Chapter 4

Growth and Culturing of Bacteria

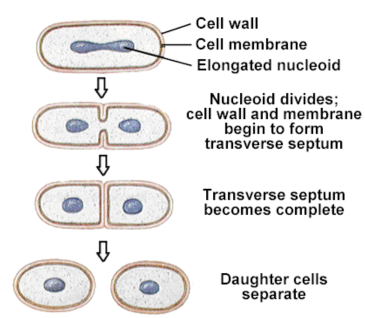
Growth of Bacteria

- ✓ Asexual reproduction does not involve sex cells.
- ✓ One cell divides into two identical daughter cells by binary fission.

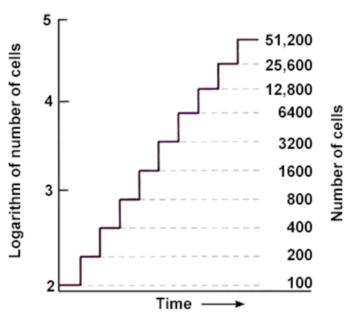
Types of reproduction

1. binary fission
2. budding
3. fragmentation

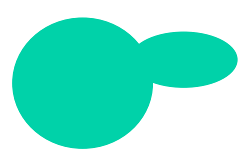
Binary Fission



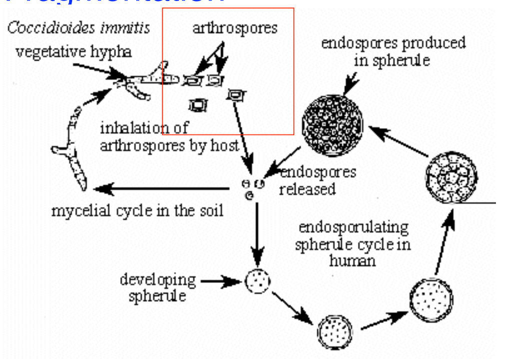
Binary fission leads to log growth



Budding



Fragmentation

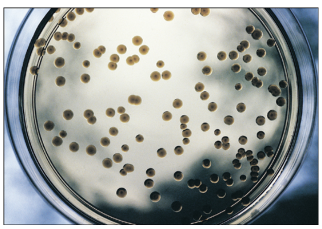


Key terms

- ✓ Generation time/Doubling time
  - Time interval required for each microbe to divide.
- ✓ Growth rate
  - Number of cell divisions per unit time.

Cultures

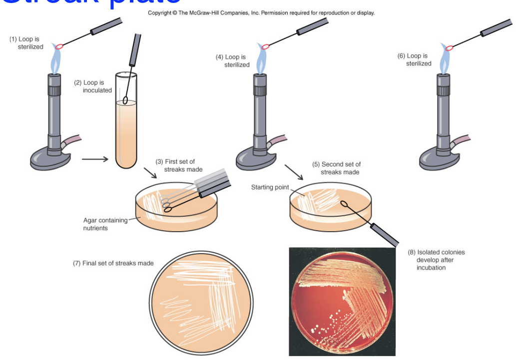
- ✓ Media
  - Liquid is broth
  - Solid is broth with **agar** added
  - Slant or Petri dish
- ✓ Agar
  - Polysaccharide extracted from red algae
    - Liquid above 95-100°C
    - Solid at 42-45°C
- ✓ Colony
  - A visible growth of bacteria that arose from a single cell
- ✓ CFU (Colony Forming Unit)
    - A unit that gives rise to a colony - used for counting



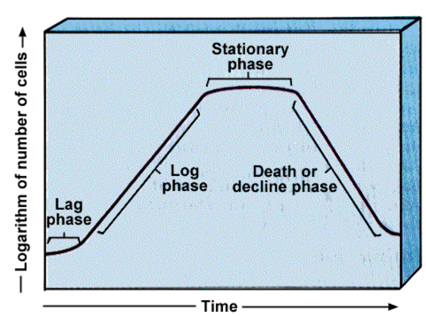
Obtaining a pure culture

- ✓ Streak plate
  - Simplest and most common
- ✓ Pour plate
  - Used in enumeration experiments too
  - We need a single colony to inoculate our culture
    - A pure culture - contains a single species of organism
    - A mixed culture - different kinds of organisms

