

## Chapter 4

### Growth and Culturing of Bacteria

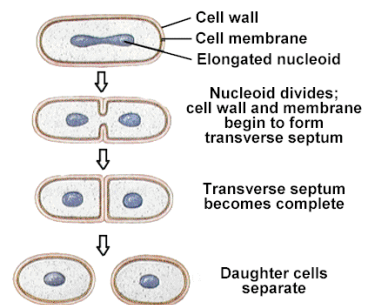
### Growth of Bacteria

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

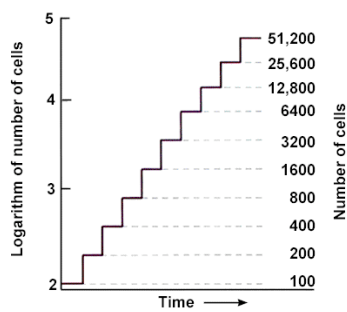
### Types of reproduction

1. binary fission
2. budding
3. fragmentation

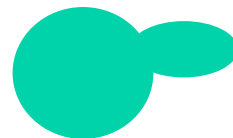
### Binary Fission



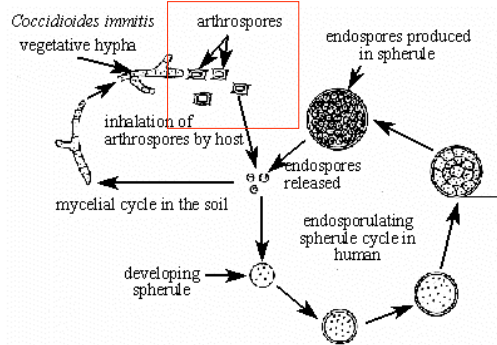
### Binary fission leads to log growth



### Budding



## Fragmentation

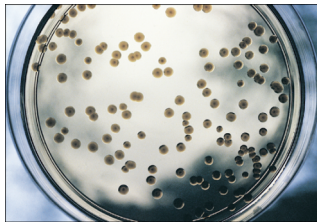


## Key terms

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

## Cultures

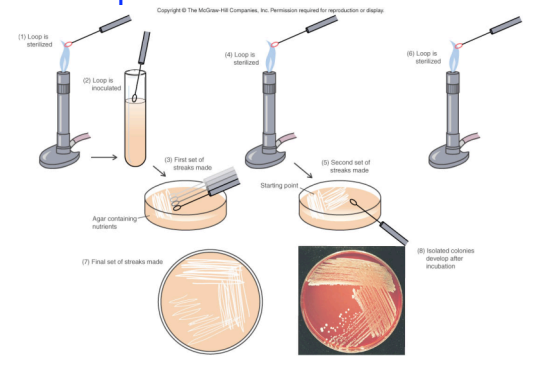
- ✓ Media
  - Liquid is broth
  - Solid is broth with agar added
  - Slant or Petri dish
- ✓ Agar
  - Polysaccharide extracted from red algae
    - ↳ Liquid above 95-100°C
    - ↳ Solid at 42-45°C
- ✓ Colony
  - A visible growth of bacteria that arose from a single cell
- ✓ CFU (Colony Forming Unit)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



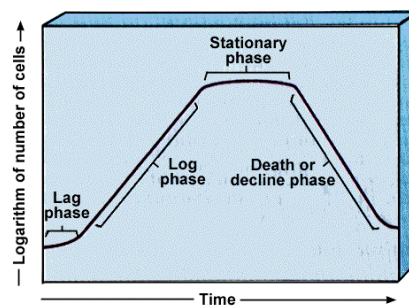
## Obtaining a pure culture

- ✓ 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

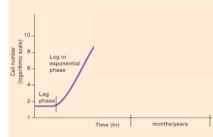
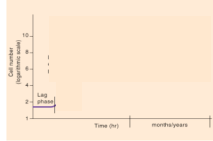


## Standard bacterial growth curve



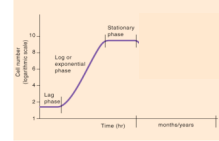
## Bacterial Growth in Laboratory Conditions

