Elements of Microbial Nutrition, Ecology, and Growth
Microbial Nutrition

**nutrition** – process by which chemical substances (nutrients) are acquired from the environment and used for cellular activities

**essential nutrients** - must be provided to an organism

Two categories of essential nutrients:

- **macronutrients** – required in large quantities; play principal roles in cell structure and metabolism
  - proteins, carbohydrates
- **micronutrients or trace elements** – required in small amounts; involved in enzyme function and maintenance of protein structure
  - manganese, zinc, nickel
Nutrients

• **Inorganic nutrients** – atom or molecule that contains a combination of atoms other than carbon and hydrogen
  – metals and their salts (magnesium sulfate, ferric nitrate, sodium phosphate), gases (oxygen, carbon dioxide) and water

• **Organic nutrients** – contain carbon and hydrogen atoms and are usually the products of living things
  – methane (CH₄), carbohydrates, lipids, proteins, and nucleic acids
Chemical Analysis of Microbial Cytoplasm

- 70% water
- Proteins
- 96% of cell is composed of 6 elements:
  - carbon
  - hydrogen
  - oxygen
  - phosphorous
  - sulfur
  - nitrogen
Sources of Essential Nutrients

• Carbon sources

• **Heterotroph** – must obtain carbon in an organic form such as proteins, carbohydrates, lipids and nucleic acids, made by other living organisms

• **Autotroph** - an organism that uses CO$_2$, an inorganic gas as its carbon source
  – not nutritionally dependent on other living things
Sources of Essential Nutrients

Nitrogen Sources

- Main reservoir is nitrogen gas (N\textsubscript{2}); 79% of earth’s atmosphere is N\textsubscript{2}.
- Nitrogen is part of the structure of proteins, DNA, RNA & ATP – these are the primary source of N for heterotrophs.
- Some bacteria & algae use inorganic N nutrients (NO\textsubscript{3}⁻, NO\textsubscript{2}⁻, or NH\textsubscript{3}).
- Some bacteria can fix N\textsubscript{2}.
- Regardless of how N enters the cell, it must be converted to NH\textsubscript{3}, the only form that can be combined with carbon to synthesis amino acids, etc.
Sources of Essential Nutrients

Oxygen Sources

- Major component of carbohydrates, lipids, nucleic acids, and proteins
- Plays an important role in structural and enzymatic functions of cell
- Component of inorganic salts (sulfates, phosphates, nitrates) and water
- $O_2$ makes up 20% of atmosphere
- Essential to metabolism of many organisms
Sources of Essential Nutrients

Hydrogen Sources

• Major element in all organic compounds and several inorganic ones (water, salts and gases)
• Gases are produced and used by microbes.
• Roles of hydrogen:
  – maintaining pH
  – forming H bonds between molecules
  – serving as the source of free energy in oxidation-reduction reactions of respiration
Phosphorous (Phosphate Sources)

- Main inorganic source is phosphate ($\text{PO}_4^{3-}$) derived from phosphoric acid ($\text{H}_3\text{PO}_4$) found in rocks and oceanic mineral deposits
- Key component of nucleic acids, essential to genetics
- Serves in energy transfers (ATP)
Sources of Essential Nutrients

Sulfur Sources

- Widely distributed in environment, rocks; sediments contain sulfate, sulfides, hydrogen sulfide gas and sulfur
- Essential component of some vitamins and the amino acids: methionine and cysteine
- Contributes to stability of proteins by forming disulfide bonds
Other Nutrients Important in Microbial Metabolism

• Potassium – essential to protein synthesis and membrane function
• Sodium – important to some types of cell transport
• Calcium – cell wall and endospore stabilizer
• Magnesium – component of chlorophyll; membrane and ribosome stabilizer
• Iron – component of proteins of cell respiration
• Zinc, copper, nickel, manganese, etc.
Growth Factors: Essential Organic Nutrients

- Organic compounds that cannot be synthesized by an organism because they lack the genetic and metabolic mechanisms to synthesize them
- Must be provided as a nutrient
  - essential amino acids, vitamins
Nutritional Types

Main determinants of nutritional type are:
- carbon source – heterotroph, autotroph
- energy source –
  - chemotroph – gain energy from chemical compounds
  - phototrophs – gain energy through photosynthesis
<table>
<thead>
<tr>
<th>Category</th>
<th>Energy Source</th>
<th>Carbon Source</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autotroph</td>
<td>Nonliving Environment</td>
<td>CO₂</td>
<td>Photosynthetic organisms, such as algae, plants, cyanobacteria</td>
</tr>
<tr>
<td></td>
<td>Sunlight</td>
<td>CO₂</td>
<td></td>
</tr>
<tr>
<td>Chemoautotroph</td>
<td>Simple inorganic chemicals</td>
<td>CO₂</td>
<td>Only certain bacteria, such as methanogens, deep sea vent bacteria</td>
</tr>
<tr>
<td>Heterotroph</td>
<td>Other Organisms or Sunlight</td>
<td>Organic</td>
<td>Protozoa, fungi, many bacteria, animals</td>
</tr>
<tr>
<td>Chemoheterotroph</td>
<td>Metabolic conversion of the nutrients from other organisms</td>
<td>Organic</td>
<td></td>
</tr>
<tr>
<td>Saprobe</td>
<td>Metabolizing the organic matter of dead organisms</td>
<td>Organic</td>
<td>Fungi, bacteria (decomposers)</td>
</tr>
<tr>
<td>Parasite</td>
<td>Utilizing the tissues, fluids of a live host</td>
<td>Organic</td>
<td>Various parasites and pathogens; can be bacteria, fungi, protozoa, animals</td>
</tr>
<tr>
<td>Photoheterotroph</td>
<td>Sunlight</td>
<td>Organic</td>
<td>Purple and green photosynthetic bacteria</td>
</tr>
</tbody>
</table>
Transport: Movement of Chemicals Across the Cell Membrane