Streak plate

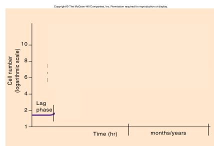


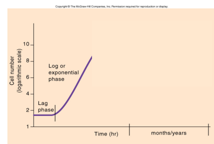
Standard bacterial growth curve



Bacterial Growth in Laboratory Conditions

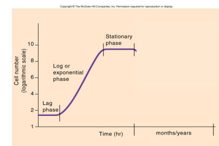
- ✓ Lag phase
  - Number of cells does not increase
  - Cells prepare for growth
    - Enzyme production
- ✓ Log phase
  - Period of exponential growth
    - Doubling of population with each generation
  - Produce primary metabolites
    - Compounds required for growth
    - Maxium growth for conditions

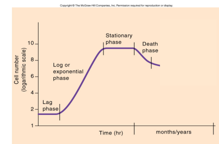




Bacterial Growth in Laboratory Conditions

- ✓ Stationary phase
  - Overall population remains relatively stable
    - Cells exhausted nutrients
    - Cell growth = cell death
      - Dying cell supply metabolites for replicating cells
    - Synthesize secondary metabolites
      - Used to enhance survival
      - antibiotics
      - Toxins
- ✓ Death phase
  - Total number of viable cells decreases
    - Decrease at constant rate
  - Death is exponential





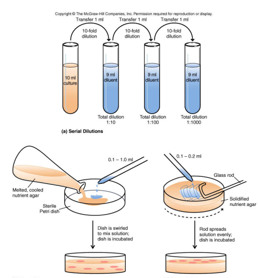
Measuring growth

1. Serial dilution and standard plate count (Viable).
   a. pour plates
   b. spread plates
2. Direct Microscopic count
   a. counting chamber
   b. Cell counting instruments
      a. Electronic cell count
      b. Flow cytometry

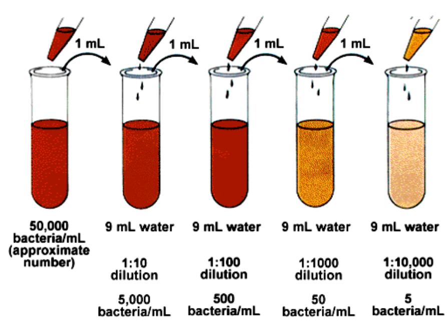
4.Most probable number (MPN)
5. Filtration
6. Turbidity
6. Other methods

Plate count/serial dilution

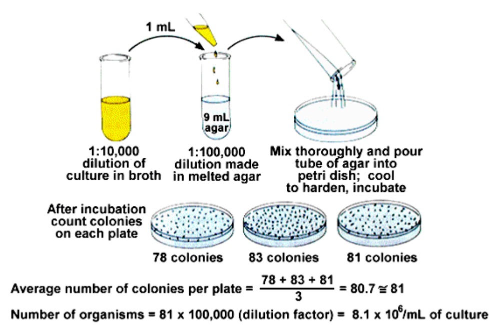
1. Relatively easy to do.
2. Some inaccuracies in counting colonies.
3. Risk of contamination.
4. Slow.



Serial Dilutions



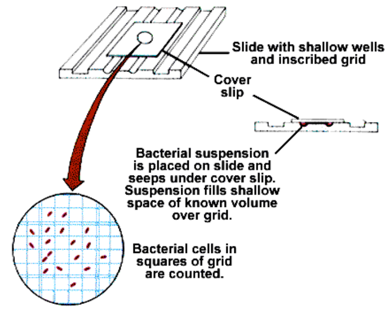
Pour plate method



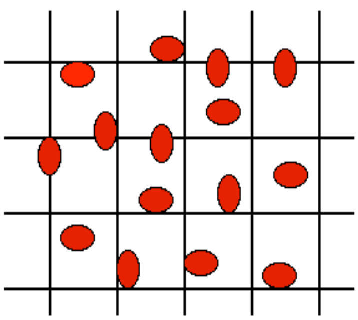
Microscopic count

1. Cannot determine viable cells
2. Time consuming
3. May need special equipment.
4. Small cells are difficult to see (inaccurate)
5. Low cell counts cause inaccuracies

