

Chapter 6: Microbial Growth

Microbial Growth:

- ✓ Refers to an increase in **cell number**, not in cell size.
- ✓ Bacteria grow and divide by **binary fission**, a rapid and relatively simple process.

Requirements for Growth

Physical Requirements

1. **Temperature:** Microbes are *loosely* classified into several groups based on their preferred temperature ranges.

A. **Psychrophiles:** “Cold-loving”. Can grow at 0°C. Two groups:

- ◆ **True Psychrophiles:** Sensitive to temperatures over 20°C. Optimum growth at 15°C or below. Found in very cold environments (North pole, ocean depths). Seldom cause disease or food spoilage.
- ◆ **Psychrotrophs:** Optimum growth at 20 to 30°C. Responsible for most low temperature food spoilage.

Requirements for Growth

Physical Requirements

1. **Temperature:**

B. **Mesophiles:** “Middle loving”. Most bacteria.

- ◆ Include most pathogens and common spoilage organisms.
- ◆ Best growth between 25 to 40°C.
- ◆ Optimum temperature commonly 37°C.
- ◆ Many have adapted to live in the bodies of animals.

Requirements for Growth

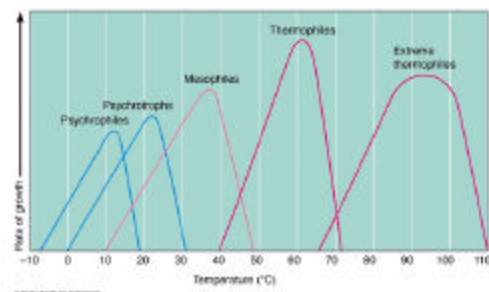
Physical Requirements

1. **Temperature:**

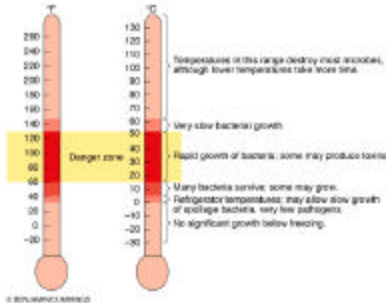
C. **Thermophiles:** “Heat loving”.

- ◆ Optimum growth between 50 to 60°C.
- ◆ Many cannot grow below 45°C.
- ◆ Adapted to live in sunlit soil, compost piles, and hot springs.
- ◆ Some thermophiles form extremely heat resistant endospores.
- ◆ **Extreme Thermophiles (Hyperthermophiles):** Optimum growth at 80°C or higher. Archaeobacteria. Most live in volcanic and ocean vents.

Growth Rates of Bacterial Groups at Different Temperatures



Food Spoilage Temperatures



Requirements for Growth

Physical Requirements

2. pH:

- ✓ Most **bacteria** prefer neutral pH (6.5-7.5).
- ✓ **Molds** and **yeast** grow in wider pH range, but prefer pH between 5 and 6.
- ✓ **Acidity** inhibits most microbial growth and is used frequently for food preservation (e.g.: pickling).
- ✓ **Alkalinity** inhibits microbial growth, but not commonly used for food preservation.
- ✓ Acidic products of bacterial metabolism interfere with growth. Buffers can be used to stabilize pH.

Requirements for Growth

Physical Requirements

2. pH: Organisms can be classified as:

A. **Acidophiles**: "Acid loving".

- ◆ Grow at very low pH (0.1 to 5.4)
- ◆ *Lactobacillus* produces lactic acid, tolerates mild acidity.

B. **Neutrophiles**:

- ◆ Grow at pH 5.4 to 8.5.
- ◆ Includes most human pathogens.

C. **Alkaliphiles**: "Alkali loving".

- ◆ Grow at alkaline or high pH (7 to 12 or higher)
- ◆ *Vibrio cholerae* and *Alkaligenes faecalis* optimal pH 9.
- ◆ Soil bacterium *Agrobacterium* grows at pH 12.

Requirements for Growth

Physical Requirements

3. **Osmotic Pressure**: Cells are 80 to 90% water.

A. **Hypertonic solutions**: High osmotic pressure removes water from cell, causing shrinkage of cell membrane (plasmolysis).

