LECTURE PRESENTATIONS

For BROCK BIOLOGY OF MICROORGANISMS, THIRTEENTH EDITION

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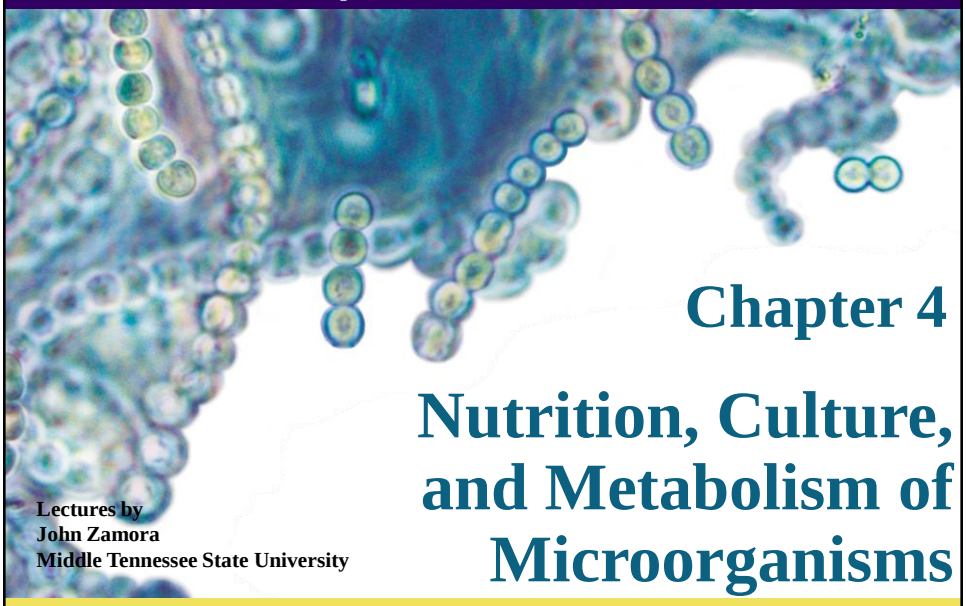

Chapter 4

Nutrition, Culture, and Metabolism of Microorganisms

Lectures by
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I. Nutrition, Culture, and Metabolism of Microorganisms

- 4.1 Nutrition and Cell Chemistry
- 4.2 Culture Media
- 4.3 Laboratory Culture

4.1 Nutrition and Cell Chemistry

- *Metabolism*
  - The sum total of all chemical reactions that occur in a cell
- *Catabolic reactions (catabolism)*
  - Energy-releasing metabolic reactions
- *Anabolic reactions (anabolism)*
  - Energy-requiring metabolic reactions
- Most knowledge of microbial metabolism is based on study of laboratory cultures

4.1 Nutrition and Cell Chemistry

- *Nutrients*
  - Supply of monomers (or precursors of) required by cells for growth
- *Macronutrients*
  - Nutrients required in large amounts
- *Micronutrients*
  - Nutrients required in trace amount

4.1 Nutrition and Cell Chemistry

- *Carbon*
  - Required by all cells
  - Typical bacterial cell ~50% carbon (by dry weight)
  - Major element in all classes of macromolecules
  - Heterotrophs use organic carbon
  - Autotrophs use inorganic carbon

4.1 Nutrition and Cell Chemistry

- *Nitrogen*
  - Typical bacterial cell ~12% nitrogen (by dry weight)
  - Key element in proteins, nucleic acids, and many more cell constituents

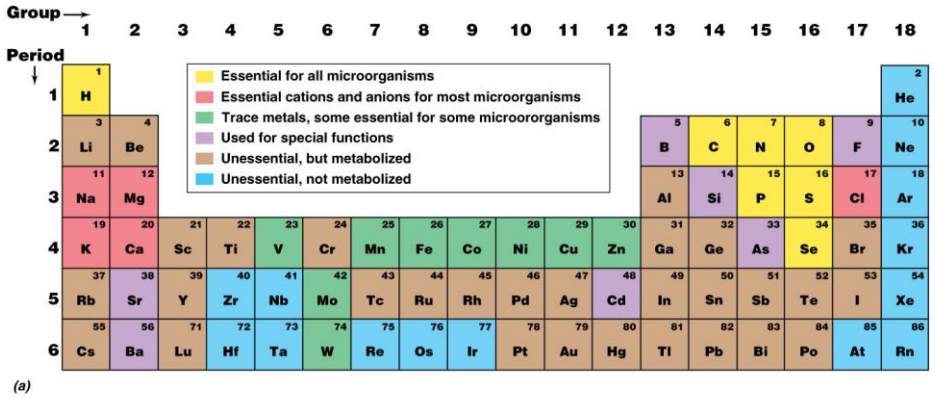
4.1 Nutrition and Cell Chemistry

- Other Macronutrients
  - *Phosphorus (P)*
    - Synthesis of nucleic acids and phospholipids
  - *Sulfur (S)*
    - Sulfur-containing amino acids (cysteine and methionine)
    - Vitamins (e.g., thiamine, biotin, lipoic acid) and coenzyme A
  - *Potassium (K)*
    - Required by enzymes for activity

4.1 Nutrition and Cell Chemistry

- Other Macronutrients (cont'd)
  - *Magnesium (Mg)*
    - Stabilizes ribosomes, membranes, and nucleic acids
    - Also required for many enzymes
  - *Calcium (Ca)*
    - Helps stabilize cell walls in microbes
    - Plays key role in heat stability of endospores
  - *Sodium (Na)*
    - Required by some microbes (e.g., marine microbes)

