Chapter 8
An Introduction to Microbial Metabolism
8.1 The Metabolism of Microbes

Metabolism – all chemical and physical workings of a cell

Two types of chemical reactions:

**Catabolism** – degradative; breaks the bonds of larger molecules forming smaller molecules; releases energy

**Anabolism** – biosynthesis; process that forms larger macromolecules from smaller molecules; requires energy input

![Figure 8.1 Simplified model of metabolism](image)
Enzymes: Catalyzing the Chemical Reactions of Life

• Enzymes are biological catalysts that increase the rate of a chemical reaction by lowering the energy of activation (the resistance to a reaction)
• The enzyme is not permanently altered in the reaction
• Enzyme promotes a reaction by serving as a physical site for specific substrate molecules to position

Insight 8.1
Enzyme Structure

• Simple enzymes – consist of protein alone
• Conjugated enzymes or holoenzymes – contain protein and nonprotein molecules
  – Apoenzyme: protein portion
  – Cofactors: nonprotein portion
    • Metallic cofactors: iron, copper, magnesium
    • Coenzymes, organic molecules: vitamins

Figure 8.2 Conjugated enzyme structure
Apoenzymes: *Specificity and the Active Site*

- Exhibits primary, secondary, tertiary, and some, quaternary structure
- Site for substrate binding is **active site**, or catalytic site

**Figure 8.3**

(a) As the polypeptide forms intrachain bonds and folds, it assumes a three-dimensional (tertiary) state that displays an active site (AS).

(b) Because each different polypeptide folds differently, each apoenzyme will have a differently shaped active site.

(c) More complex enzymes have a quaternary structure consisting of more than one polypeptide. The active sites may be formed by the junction of two polypeptides.
Apoenzymes: Specificity and the Active Site

- A temporary enzyme-substrate union occurs when substrate moves into active site – **induced fit**
- Appropriate reaction occurs; product is formed and released

**Figure 8.4 Enzyme-substrate reactions**
Cofactors: Supporting the Work of Enzymes

- Micronutrients, such as metal ions, are needed as cofactors to assist the enzyme.

- Coenzymes act as carriers to assist the enzyme in its activity, such as electron transfer.

Figure 8.5 Function of coenzymes

1. An enzyme with a coenzyme positioned to react with two substrates.

2. Coenzyme picks up a chemical group from substrate 1.

3. Coenzyme readies the chemical group for transfer to substrate 2.

4. Final action is for group to be bound to substrate 2; altered substrates are released from enzyme.
Location and Regularity of Enzyme Action

- **Exoenzymes** – transported extracellularly, where they break down large food molecules or harmful chemicals
  - Cellulase, amylase, penicillinase
- **Endoenzymes** – retained intracellularly and function there
  - Most enzymes are endoenzymes

Figure 8.6 Location of action of enzymes
Regularity of Enzyme Action

- **Constitutive enzymes** – always present, always produced in equal amounts or at equal rates, regardless of the amount of substrate.

- **Regulated enzymes** – not constantly present; production is turned on (induced) or turned off (repressed) in response to changes in the substrate concentration.
Synthesis and Hydrolysis Reactions

- **Synthesis or condensation reactions** – *anabolic reactions to form covalent bonds* between smaller substrate molecules, require ATP, release one molecule of water for each bond formed.

(a) Condensation Reaction. Forming a glycosidic bond between two glucose molecules to generate maltose requires the removal of a water molecule and energy from ATP.
Synthesis and Hydrolysis Reactions

• **Hydrolysis reactions**  
  – *catabolic reactions that break down substrates* into small molecules; requires the input of water to break bonds

(b) Hydrolysis Reaction. Breaking a peptide bond between two amino acids requires a water molecule that adds OH to one amino acid and H to the other.
Sensitivity of Enzymes to Their Environment