Counting chamber

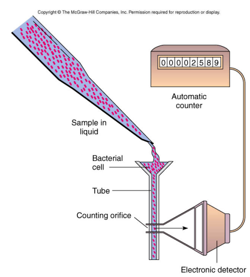


Microscopic count

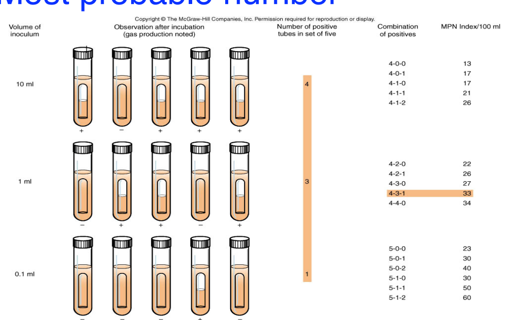


Electronic cell count

1. Cannot distinguish between viable and non-viable cells.
2. Expensive equipment
3. Difficult to calibrate
4. Coulter counter
   1. Detects electrical resistance
5. Flow cytometer
   1. Measures laser lights



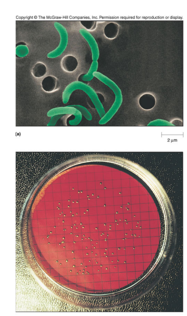
Most probable number



For testing samples that may have few organisms

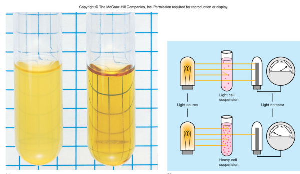
Membrane filter

1. Relatively simple to do.
2. Good for water samples.
3. Some inaccuracies in counting colonies.
4. Slow.

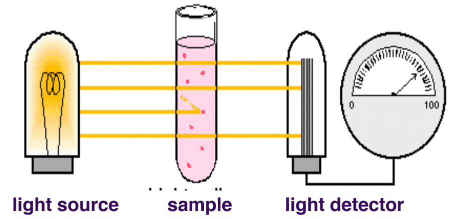


Turbidity

1. Most convenient method.
2. Quick.
3. Non-destructive.
4. Requires calibration.



Turbidity



Other methods

- Total Weight
  - Wet weight
    - Cell spun down and weighed
  - Dry weight
    - Packed cells dried at 100°C for 8-12 hr
    - Weighed
- Cell products
  - Acid production
    - pH indicator
  - Gas production
    - Durham tube traps gas
  - ATP/enzyme production
  - Dye reduction
  - Nitrogen content

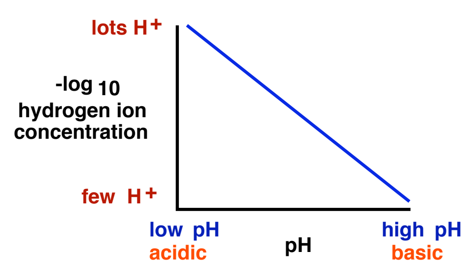
Factors Affecting Bacterial Growth

✓ pH
✓ Temperature
✓ Oxygen
✓ Water availability
✓ Hydrostatic pressure
✓ Osmotic pressure
✓ Radiation
✓ Nutritional factors

pH

✓ pH is a measure of the concentration of hydrogen ions (H^+) in a solution.
✓ Actually...

$$pH = -\log[H^+]$$



pH classification

- ✓ Acidophiles (acid-loving)
  - pH 0.1 to 5.4 (*Lactobacillus*)
    - Maintain neutral pH by pumping out protons (H^+)
- ✓ Neutrophiles
  - pH 5.4 to 8.5 (*E. coli*, *streptococcus*, most of the bacteria that cause human disease)
- ✓ Alkaliphiles (base-loving)
  - pH 7.0 to 11.5 (*Agrobacterium*, *Vibrio cholera*)
    - Maintain neutral pH by exchanging sodium (out) for external protons (in)