• **Passive transport** – does not require energy; substances exist in a gradient and move from areas of higher concentration towards areas of lower concentration
  – diffusion
  – osmosis – diffusion of water
  – facilitated diffusion – requires a carrier

• **Active transport** – requires energy and carrier proteins; gradient independent
  – active transport
  – group translocation – transported molecule chemically altered
  – bulk transport – endocytosis, exocytosis, pinocytosis
a. Inset shows a close-up of the osmotic process. The gradient goes from the outer container (higher concentration of H₂O) to the sac (lower concentration of H₂O). Some water will diffuse the opposite direction but the net gradient favors osmosis into the sac.

b. As the H₂O diffuses into the sac, the volume increases and forces the excess solution into the tube, which will rise continually.

c. Even as the solution becomes diluted, there will still be osmosis into the sac. Equilibrium will not occur because the solutions can never become equal. (Why?)
Cells with Cell Wall

**Isotonic Solution**
- Water concentration is equal inside and outside the cell, thus rates of diffusion are equal in both directions.

**Hypotonic Solution**
- Net diffusion of water is into the cell; this swells the protoplast and pushes it tightly against the wall. Wall usually prevents cell from bursting.

**Hypertonic Solution**
- Water diffuses out of the cell and shrinks the cell membrane away from the cell wall; process is known as plasmolysis.

Cells Lacking Cell Wall

**Early**
- Rates of diffusion are equal in both directions.

**Late (osmolyis)**
- Diffusion of water into the cell causes it to swell, and may burst it if no mechanism exists to remove the water.

**Early**
- Water diffusing out of the cell causes it to shrink and become distorted.

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Direction of net water movement.
Environmental Factors That Influence Microbes

- Environmental factors fundamentally affect the function of metabolic enzymes.
- Factors include:
  - temperature
  - oxygen requirements
  - pH
  - electromagnetic radiation
  - barometric pressure
3 Cardinal Temperatures

• **Minimum temperature** – lowest temperature that permits a microbe’s growth and metabolism

• **Maximum temperature** – highest temperature that permits a microbe’s growth and metabolism

• **Optimum temperature** – promotes the fastest rate of growth and metabolism
3 Temperature Adaptation Groups

1. **Psychrophiles** – optimum temperature below 15°C; capable of growth at 0°C

2. **Mesophiles** – optimum temperature 20°-40°C; most human pathogens

3. **Thermophiles** – optimum temperature greater than 45°C
Oxygen

- As oxygen is utilized it is transformed into several toxic products:
  - singlet oxygen ($O_2$), superoxide ion ($O_2^-$), peroxide ($H_2O_2$), and hydroxyl radicals ($OH^-$)
- Most cells have developed enzymes that neutralize these chemicals:
  - superoxide dismutase, catalase
- If a microbe is not capable of dealing with toxic oxygen, it is forced to live in oxygen free habitats.
Categories of Oxygen Requirement

- **Aerobe** – utilizes oxygen and can detoxify it
- **Obligate aerobe** - cannot grow without oxygen
- **Facultative anaerobe** – utilizes oxygen but can also grow in its absence
- **Microaerophilic** – requires only a small amount of oxygen
Categories of Oxygen Requirement

• **Anaerobe** – does not utilize oxygen
• **Obligate anaerobe** - lacks the enzymes to detoxify oxygen so cannot survive in an oxygen environment
• **Aerotolerant anaerobes** – do no utilize oxygen but can survive and grow in its presence
Carbon Dioxide Requirement

All microbes require some carbon dioxide in their metabolism.

• Capnophile – grows best at higher CO$_2$ tensions than normally present in the atmosphere
Lockscrew

Outer lid  Inner lid

Catalyst chamber contains palladium pellets, which scavenge excess oxygen.

Rubber gasket provides air-tight seal.

Petri dishes

Gas generator envelope. Water is added to chemicals in envelope to generate $\text{H}_2$ and $\text{CO}_2$. $\text{H}_2$ combines with oxygen in chamber to produce $\text{H}_2\text{O}$, which is visible as condensation on the walls of the chamber.