• Activity of an enzyme is influenced by the cell’s environment
• Enzymes operate under temperature, pH, and osmotic pressure of organism’s habitat
• When enzymes are subjected to changes in organism’s habitat they become unstable
  – Labile: chemically unstable enzymes
  – Denaturation: weak bonds that maintain the shape of the apoenzyme are broken
Regulation of Enzymatic Activity and Metabolic Pathways

Figure 8.9 Patterns of metabolic pathways
Direct Controls on the Actions of Enzymes

1. **Competitive inhibition** – substance that resembles the normal substrate competes with the substrate for the active site

2. **Noncompetitive inhibition** – enzymes are regulated by the binding of molecules other than the substrate away from the active site
   - **Enzyme repression** – inhibits at the genetic level by controlling synthesis of key enzymes
   - **Enzyme induction** – enzymes are made only when suitable substrates are present
Figure 8.10 Regulation of enzyme action

**Competitive Inhibition**

- Normal substrate
- Competitive inhibitor with similar shape
- Both molecules compete for the active site.
- Reaction proceeds
- Reaction is blocked because competitive inhibitor is incapable of becoming a product.

**Noncompetitive Inhibition**

- Substrate
- Active site
- Regulatory site
- Regulatory molecule (product)
- Reaction proceeds
- Reaction is blocked because binding of regulatory molecule in regulatory site changes conformation of active site so that substrate cannot enter.
Figure 8.11 Enzyme repression – feedback inhibition

1. DNA → 2. RNA → 3. Protein → 4. Folds to form functional enzyme structure

5. Enzyme + Substrate → Products

6. Excess product binds to DNA and shuts down further enzyme production.

7. DNA → 8. RNA → 9. Protein → No enzyme
8.2 The Pursuit and Utilization of Energy

- **Energy**: the capacity to do work or to cause change
- Forms of energy include
  - Thermal, radiant, electrical, mechanical, atomic, and chemical
Cell Energetics

- Cells manage energy in the form of chemical reactions that make or break bonds and transfer electrons

- **Endergonic reactions** – consume energy
  \[
  \text{Energy} + \text{A} + \text{B} \rightarrow \text{C} \quad \text{Enzyme}
  \]

- **Exergonic reactions** – release energy
  \[
  \text{X} + \text{Y} \rightarrow \text{Z} + \text{Energy} \quad \text{Enzyme}
  \]

- Energy released is temporarily stored in high energy phosphate molecules. The energy of these molecules is used in endergonic cell reactions.
A Closer Look at Biological Oxidation and Reduction

- **Redox reactions** – always occur in pairs
- There is an electron donor and electron acceptor which constitute a redox pair
- Process salvages electrons and their energy
- Released energy can be captured to phosphorylate ADP or another compound

\[
\text{Glucose} + 6\text{O}_2 \rightarrow 6\text{CO}_2 + \text{H}_2\text{O} + \text{Energy}
\]

Oxidation

Glucose

Oil Rig

Reduction
Electron and Proton Carriers

- Repeatedly accept and release electrons and hydrogen to facilitate the transfer of redox energy.
- Most carriers are coenzymes: NAD, FAD, NADP, coenzyme A, and compounds of the respiratory chain.

![Figure 8.13 NAD reduction](image-url)
Adenosine Triphosphate: ATP, Metabolic Money

• Metabolic “currency”
• Three part molecule consisting of:
  – Adenine – a nitrogenous base
  – Ribose – a 5-carbon sugar
  – 3 phosphate groups
• Removal of the terminal phosphate releases energy
• ATP utilization and replenishment is a constant cycle in active cells

Figure 8.14

Bond that releases energy when broken
Figure 8.15 Phosphorylation of Glucose by ATP

Glucose $\rightarrow$ Glucose-6-phosphate

ATP

ADP

Hexokinase
ATP can be formed by three different mechanisms:

1. **Substrate-level phosphorylation** – transfer of phosphate group from a phosphorylated compound (substrate) directly to ADP