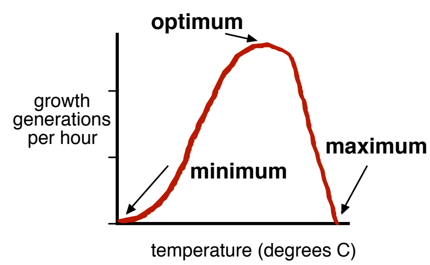
pH parameters

- ✓ Intracellular pH of cell = ~7.5
- ✓ maintains pH by expelling or bringing H^+ ions into the cell.
- ✓ Optimum pH is at about neutrality
- ✓ (pH 7).

Optimal pH for growth

✓ Bacteria	pH 6-8
✓ Fungi	pH 5-6
✓ Protozoa	pH 6.7 - 7.7
✓ Algae	pH 4-8.5

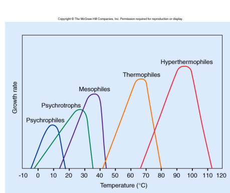
Temperature ranges



The temperature range is determined by the temperatures that the organism's enzymes function.

Temperature classification

- ✓ psychrophiles
  - cold-loving
- ✓ Psychrotrophs
  - Cold feeding
- ✓ mesophiles
  - moderate temp-loving
- ✓ thermophiles
  - heat-loving
- ✓ Hyperthermophiles



Optimal growth

- ✓ Psychrophile
  - Optimum temperature -5°C to 15°C
    - Found in Arctic and Antarctic regions
- ✓ Psychrotroph
  - 20°C to 30°C
    - Important in food spoilage
- ✓ Mesophile
  - 25°C to 45°C
    - More common
    - Disease causing
- ✓ Thermophiles
  - 45°C to 70°C
    - Common in hot springs
- ✓ Hyperthermophiles
  - 70°C to 110°C
    - Usually members of *Archaea*
    - Found in hydrothermal vents

Oxygen requirement

✓ Microorganisms require different amounts of...

✓ Oxygen

✓ Carbon dioxide

✓ Nitrogen

✓ Hydrogen

Definitions

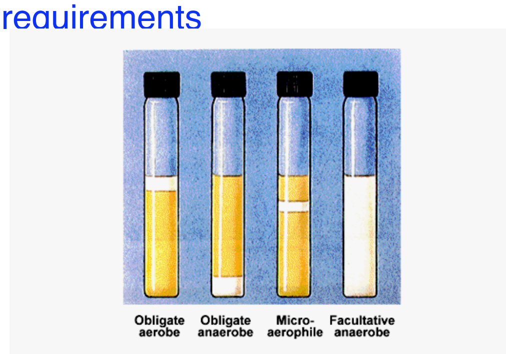
✓ Obligate

- Must have the specified environmental conditions
  - Obligate anaerobe *Bacterioides* killed by the presence of oxygen

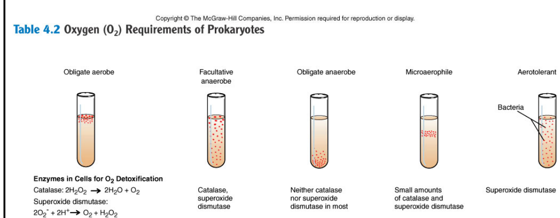
✓ Facultative

- The organism is able to adjust to and tolerate the environmental conditions, but it can live in other conditions
  - Facultative anaerobe *E. coli*

Microbes have different O2 requirements



Oxygen requirements



Aerobes

✓ 21% oxygen

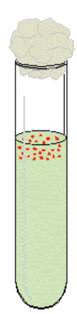
✓ efficient energy producers

✓ some require 5-10% CO_2

- *Mycobacterium tuberculosis*

✓ Obligate aerobes

- *Psuedomonas* spp.

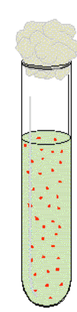


Facultative anaerobes

✓ Can grow in air with O_2 and without O_2.

