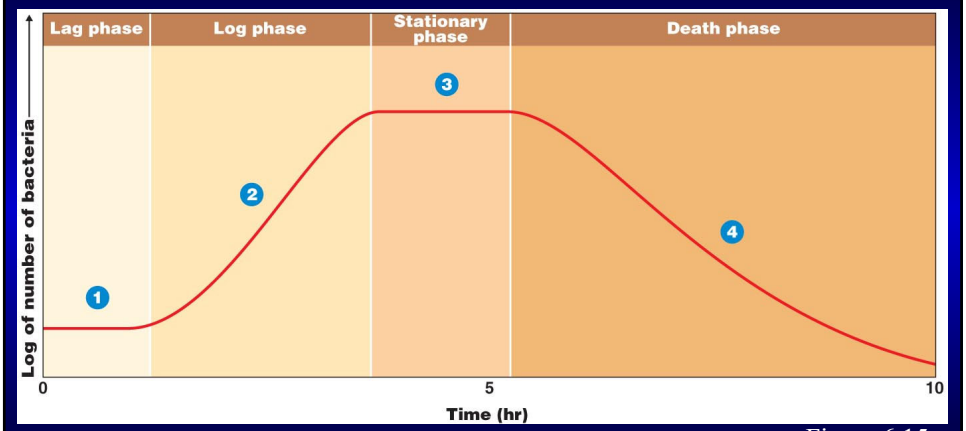

Figure 6.15

Question: What is happening to the cells at each phase??

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C. Direct Measurements of Microbial Growth

1. Plate Counts:

Perform ***serial dilutions*** of a sample.

- After incubation, count colonies on plates that have 25-250 colonies (CFUs) -- [we used 30-300 CFUs]

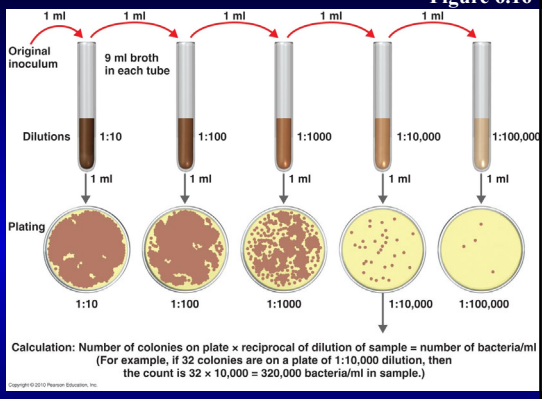

Figure 6.16

2. Cytometer count:

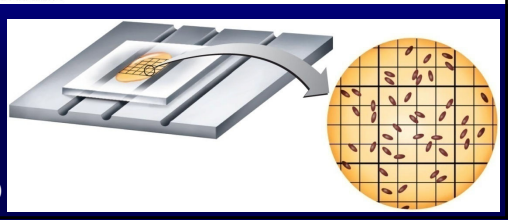

Figure 6.20

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3. Most Probable Number Test

- **Multiple tube MPN test**
- ***Based on "dilution to extinction"***
  - *Highest dilution with growth used to estimate bact. In liquid sample*
- Count positive tubes and compare to statistical MPN table.

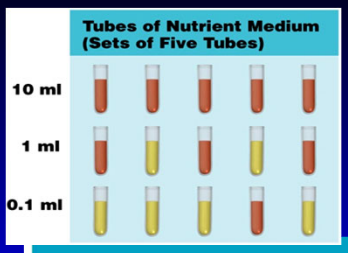

Figure 6.19

Combination of Positives	MPN Index/ 100 ml	95% Confidence Limits	
		Lower	Upper
4-2-0	22	6.8	50
4-2-1	26	9.8	70
4-3-0	27	9.9	70
4-3-1	33	10	70
4-4-0	34	14	100
5-0-0	23	6.8	70
5-0-1	31	10	70
5-0-2	43	14	100
5-1-0	33	10	100
5-1-1	46	14	120
5-1-2	63	22	150
5-2-0	49	15	150
5-2-1	70	22	170
5-2-2	94	34	230
5-3-0	79	22	220
5-3-1	110	34	250
5-3-2	140	52	400

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D. Estimating Bacterial Numbers by Indirect Methods

- ***Turbidity***

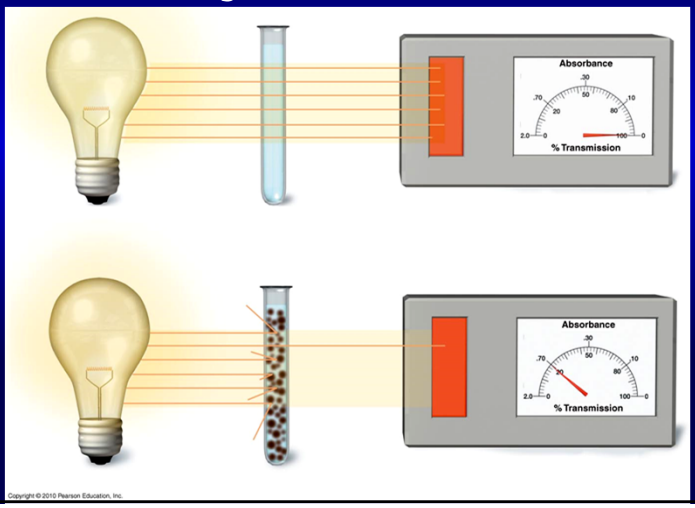

Figure 6.21

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