- ✓ 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
    - Maximum 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

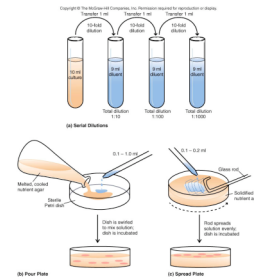


## Measuring growth

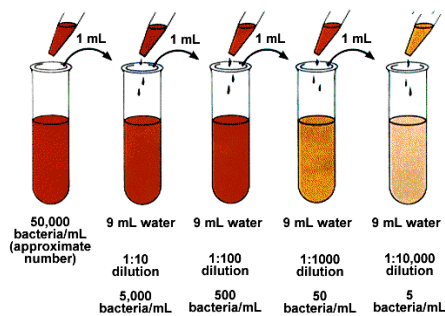
1. Serial dilution and **standard plate count (Viable)**
  - a. pour plates
  - b. spread plates
2. **Direct Microscopic** count
  - a. counting chamber
  - b. Cell counting instruments
    - a. Electronic cell count
    - b. Flow cytometry
4. Most probable number (MPN)
5. Filtration
6. Turbidity
6. Other methods

## Plate count/serial dilution

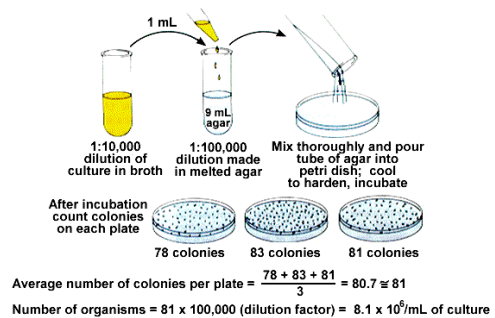
1. Relatively easy to do.
2. Some inaccuracies in counting colonies.
3. Risk of contamination.
4. Slow.