2. **Oxidative phosphorylation** – series of redox reactions occurring during respiratory pathway

3. **Photophosphorylation** – ATP is formed utilizing the energy of sunlight
8.3 Pathways of Bioenergetics

- **Bioenergetics** – study of the mechanisms of cellular energy release
- Includes catabolic and anabolic reactions
- Primary catabolism of fuels (glucose) proceeds through a series of three coupled pathways:
  1. Glycolysis
  2. Kreb’s cycle
  3. Respiratory chain, electron transport
Energy Strategies in Microorganisms

- Nutrient processing is varied, yet in many cases is based on three catabolic pathways that convert glucose to CO$_2$ and gives off energy

- **Aerobic respiration** – glycolysis, the Kreb’s cycle, respiratory chain

- **Anaerobic respiration** – glycolysis, the Kreb’s cycle, respiratory chain; molecular oxygen is not the final electron acceptor

- **Fermentation** – glycolysis, organic compounds are the final electron acceptors
Overview of Catabolic Pathways

Figure 8.17

(a) AEROBIC RESPIRATION

Glycolysis

Glucose (6 C) → 2 pyruvate (3 C) → Acetyl Co A

Electrons

O₂ is final electron acceptor.

Maximum ATP produced = 38

(b) ANAEROBIC RESPIRATION

Glycolysis

Glucose (6 C) → 2 pyruvate (3 C) → Acetyl Co A

Electrons

Nonoxygen electron acceptors (examples: SO₄²⁻, NO₃⁻, CO₃²⁻)

Maximum ATP produced = 2 – 36

(c) FERMENTATION

Glycolysis

Glucose (6 C) → 2 pyruvate (3 C) → Acetyl Co A

Electrons

Fermentation

Lactic acid → Acetaldehyde → Ethanol + CO₂

Or other alcohols, acids, gases

An organic molecule is final electron acceptor (pyruvate, acetaldehyde, etc.).

Maximum ATP produced = 2
Aerobic Respiration

- Series or enzyme-catalyzed reactions in which electrons are transferred from fuel molecules (glucose) to oxygen as a final electron acceptor

- **Glycolysis** – glucose (6C) is oxidized and split into 2 molecules of pyruvic acid (3C), NADH is generated

- **TCA/Krebs** – processes pyruvic acid and generates 3 $\text{CO}_2$ molecules, NADH and $\text{FADH}_2$ are generated

- **Electron transport chain** – accepts electrons from NADH and $\text{FADH}_2$; generates energy through sequential redox reactions called oxidative phosphorylation

The diagram illustrates the glycolysis and TCA cycles with the following key points:

- **Glycolysis**:
  - Glucose (6C) is oxidized to 2 molecules of pyruvate (3C), NADH is generated.
  - Pyruvate is converted to acetyl CoA.

- **TCA/Krebs Cycle**:
  - Acetyl CoA enters the cycle and generates $\text{CO}_2$ molecules.
  - NADH and $\text{FADH}_2$ are generated.
  - Electrons from NADH and $\text{FADH}_2$ are transferred to the electron transport chain.

- **Electron Transport Chain**:
  - Generates energy through oxidative phosphorylation.
  - Oxygen ($\text{O}_2$) is the final electron acceptor.

The maximum ATP produced is 38.
Glycolysis: The Starting Lineup

**AERobic Respiration**

Glycolysis

- **Glucose** (6 C)
- ATP
- NADH
- CO₂
- 2 pyruvate (3 C)
- Acetyl CoA
- FADH₂
- NADH
- ATP
- Electrons

Electron transport

O₂ is final electron acceptor

ATP produced = 38

First phosphorylation

- ATP
- ADP
- PO₄

Glucose

- Glucose-6-phosphate
- Fructose-6-phosphate

Second phosphorylation

- ATP
- ADP
- PO₄

Fructose-1,6-diphosphate (F-1,6-P)