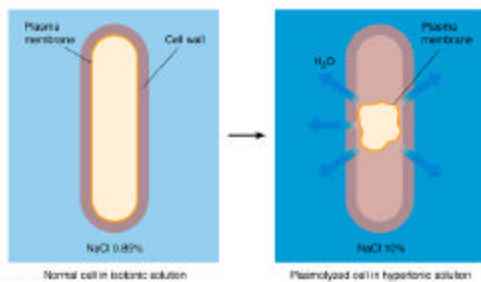
Used to control spoilage and microbial growth.

- ◆ Sugar in jelly.
- ◆ Salt on meat.

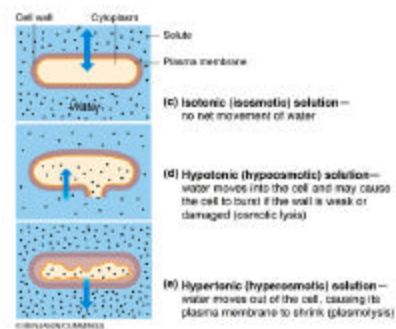
B. **Hypotonic solutions**: Low osmotic pressure causes water to enter the cell. In most cases cell wall prevents excessive entry of water. Microbe may lyse or burst if cell wall is weak.

Isotonic Versus Hypertonic Solution

Plasmolysis



Effects of Osmosis on Bacterial Cells



Requirements for Growth

Physical Requirements

3. Osmotic Pressure:

- ◆ **Halophiles**: Require moderate to large salt concentrations. Ocean water contains 3.5% salt.
 - ◆ Most bacteria in oceans.
- ◆ **Extreme or Obligate Halophiles**: Require very high salt concentrations (20 to 30%).
 - ◆ Bacteria in Dead Sea, brine vats.
- ◆ **Facultative Halophiles**: Do not require high salt concentrations for growth, but tolerate 2% salt or more.

Requirements for Growth

Chemical Requirements

1. Carbon: Makes up 50% of dry weight of cell.

- ◆ Structural backbone of all organic compounds.
- ◆ **Chemoheterotrophs**: Obtain carbon from their energy source: lipids, proteins, and carbohydrates.
- ◆ **Chemoautotrophs and Photoautotrophs**: Obtain carbon from carbon dioxide.

Requirements for Growth

Chemical Requirements

2. Nitrogen, Sulfur, and Phosphorus:

A. **Nitrogen**: Makes up 14% of dry cell weight. Used to form amino acids, DNA, and RNA.

Sources of nitrogen:

- ◆ **Protein**: Most bacteria
- ◆ **Ammonium**: Found in organic matter
- ◆ **Nitrogen gas (N₂)**: Obtain N directly from atmosphere. Important **nitrogen fixing bacteria**, live free in soil or associated with legumes (peas, beans, alfalfa, clover, etc.). Legume cultivation is used to fertilize soil naturally.
- ◆ **Nitrates**: Salts that dissociate to give NO₃⁻.

Requirements for Growth

Chemical Requirements

2. Nitrogen, Sulfur, and Phosphorus:

B. **Sulfur**: Used to form proteins and some vitamins (thiamin and biotin).

Sources of sulfur:

- ◆ **Protein**: Most bacteria
- ◆ **Hydrogen sulfide**
- ◆ **Sulfates**: Salts that dissociate to give SO₄²⁻.

C. **Phosphorus**: Used to form DNA, RNA, ATP, and phospholipids.

Sources: Mainly inorganic phosphate salts and buffers.

Requirements for Growth

Chemical Requirements

3. **Other Elements**: Potassium, magnesium, and calcium are often required as enzyme cofactors.

Calcium is required for cell wall synthesis in Gram positive bacteria.

4. Trace Elements:

Many are used as enzyme cofactors.

Commonly found in tap water.

- ◆ Iron
- ◆ Copper
- ◆ Molybdenum
- ◆ Zinc

Requirements for Growth

Chemical Requirements

5. **Oxygen**: Organisms that use molecular oxygen (O₂), produce more energy from nutrients than anaerobes.

