Chapter 7

Microbial Nutrition, Ecology, and Growth
Microbial Nutrition

Nutrition – process by which chemical substances (nutrients) are acquired from the environment and used in cellular activities.

Essential nutrients – must be provided to an organism

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, Nickel, Magnesium, Zinc
Nutrients

Organic nutrients

- contain carbon and hydrogen atoms
- usually the products of living things

Methane (CH₄), carbohydrates, lipids, proteins, nucleic acids

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), water
Chemical Analysis of Cell Contents

- 70% water
- **Proteins** - most prevalent organic compounds
- 96% of cell is composed of **6 elements**:
  - **Carbon**
  - **Hydrogen**
  - **Oxygen**
  - **Nitrogen**
  - **Phosphorous**
  - **Sulfur**

Table 7.1 on page 189
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

- **Growth factors** must be provided as a nutrient
  - Essential amino acids, vitamins
Growth Factors: Essential Organic Nutrients

- *Haemophilus influenzae* is a fastidious organism

- Grows best at 35-37°C with ~5% CO$_2$ (or in a candle-jar)

- requires hemin (X factor)

- nicotinamide-adenine-dinucleotide (NAD) also known as V factor

- prepared with heat-lysed horse/sheep blood: good source of both hemin and NAD

- NAD is released from the blood during the heating process

- hemin is available from non-hemolysed as well as hemolyzed blood cells.
Sources of Essential Nutrients

- Carbon sources

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

- **Autotroph** – an organism that uses CO₂, an inorganic gas as its carbon source
  - Not nutritionally dependent on other living things
Nutritional Types

**Carbon**
- Heterotroph
  - must obtain carbon in an organic form
  - nutritionally dependent on other life forms
  - proteins, carbohydrates, lipids, nucleic acids
- Autotroph
  - an organism that uses CO₂, an inorganic gas as its carbon source
  - not nutritionally dependent on other life forms

**Energy**
- Phototrophs
  - gain energy through photosynthesis
- Chemotroph
  - gain energy from chemical compounds
## TABLE 7.2 Nutritional Categories of Microbes by Energy and Carbon Source

<table>
<thead>
<tr>
<th>Category/Carbon Source</th>
<th>Energy Source</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autotroph/CO₂</td>
<td>Nonliving Environment</td>
<td>Photosynthetic organisms, such as algae, plants, cyanobacteria</td>
</tr>
<tr>
<td>Photoautotroph</td>
<td>Sunlight</td>
<td>Only certain bacteria, such as methanogens, deep-sea vent bacteria</td>
</tr>
<tr>
<td>Chemoautotroph</td>
<td>Simple inorganic chemicals</td>
<td></td>
</tr>
<tr>
<td>Heterotroph/Organic</td>
<td>Other Organisms or Sunlight</td>
<td>Protozoa, fungi, many bacteria, animals</td>
</tr>
<tr>
<td>Chemoheterotroph</td>
<td>Metabolic conversion of the nutrients from other organisms</td>
<td>To heterotroph or sunlight</td>
</tr>
<tr>
<td>1. Saprobe</td>
<td>Metabolizing the organic matter of dead organisms</td>
<td>Fungi, bacteria (decomposers)</td>
</tr>
<tr>
<td>2. Parasite</td>
<td>Utilizing the tissues, uids of a live host</td>
<td>Various parasites and pathogens; can be bacteria, fungi, protozoa, animals</td>
</tr>
<tr>
<td>Photoheterotroph</td>
<td>Sunlight or organic matter</td>
<td>Purple and green photosynthetic bacteria</td>
</tr>
</tbody>
</table>
Autotrophs and Their Energy Sources

• Photoautotrophs
  - Oxyenic photosynthesis: oxygen producing, chlorophyll
  - Anoxyenic photosynthesis: no oxygen produced, bacteriochlorophyll

• Chemoautotrophs (lithoautotrophs) survive totally on inorganic substances

• Methanogens, a kind of chemoautotroph, produce methane gas under anaerobic conditions