Split of F-1,6-P; subsequent reactions in duplicate

- Dihydroxyacetone Phosphate (DHAP)
- Glyceraldehyde-3-phosphate (G-3-P)

Krebs cycle or fermentation (see figures 8.20 and 8.25)

Krebs cycle or fermentation
Pyruvic Acid – A Central Metabolite

Glycolysis

Pyruvic acid

Acetyl CoA

Krebs cycle

Amino acids
Sugars
Fat metabolites

Acetaldehyde

Alcohol
Acetone
2, 3 butanediol

Acids, gas

Anabolic pathways

Respiration

Fermentation

Figure 8.19 *Pyruvic acid* is the “hub”, three different fates from here
The Krebs Cycle – A Carbon and Energy Wheel

**Figure 8.20**

Step after first arrow is the reaction that links glycolysis and the Krebs cycle and converts pyruvic acid to acetyl coenzyme A. This involves a reduction of NAD+

1. The 2C acetyl CoA molecule combines with oxaloacetic acid, forming 6C citrate, and releasing CoA.
2. Citric acid changes the arrangement of atoms to form isocitric acid.
3. Isocitric acid is converted to 5C α-ketoglutaric acid, which yield NADH and CO₂.
4. α-ketoglutaric acid Loges the second CO₂ and generates another NADH, plus 4C succinyl CoA.
5. Succinyl CoA is converted to succinic acid and Regenerates CoA. This releases energy that is captured in ATP.
6. Succinic acid loses 2 H⁺ and 2 e⁻ yielding fumaric acid and generating FADH₂.
7. Fumaric acid reacts with water to form malic acid.
8. An additional NADH is formed when malic acid is converted to oxaloacetic acid, which is the final product to enter the cycle again, by reacting with acetyl CoA.
The Respiratory Chain: Electron Transport and Oxidative Phosphorylation

- Final processing of electrons and hydrogen and the major generator of ATP
- Chain of redox carriers that receive electrons from reduced carriers (NADH and FADH$_2$)
- ETS shuttles electrons down the chain, energy is released and subsequently captured and used by **ATP synthase** complexes to produce ATP – Oxidative phosphorylation
Figure 8.21 Electron Transport and Chemiosmosis

- **Matrix (inner compartment)**
  - Cristae
  - Outer mitochondrial membrane
  - Inner mitochondrial membrane

- **Outer mitochondrial membrane**
- **Intermembrane space of matrix**
- **Outer membrane**
- **Intermembrane space (outer compartment)**

- **NADH dehydrogenase**
  - FMN
  - Coenzyme Q
  - Cytochrome c
  - Cytochrome a
  - Cytochrome a3

- **From Krebs and glycolysis**
- **2 H+**

- **ATP synthase**
- **NAD**
- **FADH2**
- **Cytochrome c1**
- **Cytochrome b**
- **Cytochrome c**
- **Cytochrome a3**
- **Cytochrome a**
- **1/2 O2**
- **H2O**
- **2 H+**

**Legend:**
- Blue: Outer compartment (intermembrane space)
- Purple: Inner compartment (mitochondrial matrix)
The Formation of ATP and Chemiosmosis

- **Chemiosmosis** – as the electron transport carriers shuttle electrons, they actively pump hydrogen ions (protons) across the membrane setting up a gradient of hydrogen ions – *proton motive force*

- Hydrogen ions diffuse back through the ATP synthase complex causing it to rotate, causing a 3-D change resulting in the production of ATP
(a) As the carriers in the mitochondrial cristae transport electrons, they also actively pump H+ ions (protons) to the intermembrane space, producing a chemical and charge gradient between the outer and inner mitochondrial compartments.