Can classify microorganism based on their oxygen requirements:

A. **Obligate Aerobes**: Require oxygen to live.

Disadvantage: Oxygen dissolves poorly in water.

Example: *Pseudomonas*, common nosocomial pathogen.

Requirements for Growth

Chemical Requirements

5. Oxygen:

B. Facultative Anaerobes: Can use oxygen, but can grow in its absence. Have complex set of enzymes.
Examples: *E. coli*, *Staphylococcus*, yeasts, and many intestinal bacteria.

C. Obligate Anaerobes: Cannot use oxygen and are harmed by the presence of toxic forms of oxygen.
Examples: *Clostridium* bacteria that cause tetanus and botulism.

Requirements for Growth

Chemical Requirements

5. Oxygen:

D. Aerotolerant Anaerobes: Can't use oxygen, but tolerate its presence. Can break down toxic forms of oxygen.
Example: *Lactobacillus* carries out fermentation regardless of oxygen presence.

E. Microaerophiles: Require oxygen, but at low concentrations. Sensitive to toxic forms of oxygen.
Example: *Campylobacter*.

Requirements for Growth

Chemical Requirements

Toxic Forms of Oxygen:

- 1. Singlet Oxygen:** Extremely reactive form of oxygen, present in phagocytic cells.
- 2. Superoxide Free Radicals (O₂⁻):** Extremely toxic and reactive form of oxygen. All organisms growing in atmospheric oxygen must produce an enzyme **superoxide dismutase (SOD)**, to get rid of them. SOD is made by aerobes, facultative anaerobes, and aerotolerant anaerobes, **but not** by anaerobes or microaerophiles.
Reaction:

$$\underset{\text{Superoxide free radicals}}{O_2^{\cdot-}} + \underset{\text{Superoxide free radicals}}{O_2^{\cdot-}} + 2H^+ \xrightarrow{\text{SOD}} \underset{\text{Hydrogen peroxide}}{H_2O_2} + O_2$$

Requirements for Growth

Chemical Requirements

3. Hydrogen Peroxide (H₂O₂): Peroxide ion is toxic and the active ingredient of several antimicrobials (e.g.: benzoyl peroxide). There are two different enzymes that break down hydrogen peroxide:

A. Catalase: Breaks hydrogen peroxide into water and O₂. Common. Produced by humans, as well as many bacteria.

$$2 \underset{\text{Hydrogen peroxide}}{H_2O_2} \xrightarrow{\text{Catalase}} 2 \underset{\text{Water}}{H_2O} + \underset{\substack{\text{Gas} \\ \text{Bubbles}}}{O_2}$$

B. Peroxidase: Converts hydrogen peroxide into water.

$$\underset{\text{Hydrogen peroxide}}{H_2O_2} + 2H^+ \xrightarrow{\text{Peroxidase}} \underset{\text{Water}}{H_2O}$$

Microbial Growth

Culture Media

Culture Medium: Nutrient material prepared for microbial growth in the laboratory.

Requirements:

- ✓ Must be sterile
- ✓ Contain appropriate nutrients
- ✓ Must be incubated at appropriate temperature

Culture: Microbes that grow and multiply in or on a culture medium.

Microbial Growth

Culture Media

Solid Media: Nutrient material that contains a solidifying agent (plates, slants, deeps).
The most common solidifier is **agar**, first used by Robert Koch.

Unique Properties of Agar:

- ✓ Melts above 95°C.
- ✓ Once melted, does not solidify until it reaches 40°C.
- ✓ Cannot be degraded by most bacteria.
- ✓ Polysaccharide made by red algae.
- ✓ Originally used as food thickener (Angelina Hesse).

Microbial Growth

Culture Media

Chemically Defined Media: Nutrient material whose *exact* chemical composition is known.

- ✓ For chemoheterotrophs, must contain organic source of carbon and energy (e.g.: glucose, starch, etc.).
- ✓ May also contain amino acids, vitamins, and other important building blocks required by microbe.
- ✓ Not widely used.
- ✓ Expensive.

Microbial Growth

Culture Media

Complex Media: Nutrient material whose *exact* chemical composition is **not** known.