Figure 7.2b, page 191
Heterotrophs and Their Energy Sources

- **Majority are chemoheterotrophs**
  - **Aerobic respiration**

- **Two categories**
  - **Saprobes**: free-living microorganisms that feed on organic detritus from dead organisms
    - **Opportunistic pathogen**
    - **Facultative parasite**
  - **Parasites**: derive nutrients from host
    - **Pathogens**
    - **Some are obligate parasites**
Parasites

Facultative parasites
- Saprobes infecting a host
- Occurs when host is compromised (chemotherapy, AIDS)
- Opportunistic pathogen

Obligate parasites
- Pathogens - can cause disease or even death
- Ectoparasites: live on the body
- Endoparasites: live in organs & tissues
- Intracellular parasites: live in the cells

Pseudomonas aeruginosa – problem in hospitals
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 toward areas of lower concentration
  - Diffusion
  - Osmosis – diffusion of water through selective permeable membrane
  - 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
Environmental Factors That Influence Microbes

- **Niche**: totality of adaptations organisms make to their habitat

- **Environmental factors affect the function of metabolic enzymes**

- **Factors include:**
  - Temperature
  - Oxygen requirements
  - pH
  - Osmotic pressure
  - 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

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

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

Thermophiles – **optimum temperature greater than 45°C**
Gas Requirements

Oxygen

- As oxygen is utilized it is transformed into several toxic products:
  - Singlet oxygen ($^1\text{O}_2$), superoxide ion ($\text{O}_2^-$), peroxide ($\text{H}_2\text{O}_2$), and hydroxyl radicals ($\text{OH}^-$)

- Most cells have developed enzymes that neutralize these chemicals:
  - Superoxide dismutase, catalase

Catalase converts $\text{H}_2\text{O}_2$ to water and oxygen,

catalase test useful to distinguish staphylococci from enterococci and streptococci
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

- **Anaerobe** – does not utilize oxygen

- **Obligate anaerobe** – lacks the enzymes to detoxify oxygen so cannot survive in an oxygen environment

- **Aerotolerant anaerobes** – do not utilize oxygen but can survive and grow in its presence
Oxygen-related growth zones in a standing test tube

- **High oxygen** (300 μM O₂)
- **Low oxygen** (50 μM O₂)
- **“No” oxygen** (5 μM O₂)

**Growth zones**
- **Aerobic**
- **Facultative**
- **Microaerophilic**
- **Anaerobic**

*Microbiology: An Evolving Science, Third Edition  Figure 5.19  Copyright © 2014 W. W. Norton & Company, Inc.*
Sergei N. Winogradsky was one of the first microbiologists to study the organisms found in complex biofilm communities. One of the strategies he used to isolate organisms from nature was a miniature model of a pond cross section that is now called a Winogradsky column. It is a simple device for constructing a stratified ecosystem and provides a visual example of various modes of metabolism and zonation in the microbial world. It is a classic demonstration of the metabolic diversity of prokaryotes.

A soil or sediments sample is collected from nearly any source and amended with a variety of compounds such as carbon, sulfur, iron, and/or calcium. The mixture is added to a clear container and topped with water; the container is tightly capped to prevent evaporation. The column is incubated for weeks to months in well-lit conditions, thereby establishing gradients of oxygen, nutrients, and light. Different microbial taxa are adapted to different niches within these overlapping gradients, creating a stratified ecosystem defined by metabolic potential.

All life on Earth can be categorized according to an organism’s carbon and energy source. Energy can be obtained from light reactions (phototrophs) or chemical oxidations (chemolithoautotrophs), and carbon for cellular synthesis can be obtained from carbon dioxide (autochthonous) or from preformed organic compounds (heterotrophs). These categories combined form the four basic life strategies and can be found among the bacteria within a single Winogradsky column: phototrophs, photolithoautotrophs, chemolithoautotrophs, and chemoheterotrophs. Depending on conditions, Winogradsky columns can enrich for many different types of bacteria. The illustration above lists some common examples.
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

- *Neisseria* (gonorrhea, meningitis)

- *Brucella* (undulant fever)
Effects of pH

- Majority of microorganisms grow at a pH between 6 and 8 (neutrophiles)

- Acidophiles – grow at extreme acid pH
  *Euglena mutabilis*- alga grows between pH 0-1