Anaerobic indicator strip (Methylene blue becomes colorless in absence of $\text{O}_2$.)
Effects of pH

- Majority of microorganisms grow at a pH between 6 and 8
- **Obligate acidophiles** – grow at extreme acid pH
- **Alkalinoophiles** – grow at extreme alkaline pH
Osmotic Pressure

- Most microbes exist under hypotonic or isotonic conditions
- **Halophiles** – require a high concentration of salt
- **Osmotolerant** – do not require high concentration of solute but can tolerate it when it occurs
Other Environmental Factors

• **Barophiles** – can survive under extreme pressure and will rupture if exposed to normal atmospheric pressure
Ecological Associations Among Microorganisms

- **Symbiotic** – organisms live in close nutritional relationships; required by one or both members
  - **mutualism** – obligitory, dependent; both members benefit
  - **commensalism** – commensal member benefits, other member not harmed
  - **parasitism** – parasite is dependent and benefits; host is harmed
Ecological Associations Among Microorganisms

• Non-symbiotic – organisms are free-living; relationships not required for survival
  – synergism – members cooperate and share nutrients
  – antagonism – some member are inhibited or destroyed by others
Interrelationships Between Microbes and Humans

• Human body is a rich habitat for symbiotic bacteria, fungi, and a few protozoa - normal microbial flora
• Commensal, parasitic, and synergistic
Microbial Biofilms

• **Biofilms** result when organisms attach to a substrate by some form of extracellular matrix that binds them together in complex organized layers

• Dominate the structure of most natural environments on earth

• Communicate and cooperate in the formation and function of biofilms – **quorum sensing**
The Study of Microbial Growth

- Microbial growth occurs at two levels: growth at a cellular level with increase in size, and increase in population.
- Division of bacterial cells occurs mainly through **binary fission** (transverse)
  - parent cell enlarges, duplicates its chromosome, and forms a central transverse septum dividing the cell into two daughter cells.
(a) A young cell at early phase of cycle.

(b) A parent cell prepares for division by enlarging its cell wall, cell membrane, and overall volume. Midway in the cell, the wall develops notches that will eventually form the transverse septum, and the duplicated chromosome becomes affixed to a special membrane site.

(c) The septum wall grows inward, and the chromosomes are pulled toward opposite cell ends as the membrane enlarges. Other cytoplasmic components are distributed (randomly) to the two developing cells.

(d) The septum is synthesized completely through the cell center, and the cell membrane patches itself so that there are two separate cell chambers.

(e) At this point, the daughter cells are divided. Some species will separate completely as shown here, while others will remain attached, forming chains or doublets, for example.
Rate of Population Growth

• Time required for a complete fission cycle is called the generation, or doubling time.

• Each new fission cycle increases the population by a factor of 2 – exponential or logarithmic growth.

• Generation times vary from minutes to days.
<table>
<thead>
<tr>
<th>Number of cells</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>32</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of generations</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Exponential value</td>
<td>$2^1$</td>
<td>$2^2$</td>
<td>$2^3$</td>
<td>$2^4$</td>
<td>$2^5$</td>
<td></td>
</tr>
</tbody>
</table>

![Diagram showing exponential growth](image)

- **Log of number of cells**
- **Number of cells**
- **Time**

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Rate of Population Growth

• Equation for calculating population size over time:

\[ N_f = (N_i)2^n \]

\( N_f \) is total number of cells in the population. 
\( N_i \) is starting number of cells. 
Exponent \( n \) denotes generation time. 
\( 2^n \) number of cells in that generation
The Population Growth Curve

- In laboratory studies, populations typically display a predictable pattern over time – **growth curve**.
- Stages in the normal growth curve:
  1. **Lag phase** – “flat” period of adjustment, enlargement; little growth
  2. **Exponential growth phase** – a period of maximum growth will continue as long as cells have adequate nutrients and a favorable environment
  3. **Stationary phase** – rate of cell growth equals rate of cell death caused by depleted nutrients and O\textsubscript{2}, excretion of organic acids and pollutants
  4. **Death phase** – as limiting factors intensify, cells die exponentially in their own wastes
The graph illustrates the growth phases of a microbial culture over time. The x-axis represents time in hours, ranging from 0 to 40. The y-axis represents the logarithm (10^x) of viable cells, ranging from 0 to 10.

1. **Lag phase**: This phase begins at hour 0 and shows a low but rising population of viable cells, indicating that the cells are adapting to the new environment.
2. **Exponential growth phase**: This phase starts after the lag phase and is characterized by a rapid increase in the population of viable cells. The graph shows a steep upward curve.
3. **Stationary phase**: As the exponential growth phase progresses, the population reaches a plateau, indicating that the growth rate becomes constant. This is followed by a decline in the number of viable cells.
4. **Death phase**: The population of viable cells begins to decrease as the culture enters the death phase.

The final outcome of the culture varies with the specific conditions of the culture setup. The total cells in the population, live and dead, are shown at each phase.

Legend:
- **Few cells**
- **Live cells**
- **Dead cells (not part of count)**

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Methods of Analyzing Population Growth

- Turbidometry – most simple
- Degree of cloudiness, turbidity, reflects the relative population size
- Enumeration of bacteria:
  - viable colony count
  - direct cell count – count all cells present; automated or manual