- ✓ Widely used for heterotrophic bacteria and fungi.
- ✓ Made of extracts from yeast, meat, plants, protein digests, etc.
- ✓ Composition may vary slightly from batch to batch.
- ✓ Energy, carbon, nitrogen, and sulfur requirements are primarily met by protein fragments (**peptones**).
- ✓ Vitamins and organic growth factors provided by meat and yeast extracts.
- ✓ Two forms of complex media:
 - **Nutrient broth:** Liquid media
 - **Nutrient agar:** Solid media

Microbial Growth

Culture Media

Anaerobic Growth Media: Used to grow anaerobes that might be killed by oxygen.

- ✓ Reducing media
- ✓ Contain ingredients that chemically combine with oxygen and remove it from the medium.
Example: Sodium thioglycolate
- ✓ Tubes are heated shortly before use to drive off oxygen.
- ✓ Plates must be grown in oxygen free containers (anaerobic chambers).

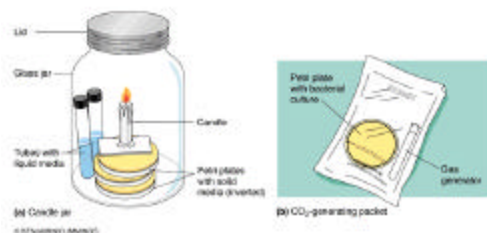
Microbial Growth

Culture Media

Special Culture Techniques: Used to grow bacteria with unusual growth requirements.

- ✓ **Bacteria that do not grow on artificial media:**
 - *Mycobacterium leprae* (leprosy): Grown in armadillos.
 - *Treponema pallidum* (syphilis): Grown in rabbit testicles.
 - Obligate intracellular bacteria (ricketsias and chlamydias): Only grow in host cells.
- ✓ **Bacteria that require high or low CO₂ levels:**
 - **Capnophiles:** Grow better at high CO₂ levels and low O₂ levels. Similar to environment of intestinal tract, respiratory tract, and other tissues.

Equipment for Producing CO₂ Rich Environments



Microbial Growth

Culture Media

Selective Media: Used to suppress the growth of unwanted bacteria and encourage the growth of desired microbes.

- ✓ **Saboraud's Dextrose Agar:** pH of 5.6 discourages bacterial growth. Used to isolate fungi.
- ✓ **Brilliant Green Agar:** Green dye selectively inhibits gram-positive bacteria. Used to isolate gram-negative *Salmonella*.
- ✓ **Bismuth Sulfite Agar:** Used to isolate *Salmonella typhi*. Inhibits growth of most other bacteria.

Microbial Growth

Culture Media

Differential Media: Used to *distinguish* colonies of a desired organism.

- ✓ **Blood Agar:** Used to distinguish bacteria that destroy red blood cells (**hemolysis**). Hemolysis appears as an area of clearing around colony.
Example: *Streptococcus pyogenes*.

Microbial Growth

Culture Media

Both Selective and Differential Media: Used both to *distinguish* colonies of a desired organism, and *inhibit* the growth of other microbes.

- ✓ **Mannitol Salt Agar:** Used to distinguish and select for *Staphylococcus aureus*.
 - High salt (7.5% NaCl) discourages growth of other organisms.
 - pH indicator changes color when mannitol is fermented to acid.

Microbial Growth

Culture Media

Both Selective and Differential Media: Used both to *distinguish* colonies of a desired organism, and *inhibit* the growth of other microbes.

- ✓ **MacConkey Agar:** Used to distinguish and select for *Salmonella*.
 - Bile salts and crystal violet discourage growth of gram-positive bacteria.
 - Lactose plus pH indicator: Lactose fermenters produce pink or red colonies, nonfermenters are colorless.

Microbial Growth

Culture Media

Enrichment Culture: Used to favor the growth of a microbe that may be found in very small numbers.

- ◆ Unlike selective medium, does not necessarily suppress the growth of other microbes.
- ◆ Used mainly for fecal and soil samples.
- ◆ After incubation in enrichment medium, greater numbers of the organisms, increase the likelihood of positive identification.

Microbial Growth

Obtaining Pure Cultures

Pure Culture: Contains a *single microbial species*.