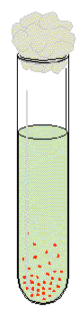
✓ In anaerobic conditions these organisms ferment carbon sources to produce energy.

✓ *E.coli*

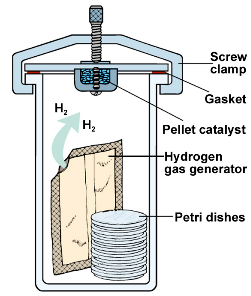


Anaerobes

- ✓ Oxygen may be toxic.
- ✓ Enzymes detoxify O_2 and O_2-related molecules.
- ✓ Grow well in atmosphere containing CO_2 and H_2.
- ✓ *Clostridium sp.*

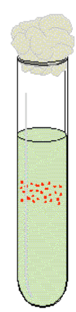


Anaerobes



Microaerophiles

- ✓ Cannot withstand 21% O_2-- prefer 1-15%.
- ✓ *Campylobacter jejuni*



Water availability

- ✓ Most single celled organisms live a few hours without moisture
  - Exceptions are spores and spore-forming organisms

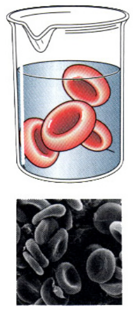
Hydrostatic Pressure

- ✓ Barophiles are pressure-dependent microorganisms.
  - Live at different water depths
    - 50 meters of water = 32X atmospheric pressure
    - Pressure keeps their enzyme together and functional

Osmosis

- ✓ Movement of water across a selectively permeable membrane in response to a concentration gradient.
- ✓ Movement of water is towards the zone of high solute concentration.
- ✓ The pressure associated with the movement of water is called osmotic pressure.

Isotonic

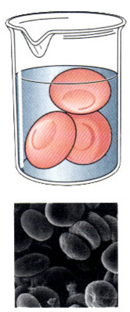


- ✓ Solute concentration is the same on both sides of the plasma membrane.
- ✓ Movement of water is equal in both directions.

Hypertonic

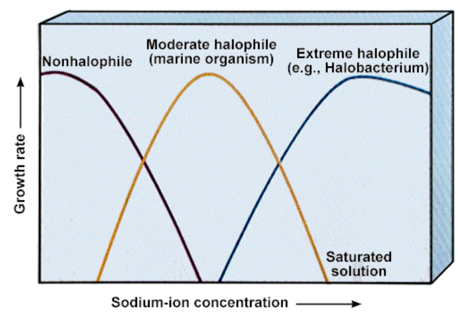
- ✓ Solute concentration is greater on the outside of cell.
- ✓ Movement of water is towards the greater solute concentration.
- ✓ Water moves OUT of the cell.

Hypotonic



- ✓ Solute concentration is greater on the inside of cell.
- ✓ Movement of water is towards the greater solute concentration.
- ✓ Water moves INTO the cell.

Halophile (salt-loving)



Organisms require a variety of chemical elements as nutrients?

Table 4.3 Representative Functions of the Major Elements

Chemical	Function
Carbon, oxygen, and hydrogen	Component of cellular constituents including amino acids, lipids, nucleic acids, and sugars.
Nitrogen	Component of amino acids and nucleic acids.
Sulfur	Component of some amino acids.
Phosphorus	Component of nucleic acids, membrane lipids, and ATP.
Potassium, magnesium, and calcium	Required for the functioning of certain enzymes; additional functions as well.
Iron	Part of certain enzymes.

The Chemical Composition of the Cell

The Chemical Composition of the Cell

90% water

10% macromolecules

inorganic molecules

biosynthetic precursors

Nutritional Factors on Growth

- ✓ Required elements
  - Major elements
    - Carbon (C), oxygen (O), hydrogen (H), nitrogen (N), sulfur (S), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca) and iron (Fe)
      - Essential components for macromolecules
  - Organisms classified based on carbon usage
    - Heterotrophs
      - Use organism carbon as nutrient source
    - Autotrophs
      - Use inorganic carbon (CO_2) as carbon source
  - Trace elements
    - Cobalt (Co), zinc (Zn), copper (Cu), molybdenum (Mo) and manganese (Mn)
      - Required in minute amounts