## Serial Dilutions



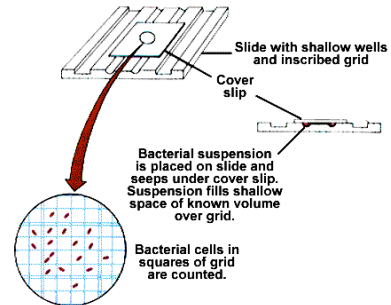
## Pour plate method



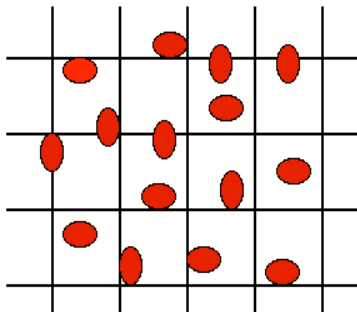
## Microscopic count

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

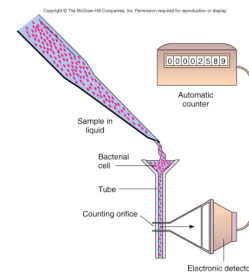


## Microscopic count

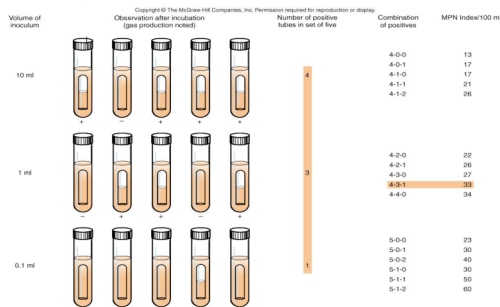


## Electronic cell count

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



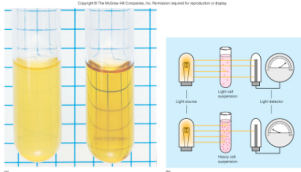
## Membrane filter

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

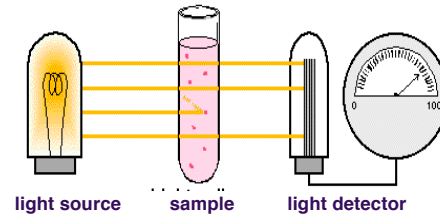


## Turbidity

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

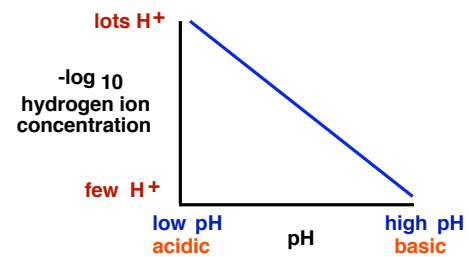
## Factors Affecting Bacterial Growth

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

## pH

- ✓ pH is a measure of the concentration of hydrogen ions (H<sup>+</sup>) in a solution.
- ✓ Actually...

$$\text{pH} = -\log[\text{H}^+]$$



## pH classification

- ✓ Acidophiles (acid-loving)
  - pH 0.1 to 5.4 (*Lactobacillus*)
    - Maintain neutral pH by pumping out protons (H<sup>+</sup>)
- ✓ 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)

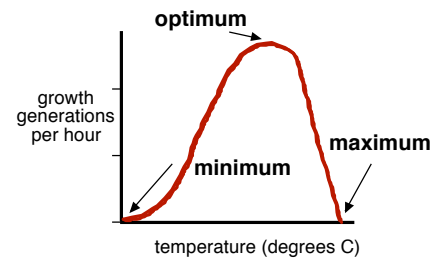
## pH parameters

- ✓ Intracellular pH of cell = ~7.5
- ✓ maintains pH by expelling or bringing H<sup>+</sup> 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     |

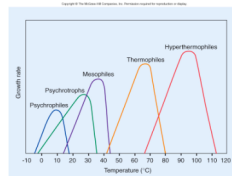
## Temperature ranges



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

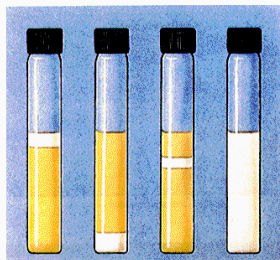
## Oxygen requirement

- ✓ 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*

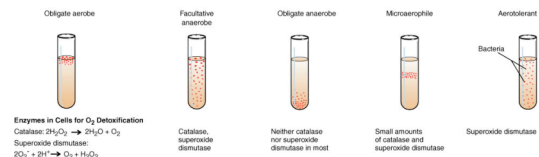
## Microbes have different O<sub>2</sub> requirements



Obligate aerobe    Obligate anaerobe    Microaerophile    Facultative anaerobe

## Oxygen requirements

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Table 4.2 Oxygen (O<sub>2</sub>) Requirements of Prokaryotes



Enzymes in Cells for O<sub>2</sub> Detoxification  
Catalase:  $2H_2O_2 \rightarrow 2H_2O + O_2$   
Superoxide dismutase:  
 $2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$

Catalase, superoxide dismutase

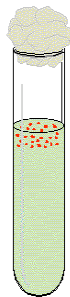
Neither catalase nor superoxide dismutase in most

Small amounts of catalase and superoxide dismutase

Superoxide dismutase

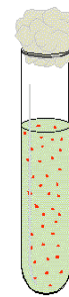
## Aerobes

- ✓ 21% oxygen
- ✓ efficient energy producers
- ✓ some require 5-10% CO<sub>2</sub>
  - *Mycobacterium tuberculosis*
- ✓ Obligate aerobes
  - *Pseudomonas* spp.



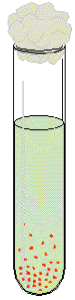
## Facultative anaerobes

- ✓ Can grow in air with O<sub>2</sub> and without O<sub>2</sub>.
- ✓ In anaerobic conditions these organisms ferment carbon sources to produce energy.
- ✓ *E. coli*