Most clinical and environmental specimens contain several different microorganisms.

To obtain a pure culture, individual organisms must be *isolated*.

The most common method of isolation is the **streak plate**, in which a sterile loop is inserted into a sample and streaked onto a plate in a pattern, to obtain individual colonies

Colony: A group of descendants of an original cell.

Microbial Growth

Growth of Bacterial Cultures

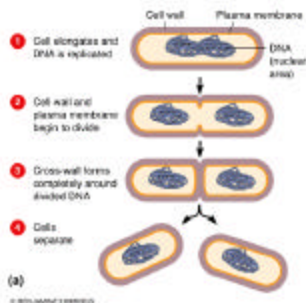
Bacterial Division: Occurs mainly by binary fission. A few bacterial species reproduce by budding.

Generation Time: Time required for a cell to divide, *and* its population to double.

Generation time varies considerably:

- ◆ *E. coli* divides every 20 minutes.
- ◆ Most bacteria divide every 1 to 3 hours.
- ◆ Some bacteria require over 24 hours to divide.

Bacterial Growth: Binary Fission



Microbial Growth

Growth of Bacterial Cultures

Logarithmic Representation of Bacterial Growth:

We can express the number of cells in a bacterial generation as 2^n , where n is the number of doublings that have occurred.

Arithmetic Numbers, Number of Cells	Numbers Expressed as a Power of 2	Visual Representation of Numbers
1	2^0	•
2	2^1	••
4	2^2	••••
8	2^3	••••••
16	2^4	••••••••
32	2^5	••••••••••

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Microbial Growth Phases of Growth

Bacterial Growth Curve: When bacteria are inoculated into a liquid growth medium, we can plot of the number of cells in the population over time.

Four phases of Bacterial Growth:

1. Lag Phase:

- ✓ Period of adjustment to new conditions.
- ✓ Little or no cell division occurs, population size doesn't increase.
- ✓ Phase of intense metabolic activity, in which individual organisms grow in size.
- ✓ May last from one hour to several days.

Microbial Growth Phases of Growth

Four phases of Bacterial Growth:

2. Log Phase:

- ✓ Cells begin to divide and generation time reaches a constant minimum.
- ✓ Period of most rapid growth.
Number of cells produced > Number of cells dying
- ✓ Cells are at highest metabolic activity.
- ✓ Cells are *most susceptible* to adverse environmental factors at this stage.
 - Radiation
 - Antibiotics

Microbial Growth Phases of Growth

Four phases of Bacterial Growth:

3. Stationary Phase:

- ✓ Population size begins to stabilize.
Number of cells produced = Number of cells dying
- ✓ Overall cell number does not increase.
- ✓ Cell division begins to slow down.
- ✓ Factors that slow down microbial growth:
 - Accumulation of toxic waste materials
 - Acidic pH of media
 - Limited nutrients
 - Insufficient oxygen supply

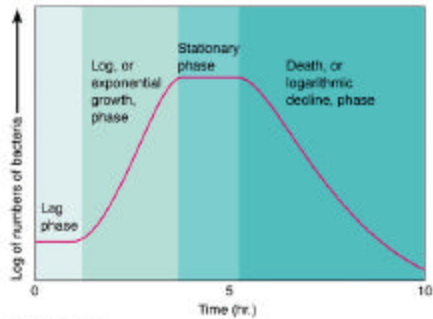
Microbial Growth Phases of Growth

Four phases of Bacterial Growth:

4. Death or Decline Phase:

- ✓ Population size begins to decrease.
Number of cells dying > Number of cells produced
- ✓ Cell number decreases at a logarithmic rate.
- ✓ Cells lose their ability to divide.
- ✓ A few cells may remain alive for a long period of time.

Four Phases of Bacterial Growth Curve



Measuring Microbial Growth

Direct Methods of Measurement

1. Plate count:

- ✓ Most frequently used method of measuring bacterial populations.
- ✓ Inoculate plate with a sample and count number of colonies.

Assumptions:

- Each colony originates from a single bacterial cell.
- Original inoculum is homogeneous.
- No cell aggregates are present.