Nutritional Factors on Growth

- ✓ Growth factors
  - Some bacteria cannot synthesize some cell constituents
    - These must be added to growth environment
      - Referred to as growth factors
  - Some need very few while others require many
      - These termed fastidious

Nutritional Types of Microorganisms

Table 5.1 Sources of Carbon, Energy, and Electrons

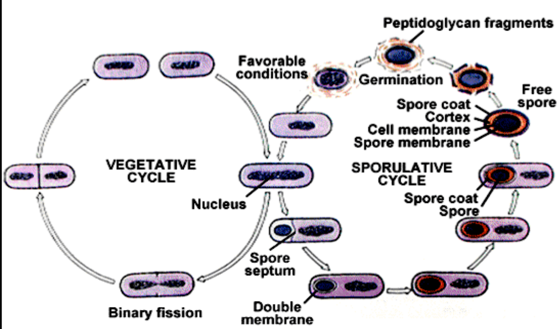
Carbon Sources	
Autotrophs	CO_2 sole or principal biosynthetic carbon source (*pp. 207–8*)[a]
Heterotrophs	Reduced, preformed, organic molecules from other organisms (*chapters 9 and 10*)
Energy Sources	
Phototrophs	Light (*pp. 195–201*)
Chemotrophs	Oxidation of organic or inorganic compounds (*chapter 9*)
Electron Sources	
Lithotrophs	Reduced inorganic molecules (*pp. 193–94*)
Organotrophs	Organic molecules (*chapter 9*)

[a]For each category, the location of material describing the participating metabolic pathways is given within the parentheses.

Carbon and Energy Sources

	carbon source	energy source
chemoautotrophs	CO_2	inorganic molecules
chemoheterotrophs	organic molecules	organic molecules
photoautotrophs	CO_2	light
photoheterotrophs	organic molecules	light
Chemolithoautotroph	CO2	inorganic (rocks)
Chemoorganoheterotrophs	organic	organic

Sporulation



Culture Media

broth

- a liquid culture medium

agar

- solid, gelled medium contained in Petri dishes or in tubes as slants.
- typical concentration of 15 grams/liter
- melts at 95-100ºC, gels at 42 -45ºC

Complex media

- ✓ Used routinely for growing most microorganisms.
- ✓ Contain plant and animal extracts.
- ✓ Are usually chemically undefined

Example of Complex Medium

- ✓ Luria Broth
  - In 1000 milliliters of water:
  - 10 g tryptone
  - 5 g yeast extract
  - 10 g sodium chloride

Defined or synthetic media

- ✓ Defined or synthetic media
- ✓ Contain precise quantities of organic and inorganic nutrients.

Example of Defined Medium

Leuconostoc mesenteroides medium

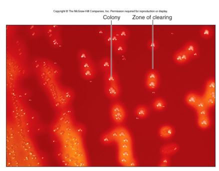
25 g glucose	10mg $FeSO_4$
3g NH4Cl	20mg $MnSO_4$
0.6g $KH2PO_4$	10mg NaCl
0.6g $K2HPO_4$	amino acids
0.2g $MgSO_4$	vitamins

purines and pyrimidines

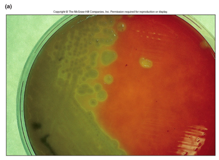
Special Classes of Media

These are subsets of both complex and defined media.

- ✓ selective media
  - Inhibit some while allowing some to grow
    - Thayer-Martin agar
      - For isolation of *Neisseria gonorrhoeae*
    - EMB agar
      - Isolation of Gram Negative
- ✓ differential media
  - Distinguish between two different kind of bacteria
    - Blood agar
      - Hemolysis - break down of RBC
    - EMB
      - Differentiate E. coli from other Gram negatives
- ✓ enrichment media



(a)



(b)

Anaerobe media

- ✓ Some microbes can tolerate either little or no oxygen.
- ✓ Growth can occur in agar-deeps, or in medium containing reducing compounds that limit the amount of oxygen in the medium.
- ✓ Contain thioglycolate (captures oxygen)