- Alkalinophiles – grow at extreme alkaline pH
  *Proteus* sp. metabolizes urea in urine to create alkaline conditions to colonize urinary tract
Classification of organisms according to their optimum growth pH

<table>
<thead>
<tr>
<th>Growth pH</th>
<th>[H⁺] (molarity)</th>
<th>pH</th>
<th>pOH</th>
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<tr>
<td>10⁻³</td>
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<tr>
<td>10⁻¹³</td>
<td>10⁻¹⁴</td>
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<tr>
<td>10⁻¹⁴</td>
<td></td>
<td>14</td>
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</tr>
</tbody>
</table>

- **Acidophiles**
- **Neutralophiles**
- **Alkaliphiles**

*Intracellular levels compatible with life*
Osmotic Pressure

- Most microbes exist under hypotonic or isotonic conditions

- **Halophiles** – require a high concentration of salt
  *Halobacterium* grows in 9-25% NaCl solutions

- **Osmotolerant** – do not require high concentration of solute but can tolerate it when it occurs
  *Staphylococcus aureus* can grow in 0.1-20% NaCl media

High salt & sugar concentrations used to preserve food like jellies & brine could support microbial growth

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

Microbial Associations

**Symbiotic**
- Organisms live in close nutritional relationships; required by one or both members.

**Mutualism**
- Obligatory, dependent; both members benefit.

**Commensalism**
- The commensal benefits; other member not harmed.

**Parasitism**
- Parasite is dependent and benefits; host harmed.

**Nonsymbiotic**
- Organisms are free-living; relationships not required for survival

**Synergism**
- Members cooperate and share nutrients.

**Antagonism**
- Some members are inhibited or destroyed by others.

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Ecological Associations

- **Symbiotic** – two organisms live together in a close partnership

  - **Mutualism** – obligatory, dependent; both members benefit

  - **Commensalism** – commensal member benefits, other member neither harmed nor benefited

  - **Parasitism** – parasite is dependent and benefits; host is harmed
Ecological Associations

- **Non-symbiotic** – organisms are free-living; relationships not required for survival

  - **Synergism** – members cooperate to produce a result that none of them could do alone

  - **Antagonism** – actions of one organism affect the success or survival of others in the same community (competition)

- **Antibiosis** – example of antagonism: antibiotics
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 relationships

HUMAN MICROBIOME PROJECT (HMP)
Researchers in the HMP are sampling and analyzing the genome of microbes from five sites on the human body.

http://commonfund.nih.gov/hmp/index

https://www.bcm.edu/departments/molecular-virology-and-microbiology/microbiome
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.
Biofilm Formation and Quorum Sensing

1. Free-swimming cells settle on a surface and remain there.
2. Cells synthesize a sticky matrix that holds them tightly to the substrate.
3. When biofilm grows to a certain density (quorum), the cells release inducer molecules that can coordinate a response.
4. Enlargement of one cell to show genetic induction. Inducer molecule stimulates expression of a particular gene and synthesis of a protein product, such as an enzyme.
5. Cells secrete their enzymes in unison to digest food particles.
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
Binary Fission

1. A young cell at early phase of cycle

2. 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.

3. 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.

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

5. 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

• Generation or doubling time: Time required for a complete fission cycle
• Each new fission cycle increases the population by a factor of 2 – exponential growth
• Generation times vary from minutes to days

(a)

<table>
<thead>
<tr>
<th>Number of cells</th>
<th>1</th>
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<th>4</th>
<th>8</th>
<th>16</th>
<th>32</th>
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</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>

(b)

<table>
<thead>
<tr>
<th>Time</th>
<th>Log of number of cells using the power of 2</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>1.5</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>2.4</td>
</tr>
</tbody>
</table>

4500*
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
Assuming Staphylococcus aureus has a doubling time of 20 minutes, if there are 10 cells at the beginning of the experiment, how many will there be in 4 hours?

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

\[ \begin{align*} 
N_i &= 10 \\
n &= 4 \text{ h} = 240/20 = 12 \\
2^n &= 2^{12} = 4,096 \\
N_f &= 10 \times 4,096 = 40,096 
\end{align*} \]

Table with powers: Table A.2
*Escherichia coli* has a doubling time of 20 minutes. If there are 5 cells at the beginning of the experiment, how many will there be in 3 hours?

