Definitions

- **Microbial growth** – an increase in number of cells (microbes), not in cell size
- _________ – an aggregation of cells arising from single parent cell
- Metabolism gives the cell the __________ for reproduction
- Reproduction results in growth
Growth Requirements: Physical

- Physical Requirements
  - Temperature
  - pH
  - ___________ Pressure
  - Hydrostatic Pressure
Temperature

• Effect of temperature on proteins

• Effect of temperature on lipid-containing membranes of cells and organelles
  • If too low, membranes become ________ and fragile
  • If too high, membranes become too fluid and cannot contain the cell or organelle
Figure 6.4

Effects of Temperature on Growth

Minimum growth temperature
Optimum growth temperature
Maximum growth temperature
Effects of Temperature on Growth

Figure 6.4
Catagories of Microbes Based on Temperature Range

Figure 6.5
Psychrophiles

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

Temperatures in this range destroy most microbes, although lower temperatures take more time.

Very slow bacterial growth.

Rapid growth of bacteria; some may produce toxins.

Many bacteria survive; some may grow.
Refrigerator temperatures; may allow slow growth of spoilage bacteria, very few pathogens.
No significant growth below freezing.
The Requirements for Growth: Physical Requirements

- pH
  - $\text{H}^+$ and $\text{OH}^-$ interfere with H bonding in proteins and nucleic acids
  - Most bacteria & protozoa grow between pH 6.5 & 7.5
    - ____________
  - **Acidophiles** grow in acidic environments
    - Molds and yeasts grow between pH 5 and 6
  - **Alkaliphiles** live in basic environments (soils & water) pH 9 to pH 11.5
The Requirements for Growth: Physical Requirements

• **Water Requirements**

• **Osmotic Pressure** – Pressure exerted on a semi-permeable membrane by a solution containing solutes that cannot freely cross membrane.
  
  • related to concentration of dissolved molecules and ions in a solution

• Metabolic reactions take place in water

• Most cells die in absence of water
The Requirements for Growth: Physical Requirements

Figure 6.4
**Physical Effects of Water**

- **Hypertonic environments** - [solute] higher or lower?
  - increased salt or sugar $\rightarrow$ plasmolysis
  - Extreme or *obligate* ____________ require high osmotic pressure – up to 30% salt
    - *Facultative* halophiles tolerate high osmotic pressure

- **Hypotonic solutions** - lower solute concentrations
  - cells will swell and burst
Hydrostatic Pressure

• Water exerts pressure in proportion to its depth
  • For every addition of ______, water pressure increases 1 atm

• Organisms that live under extreme pressure are barophiles
  • Their membranes and enzymes depend on this pressure to maintain their three-dimensional, functional shape
Growth Requirements – Chemical Requirements

- Nutrients for
  - energy needs
  - to build ________ molecules
  - to build cellular structures
- Most common nutrients have the following elements –
  - ________________________________?
- Microbes obtain nutrients from variety of sources
The Requirements for Growth: Chemical Requirements

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

- **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
The Requirements for Growth: Chemical Requirements

- **Sulfur**
  - Some amino acids - disulfide bonds (protein ____ structure level?)
  - In vitamins - thiamine, biotin
  - Most bacteria decompose proteins
  - Some bacteria use $\text{SO}_4^{2-}$ or $\text{H}_2\text{S}$

- **Phosphorus**
  - In ____________, ATP, and phospholipid membranes
  - $\text{PO}_4^{3-}$ is a source of phosphorus
The Requirements for Growth: Chemical Requirements

- **Trace Elements**
  - Inorganic elements required in small amounts
  - Usually as enzyme cofactors

- **Growth factors** – organic chemicals that cannot be synthesized by certain organisms
Sources of Carbon & Energy

- Organisms categorized into **two groups** based on source of carbon
  - **Autotrophs** - Those using an *inorganic* carbon source (carbon dioxide).
  - **Heterotrophs** - Those catabolizing reduced *organic* carbon molecules (proteins, carbohydrates, amino acids, and fatty acids).
Organisms categorized into two groups based on whether they use chemicals or light as source of energy

- **Chemotrophs** - Those that acquire energy from redox reactions involving inorganic and organic chemicals.