(b) The distribution of electric potential and the concentration gradient of protons across the membrane drive the synthesis of ATP by ATP synthase. The rotation of this enzyme couples Diffusion of H+ to the inner compartment with the bonding of ADP and P_i. The final event of electron transport is the reaction of the electrons with the H+ and O_2 to form metabolic H_2O. This step is catalyzed by cytochrome oxidase (cytochrome aa_3).
Enlarged view of bacterial cell envelope to show the relationship of electron transport and ATP synthesis. Bacteria have the ETS and ATP synthase stationed in the cell membrane. ETS carriers transport H+ and electrons from the cytoplasm to the exterior of the membrane. Here, it is collected to create a gradient just as it occurs in mitochondria.
The Terminal Step of Electron Transport

• Oxygen accepts 2 electrons from the ETS and then picks up 2 hydrogen ions from the solution to form a molecule of water. Oxygen is the final electron acceptor

\[ 2H^+ + 2e^- + \frac{1}{2}O_2 \rightarrow H_2O \]
Figure 8.23 Theoretic ATP Yield for Aerobic Respiration

Glucose

| Glycolysis |
|-----------------|-----------------|-----------------|
| Fructose 1, 6-bis PO<sub>4</sub> | 2 Glyceraldehyde 3 PO<sub>4</sub> | 2 Pyruvate |

- **2 NADHs** Oxidative phosphorylation → 6 ATPs
- **2 ATPs** Substrate-level phosphorylation
- **2 NADHs** in ETS → 6 ATPs

2 Acetyl-CoA

- **6 NADHs** in ETS → 18 ATPs
- **2 FADH<sub>2</sub>s** in ETS → 4 ATPs

2 Krebs cycles

- **2 ATPs** Substrate-level phosphorylation

Total aerobic yield: **38 ATP maximum**
Anaerobic Respiration

• Functions like aerobic respiration except it utilizes oxygen containing ions, rather than free oxygen, as the final electron acceptor
  – Nitrate (NO$_3^-$) and nitrite (NO$_2^-$)
• Most obligate anaerobes use the H$^+$ generated during glycolysis and the Kreb’s cycle to reduce some compound other than O$_2$
The Importance of Fermentation

- Incomplete oxidation of glucose or other carbohydrates in the absence of oxygen
- Uses organic compounds as terminal electron acceptors
- Yields a small amount of ATP
- Production of ethyl alcohol by yeasts acting on glucose
- Formation of acid, gas, and other products by the action of various bacteria on pyruvic acid

An organic molecule is final electron acceptor (pyruvate, acetaldehyde, etc.).

Maximum ATP produced = 2
Figure 8.24 Alcoholic & Acidic Fermentation

System: Yeasts

Glucose → NAD+ → NADH

System: Homolactic bacteria

Human muscle

Glycolysis

Pyruvic acid

Acetaldehyde + H → NADH

Ethyl alcohol

Lactic acid

CO2
Figure 8.25 Products of Pyruvate Fermentation

Pyruvate

- Butyric acid (Clostridium)
- Acetoin (Propionibacterium)
- Ethanol (Yeast)
- CO₂ + H₂ (Proteus)
- Formic acid
- Lactic acid (Streptococcus, Lactobacillus)
- Oxaloacetic acid
- Acetoacetic acid
- Acetoin
- Oxaloacetic acid
- Acetylmethylcarbinol
- 2,3 butanediol
- Succinic acid
- Propionic acid
- Acetyl CoA
- Acetic acid (Acetobacterium)
- NADH

Enterobacter

Propionibacterium

Mixed acids

Escherichia, Shigella

Enterobacteriaceae
8.4 Biosynthesis and the Crossing Pathways of Metabolism

- Many pathways of metabolism are bi-directional or **amphibolic**
Biosynthesis and the Crossing Pathways of Metabolism

• Catabolic pathways contain molecular intermediates (metabolites) that can be diverted into anabolic pathways
  – Pyruvic acid can be converted into amino acids through amination
  – Amino acids can be converted into energy sources through deamination
  – Glyceraldehyde-3-phosphate can be converted into precursors for amino acids, carbohydrates, and fats