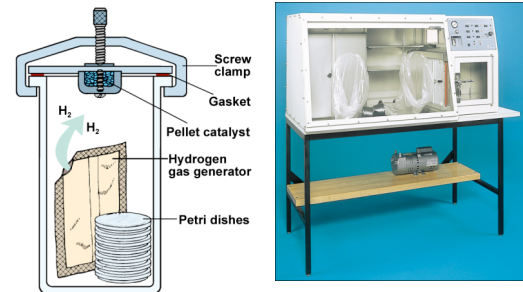


## Anaerobes

- ✓ 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.*

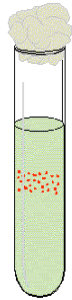


## Anaerobes



## 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

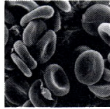
## Hydrostatic Pressure

- ✓ **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

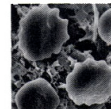
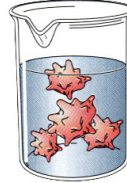
- ✓ Movement of water across a selectively permeable membrane in response to a concentration gradient.
- ✓ Movement of water is towards the zone of high solute concentration.
- ✓ The pressure associated with the movement of water is called osmotic pressure.

## Isotonic



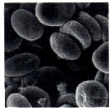
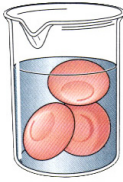
- ✓ Solute concentration is the same on both sides of the plasma membrane.
- ✓ Movement of water is equal in both directions.

## Hypertonic



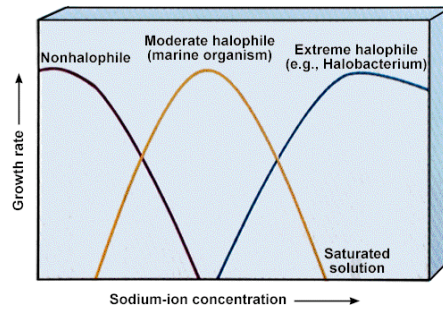
- ✓ Solute concentration is greater on the outside of cell.
- ✓ Movement of water is towards the greater solute concentration.
- ✓ Water moves OUT of the cell.

## 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<sub>2</sub>) 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

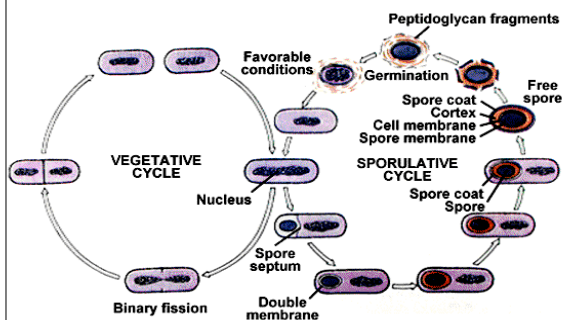
Carbon Sources	
Autotrophs	CO <sub>2</sub> sole or principal biosynthetic carbon source (pp. 207–8) <sup>a</sup>
Heterotrophs	Reduced, preformed, organic molecules from other organisms (chapters 9 and 10)
Energy Sources	
Phototrophs	Light (pp. 195–201)
Chemotrophs	Oxidation of organic or inorganic compounds (chapter 9)
Electron Sources	
Lithotrophs	Reduced inorganic molecules (pp. 193–94)
Organotrophs	Organic molecules (chapter 9)

<sup>a</sup>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 <sub>2</sub>	inorganic molecules
chemoheterotrophs	organic molecules	organic molecules
photoautotrophs	CO <sub>2</sub>	light
photoheterotrophs	organic molecules	light
Chemolithoautotroph	CO <sub>2</sub>	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 95-100°C, gels at 42 -45°C

## 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.

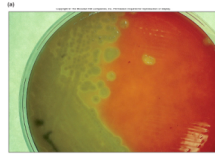
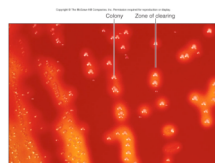
## Example of Defined Medium

- Leuconostoc mesenteroides medium
- |                                      |                        |
|--------------------------------------|------------------------|
| 25 g glucose                         | 10mg FeSO <sub>4</sub> |
| 3g NH <sub>4</sub> Cl                | 20mg MnSO <sub>4</sub> |
| 0.6g KH <sub>2</sub> PO <sub>4</sub> | 10mg NaCl              |
| 0.6g K <sub>2</sub> HPO <sub>4</sub> | amino acids            |
| 0.2g MgSO <sub>4</sub>               | 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



## 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)