- **Phototrophs** - Those that use light as their energy source.
# Four Basic Groups of Organisms

<table>
<thead>
<tr>
<th>Carbon Source</th>
<th>Energy Source</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Light (photo-)</strong></td>
<td><strong>Chemical compounds (chemo-)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Carbon dioxide (auto-)</strong></td>
<td><strong>Photoautotrophs</strong></td>
<td><strong>Chemoautotrophs</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Plants, algae, and cyanobacteria use H₂O to reduce CO₂, producing O₂ as a byproduct</td>
<td>- Hydrogen, sulfur, and nitrifying bacteria</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Photosynthetic green sulfur and purple sulfur bacteria do not use H₂O nor produce O₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organic compounds (hetero-)</td>
<td><strong>Photoheterotrophs</strong></td>
<td><strong>Chemoheterotrophs</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Green nonsulfur and purple nonsulfur bacteria</td>
<td>- Aerobic respiration: most animals, fungi, and protozoa, and many bacteria</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Anaerobic respiration: some animals and bacteria</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Fermentation: some bacteria and yeasts</td>
<td></td>
</tr>
</tbody>
</table>
Oxygen Requirements

- Organisms need and tolerate oxygen to different degrees
- Because of this they are classified into the following groups:
  - **Obligate Aerobes** – require oxygen → undergo aerobic respiration
  - **Microaerophiles** – aerobes that require lower levels of oxygen
    - levels from 2-10%
    - have a limited ability to detoxify hydrogen peroxide and superoxide radicals
  - **Facultative Anaerobes** – can maintain life via fermentation or anaerobic respiration or by aerobic respiration
  - **Aerotolerant anaerobes** – do not use aerobic metabolism but have some enzymes that detoxify oxygen’s poisonous forms
  - **Obligate Anaerobes** – do not use aerobic metabolism → oxygen is toxic to them
The Requirements for Growth: Chemical Requirements

- **Oxygen** \( (O_2) \)

<table>
<thead>
<tr>
<th>obligate aerobes</th>
<th>Faultative anaerobes</th>
<th>Obligate anaerobes</th>
<th>Aerotolerant anaerobes</th>
<th>Microaerophiles</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Copyright © 2004 Pearson Education, Inc. publishing as Benjamin Cummings
Oxygen Requirements

• Oxygen is **essential** for obligate aerobes (final electron acceptor in ETC)

• Oxygen is **deadly** for obligate anaerobes

• How can this be true?
  
  • Gaseous \( \text{O}_2 \) and oxygen covalently bound in compounds is not poisonous

  • The forms of oxygen that are ________ are excellent oxidizing agents

    • Resulting chain of oxidations causes irreparable damage to cells by oxidizing compounds such as proteins and lipids
Toxic Forms of Oxygen

• **Singlet oxygen**: $O_2$ with electrons boosted to a higher-energy state
  - Occurs during photosynthesis $\rightarrow$ carotenoids

• **Superoxide free radicals**: $O_2^-$
  - formed during incomplete reduction of oxygen in aerobic and anaerobic respiration
  - So reactive that _________ produce superoxide dismutases to detoxify them
  - Anaerobes lack superoxide dismutase $\rightarrow$ die in the presence of oxygen

\[
O_2^- + 2H^+ \xrightarrow{\text{superoxide dismutase}} H_2O_2 + O_2
\]
Toxic Forms of Oxygen

• Peroxide anion: \( O_2^{2-} \)
  • formed during reactions catalyzed by superoxide dismutase and other reactions
  • Aerobes contain either __________ or peroxidase to detoxify peroxide anion
  • Obligate anaerobes??

Where does this occur in an Eukaryotic cell?
Toxic Forms of Oxygen

• Hydroxyl radical (OH•)
  • results from *ionizing radiation* and from incomplete reduction of hydrogen peroxide
  • The __________ of the four toxic forms of oxygen
  • Not a threat to aerobes due to action of catalase and peroxidase
• Vitamins for aerobes
Nitrogen Requirements

• Anabolism often ceases due to insufficient nitrogen
  • Nitrogen is needed for proteins and nucleotides

• Nitrogen acquired from organic and inorganic nutrients
  • all cells recycle nitrogen from amino acids & nucleotides
  • In what subunit of a nucleotide would you find nitrogen?

• Nitrogen fixation
  • $\text{N}_2 \rightarrow \text{ammonia}$ (reduction)
    • Makes nitrogen available in a usable form
  • Preformed by certain bacteria
  • Essential to life on Earth.
Culturing Microorganisms

- **Culture** - Microbes growing in/on culture medium
- **Inoculum** - Introduction of microbes into medium
- **Culture Medium** - Nutrients prepared for microbial growth
- **Sterile** - _______________
Obtaining Pure Cultures

- **Cultures** composed of cells arising from a single progenitor
- A **pure culture** contains only one species or strain
- **Colony** -
  - A colony is often called a colony-forming unit (CFU)
- **Aseptic technique** - used to prevent contamination of sterile substances or objects
  - And __________?
- Two common isolation techniques
  - **Streak Plates**
  - **Pour Plates**
Streak Plate Method