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

\[ N_i = 5 \]
\[ n = 3 \text{ h} = \frac{180}{20} = 9 \]
\[ 2^n = 2^9 = 512 \]

\[ N_f = 10 \times 512 = 5120 \]
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

The final outcome varies with the culture.
The Population Growth Curve

Stages in the normal growth curve:

1. **Lag phase**

2. **Exponential growth phase** – a period of maximum growth will continue as long as cells have adequate nutrients and a favorable environment.

Total cells in population, live and dead, at each phase:
- **Green**: Few cells
- **Blue**: Live cells
- **Red**: Dead cells (not part of count)

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The Population Growth Curve

Stages in the normal growth curve:

1. Lag phase
2. Exponential growth phase
3. Stationary phase – rate of cell growth equals rate of cell death caused by depleted nutrients and O$_2$, excretion of organic acids and pollutants

Total cells in population, live and dead, at each phase

Logrithm (10$^n$) of Viable Cells

Hours

The final outcome varies with the culture.

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The Population Growth Curve

Stages in the normal growth curve:

1. **Lag phase**
2. **Exponential growth phase**
3. **Stationary phase**
4. **Death phase** – as limiting factors intensify, cells die exponentially

The final outcome varies with the culture.

Logarithm ($10^n$) of Viable Cells vs. Hours

- Lag phase
- Exponential growth phase
- Stationary phase
- Death phase

Total cells in population, live and dead, at each phase:
- Few cells
- Live cells
- Dead cells (not part of count)
Methods of Analyzing Population Growth

- **Turbidometry** – *most simple*

- **Degree of cloudiness, turbidity, reflects the relative population size**
Methods of Analyzing Population Growth

- Enumeration of bacteria:
  - Viable colony count
  - Direct cell count – count all cells present; automated or manual
Viable Plate Count

Samples taken at equally spaced intervals

500 ml

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>60</th>
<th>120</th>
<th>180</th>
<th>240</th>
<th>300</th>
<th>360</th>
<th>420</th>
<th>480</th>
<th>540</th>
<th>600</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Sample is diluted in liquid agar medium and poured or spread over surface of solidified medium.

Flask inoculated

Plates are incubated, colonies are counted

<table>
<thead>
<tr>
<th>Number of colonies (CFU) per 0.1 ml</th>
<th>&lt;1*</th>
<th>2</th>
<th>4</th>
<th>7</th>
<th>13</th>
<th>23</th>
<th>45</th>
<th>80</th>
<th>135</th>
<th>230</th>
</tr>
</thead>
</table>

Total estimated cell population in flask

| <10,000 | 5,000 | 20,000 | 35,000 | 65,000 | 115,000 | 225,000 | 400,000 | 675,000 | 1,150,000 |

*Only means that too few cells are present to be assayed.
a. Withdraw 1 ml of the sample
b. Dispense the 1 ml of sample into the tube
c. Using the same procedure, withdraw 1 ml from the first dilution tube and dispense into the second dilution tube. Continue doing this from tube to tube until the dilution is completed.

2. Transfer **1 ml from each of the last three dilution tubes** onto the surface of the corresponding agar plates. 3. Incubate the agar plates at 37°C for 48 hours. 4. Choose a plate that appears to have between **30 and 300 colonies**.
Tube 1 contains 9 mL of sterile media; you will add 1 mL of the undiluted bacterial suspension to yield a total volume of 10 mL.

$$\frac{1}{9 \text{ mL} + 1 \text{ mL}} \rightarrow \frac{1}{10 \text{ mL}} \rightarrow 1 \times 10^{-1} \rightarrow 1:10 \text{ dilution}$$

Tube 2 contains 9 mL of sterile media; you will add 1 mL of the 1:10 diluted bacterial suspension to yield a total volume of 10 mL.

$$\frac{1}{9 \text{ mL} + 1 \text{ mL}} \rightarrow \frac{1}{10 \text{ mL}} \times 1 \rightarrow 1 \times 10^{-2} \rightarrow 1:100 \text{ dilution}$$