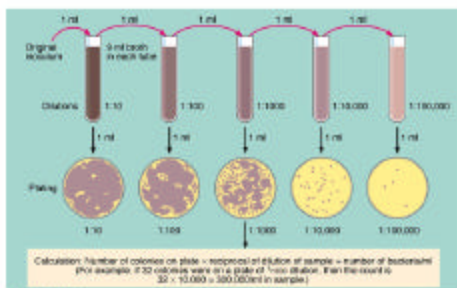
Advantages:

- Measures **viable** cells

Disadvantages:

- Takes 24 hours or more for visible colonies to appear.
- Only counts between 25 and 250 colonies are accurate.
- Must perform **serial dilutions** to get appropriate numbers/plate.

Serial Dilutions are Used with the Plate Count Method to Measure Numbers of Bacteria



Measuring Microbial Growth

Direct Methods of Measurement

1. Plate count (continued):

A. Pour Plate:

- ✓ Introduce a 1.0 or 0.1 ml inoculum into an **empty** Petri dish.
- ✓ Add liquid nutrient medium kept at 50°C.
- ✓ Gently mix, allow to solidify, and incubate.

Disadvantages:

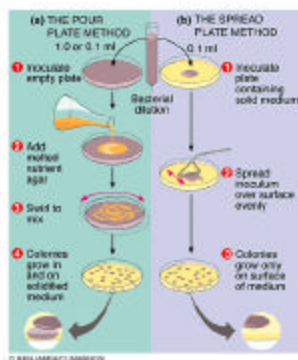
- Not useful for heat sensitive organisms.
- Colonies appear under agar surface.

B. Spread Plate:

- ✓ Introduce a 0.1 ml inoculum onto the **surface** of Petri dish.
- ✓ Spread with a sterile glass rod.

- ✓ **Advantages:** Colonies will be on surface and not exposed to melted agar.

Pour Plates versus Spread Plates



Measuring Microbial Growth

Direct Methods of Measurement

2. Filtration:

- ✓ Used to measure small quantities of bacteria.
 - **Example:** Fecal bacteria in a lake or in ocean water.
- ✓ A large sample (100 ml or more) is filtered to retain bacteria.
- ✓ Filter is transferred onto a Petri dish.
- ✓ Incubate and count colonies.

Measuring Microbial Growth

Direct Methods of Measurement

3. Most Probable Number (MPN):

- ✓ Used mainly to measure bacteria that will not grow on solid medium.
- ✓ Dilute a sample repeatedly and inoculate several broth tubes for each dilution point.
- ✓ Count the number of positive tubes in each set.
- ✓ **Statistical method:** Determines 95% probability that a bacterial population falls within a certain range.

Measuring Microbial Growth

Direct Methods of Measurement

4. Direct Microscopic Count:

- ✓ A specific volume of a bacterial suspension (0.01 ml) is placed on a microscope slide with a special grid.
- ✓ Stain is added to visualize bacteria.
- ✓ Cells are counted and multiplied by a factor to obtain concentration.

Advantages:

- No incubation time required.

Disadvantages:

- Cannot always distinguish between live and dead bacteria.
- Motile bacteria are difficult to count.
- Requires a high concentration of bacteria (10 million/ml).

Measuring Microbial Growth

Indirect Methods of Measurement

1. Turbidity:

- ✓ As bacteria multiply in media, it becomes turbid.
- ✓ Use a spectrophotometer to determine % transmission or absorbance.
- ✓ Multiply by a factor to determine concentration.

Advantages:

- No incubation time required.

Disadvantages:

- Cannot distinguish between live and dead bacteria.
- Requires a high concentration of bacteria (10 to 100 million cells/ml).

Measuring Microbial Growth

Indirect Methods of Measurement

2. Metabolic Activity:

- ✓ As bacteria multiply in media, they produce certain products:
 - Carbon dioxide
 - Acids
- ✓ Measure metabolic products.
- ✓ Expensive

3. Dry Weight:

- ✓ Bacteria or fungi in liquid media are centrifuged.
- ✓ Resulting cell pellet is weighed.
- ✓ Doesn't distinguish live and dead cells.