Quadrant Streak Plate
Streak Plate Method
Streak Plate
Pour Plate Method

Sequential inoculations

Initial sample

1.0 ml
9 ml broth
9 ml broth
9 ml broth

1.0 ml to each petri dish, add 9 ml warm agar, swirl gently to mix

Sub-surface colonies
Fewer colonies

Copyright © 2004 Pearson Education, Inc. publishing as Benjamin Cummings
Pour Plate Method

Figure 6.9
# Clinical Sampling

## Table 6.3: Clinical Specimens and the Methods Used to Collect Them

<table>
<thead>
<tr>
<th>Type or Location of Specimen</th>
<th>Collection Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin, accessible mucous membrane</td>
<td>Sterile swab brushed across the surface; care should be taken not to contact neighboring tissues</td>
</tr>
<tr>
<td>(including eye, outer ear, nose, throat, vagina, cervix, urethra) or open wounds</td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>Needle aspiration from vein, anticoagulants are included in the specimen transfer tube</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>Needle aspiration from subarachnoid space of spinal column</td>
</tr>
<tr>
<td>Stomach</td>
<td>Intubation, which involves inserting a tube into the stomach, often via a nostril</td>
</tr>
<tr>
<td>Urine</td>
<td>In aseptic collection, a catheter is inserted into the bladder through the urethra; in the “clean catch” method, initial urination washes the urethra, and the specimen is midstream urine</td>
</tr>
<tr>
<td>Lungs</td>
<td>Collection of sputum either dislodged by coughing or acquired via a catheter</td>
</tr>
<tr>
<td>Diseased tissue</td>
<td>Surgical removal (biopsy)</td>
</tr>
</tbody>
</table>
Culture Media

- **Chemically Defined Media**: Exact chemical composition is known

- **Complex Media**: Exact chemical composition of some part are known while others are unknown (extracts of yeasts, meat, or plants)
  - Nutrient broth
  - Nutrient agar
## Table 6.2: A Chemically Defined Medium for Growing a Typical Chemoheterotroph, Such as *E. coli*

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Ammonium phosphate, monobasic (NH₄H₂PO₄)</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Sodium chloride (NaCl)</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Magnesium sulfate (MgSO₄ · 7H₂O)</td>
<td>0.2 g</td>
</tr>
<tr>
<td>Potassium phosphate, dibasic (K₂HPO₄)</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Water</td>
<td>1 liter</td>
</tr>
</tbody>
</table>

## Table 6.4: Composition of Nutrient Agar, a Complex Medium for the Growth of Heterotrophic Bacteria

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone (partially digested protein)</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Beef extract</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>8.0 g</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0 g</td>
</tr>
<tr>
<td>Water</td>
<td>1 liter</td>
</tr>
</tbody>
</table>
Agar

- Complex polysaccharide
- Used as solidifying agent
  - in Petri plates, slants, and deeps
- Generally not metabolized by microbes
- Liquefies at 100°C
- Solidifies ~40°C

What was used before agar?
Culture Media

• Types of general culture media
  • Defined media
  • Complex media
  • Selective media
  • Differential media
  • Enriched Media
  • Anaerobic media
Selective Media

- Selects _______ the growth of some microbes while selecting for the growth of other microbes.
Differential Media

- Make it easy to distinguish colonies of different microbes.
  Allows for differentiation between different types of microbes (usually based on color)
Enrichment Media

- Encourages growth of desired microbe
- These are usually fastidious bacteria

Chocolate agar
Anaerobic Culture Methods

- Anaerobic jar
Anaerobic Culture Methods

• Reducing media
  • Contain chemicals (thioglycollate or oxyrase) that combine free $O_2$
  • Heated to drive off $O_2$
Anaerobic Culture Methods

- Anaerobic chamber

Figure 6.6
Capnophiles require high CO₂

- Candle jar

- CO₂-packet
Preserving Cultures

• Refrigeration:

• Freezing:

• Deep-freezing: -70° to -95°C

• Lyophilization (freeze-drying): Frozen (-54° to -72°C) and dehydrated in a vacuum
Reproduction of Microbes

- Prokaryotes
  - Binary fission
- Eukaryotes
  - Sexual and Asexual (mitosis, meiosis, budding, conidiospores, fragmentation of filaments, etc.)
Binary Fission

1. Cell elongates and DNA is replicated
   - Cell wall
   - Plasma membrane
   - DNA (nuclear area)

2. Cell wall and plasma membrane begin to divide
   - Cell wall
   - Partially formed cross-wall
   - DNA (nuclear area)

3. Cross-wall forms completely around divided DNA

4. Cells separate

(a) A diagram of the sequence of cell division.

(b) A thin section of a cell of *Bacillus licheniformis* starting to divide.
Growth of Microbial Populations
Growth of Microbial Populations

Figure 6.17

1. Cytoplasmic membrane
2. Replicated chromosome
3. Septum
4. Completed septum
5. Replicated chromosome

30 minutes
60 minutes
90 minutes
120 minutes
Arithmetic Versus Logarithmic Growth

(a) Species A

(b) Species B

Figure 6.18
<table>
<thead>
<tr>
<th>Generation Number</th>
<th>Number of Cells</th>
<th>$\log_{10}$ of Number of Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>5 ($2^5$) = 32</td>
<td>1.51</td>
<td></td>
</tr>
<tr>
<td>10 ($2^{10}$) = 1,024</td>
<td>3.01</td>
<td></td>
</tr>
<tr>
<td>15 ($2^{15}$) = 32,768</td>
<td>4.52</td>
<td></td>
</tr>
<tr>
<td>16 ($2^{16}$) = 65,536</td>
<td>4.82</td>
<td></td>
</tr>
<tr>
<td>17 ($2^{17}$) = 131,072</td>
<td>5.12</td>
<td></td>
</tr>
<tr>
<td>18 ($2^{18}$) = 262,144</td>
<td>5.42</td>
<td></td>
</tr>
<tr>
<td>19 ($2^{19}$) = 524,288</td>
<td>5.72</td>
<td></td>
</tr>
<tr>
<td>20 ($2^{20}$) = 1,048,576</td>
<td>6.02</td>
<td></td>
</tr>
</tbody>
</table>
Phases of Microbial Growth

<table>
<thead>
<tr>
<th>Generation Number</th>
<th>Number of Cells</th>
<th>Log_{10} of Number of Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>5 (2^0)</td>
<td>32</td>
<td>1.51</td>
</tr>
<tr>
<td>10 (2^1)</td>
<td>1,024</td>
<td>3.01</td>
</tr>
<tr>
<td>15 (2^2)</td>
<td>32,768</td>
<td>4.52</td>
</tr>
<tr>
<td>16 (2^4)</td>
<td>65,536</td>
<td>4.82</td>
</tr>
<tr>
<td>17 (2^4)</td>
<td>131,072</td>
<td>5.12</td>
</tr>
<tr>
<td>18 (2^5)</td>
<td>262,144</td>
<td>5.42</td>
</tr>
<tr>
<td>19 (2^5)</td>
<td>524,288</td>
<td>5.72</td>
</tr>
<tr>
<td>20 (2^6)</td>
<td>1,048,576</td>
<td>6.02</td>
</tr>
</tbody>
</table>

Log, or exponential growth, phase

Stationary phase

Death, or logarithmic decline, phase

Lag phase
Measuring Microbial Growth

• Direct Methods
  • Microscopic counts
  • (Viable) Plate Counts
  • Membrane __________
  • Electronic Counters
  • Most Probable Number
**Direct Measurements of Microbial Growth**

Direct Microscope Count

1. Bacterial suspension is added here and fills the shallow volume over the squares by capillary action.

2. Microscopic count: All cells in several large squares are counted, and the numbers are averaged. The large square shown here has 14 bacterial cells.

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

\[
\frac{14}{8 \times 10^{-7}} = 17,500,000
\]
Direct Measurements of Microbial Growth

- Plate Counts: Perform serial dilutions of a sample

![Diagram of serial dilutions process](Figure 6.15, top portion)
Plate Count

- Inoculate Petri plates from serial dilutions

Figure 6.16
Plate Count

- After incubation, count colonies on plates that have 25-250 colonies (CFUs)

![Diagram of plate count process with dilutions and plating](image)
Membrane Filtration

Sample to be filtered

Membrane filter retains cells

To vacuum

Membrane transferred to culture medium

Incubation

Colonies

Figure 6.22a
Direct Measurements of Microbial Growth

• Filtration
**Most Probable Number**

Inoculate 1.0 ml into each of 5 tubes.

- Phenol red, pH color indicator, added.
- Incubate.

**Results**
- 4 tubes positive
- 2 tubes positive
- 1 tube positive
Measuring Microbial Growth

- Indirect Methods
  - Metabolic Activity
  - Dry Weight
  - Turbidity
Turbidity and Spectrophotometric Measurement
Turbidity and Spectrophotometric Measurement

![Image of spectrophotometer with labeled components: light source, uninoculated tube, light-sensitive detector, spectrophotometer, inoculated broth culture, scattered light that does not reach reflector.]