Figure 4.1 Elemental and macromolecular composition of a bacterial cell



4.1 Nutrition and Cell Chemistry

- Micronutrients: *Iron*
  - Key component of cytochromes and FeS proteins involved in electron transport
  - Under anoxic conditions, generally ferrous (Fe^{2+}) form; soluble
  - Under oxic conditions: generally ferric (Fe^{3+}) form; exists as insoluble minerals
  - Cells produce *siderophores* (iron-binding agents) to obtain iron from insoluble mineral form (Figure 4.2)

Figure 4.2 Mechanism of hydroxamate siderophores

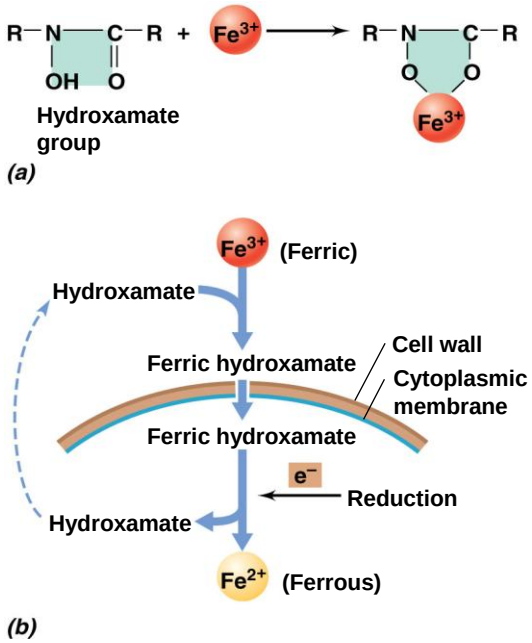


Table 4.1 Micronutrients (trace elements) needed by microorganisms

Table 4.1 *Micronutrients (trace elements) needed by microorganisms*[a]

Element	*Cellular function or molecule of which a part*
Boron (B)	Autoinducer for quorum sensing in bacteria; also found in some polyketide antibiotics
Chromium (Cr)	Possible but not proven component for glucose metabolism (necessary in mammals)
Cobalt (Co)	Vitamin B_{12}; transcarboxylase (only in propionic acid bacteria)
Copper (Cu)	In respiration, cytochrome *c* oxidase; in photosynthesis, plastocyanin, some superoxide dismutases
Iron (Fe)[b]	Cytochromes; catalases; peroxidases; iron–sulfur proteins; oxygenases; all nitrogenases
Manganese (Mn)	Activator of many enzymes; component of certain superoxide dismutases and of the water-splitting enzyme in oxygenic phototrophs (photosystem II)
Molybdenum (Mo)	Certain flavin-containing enzymes; some nitrogenases, nitrate reductases, sulfite oxidases, DMSO-TMAO reductases; some formate dehydrogenases
Nickel (Ni)	Most hydrogenases; coenzyme F_{430} of methanogens; carbon monoxide dehydrogenase; urease
Selenium (Se)	Formate dehydrogenase; some hydrogenases; the amino acid selenocysteine
Tungsten (W)	Some formate dehydrogenases; oxotransferases of hyperthermophiles
Vanadium (V)	Vanadium nitrogenase; bromoperoxidase
Zinc (Zn)	Carbonic anhydrase; alcohol dehydrogenase; RNA and DNA polymerases; and many DNA-binding proteins

[a]Not every micronutrient listed is required by all cells; some metals listed are found in enzymes or cofactors present in only specific microorganisms.
[b]Needed in greater amounts than other trace metals.

4.1 Nutrition and Cell Chemistry

- Micronutrients: *Growth Factors*
  - Organic compounds required in small amounts by certain organisms
    - Examples: vitamins, amino acids, purines, pyrimidines
  - *Vitamins*
    - Most commonly required growth factors
    - Most function as coenyzmes

4.2 Culture Media

- *Culture Media*
  - Nutrient solutions used to grow microbes in the laboratory
- Two broad classes
  - *Defined media*: precise chemical composition is known
  - *Complex media*: composed of digests of chemically undefined substances (e.g., yeast and meat extracts)

4.2 Culture Media

- *Selective Media*
  - Contains compounds that selectively inhibit growth of some microbes but not others
- *Differential Media*
  - Contains an indicator, usually a dye, that detects particular chemical reactions occurring during growth

4.2 Culture Media

- For successful cultivation of a microbe, it is important to know the nutritional requirements and supply them in proper form and proportions in a culture medium

4.3 Laboratory Culture

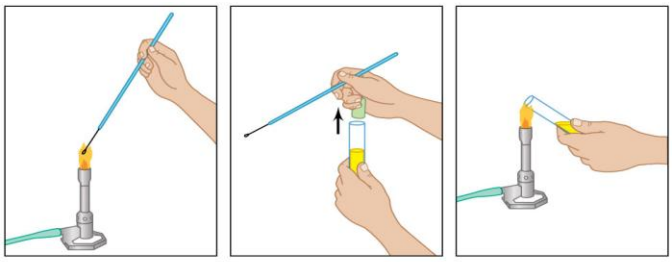
- *Pure culture*: culture containing only a single kind of microbe
- *Contaminants*: unwanted organisms in a culture
- Cells can be grown in liquid or solid culture media
  - Solid media are prepared by addition of a gelling agent (*agar* or gelatin)
  - When grown on solid media, cells form isolated masses (*colonies*)

4.3 Laboratory Culture

- Microbes are everywhere
  - Sterilization of media is critical
  - Aseptic technique should be followed (Figure 4.4)

PLAY Animation: Aseptic Transfer and the Streak Plate Method

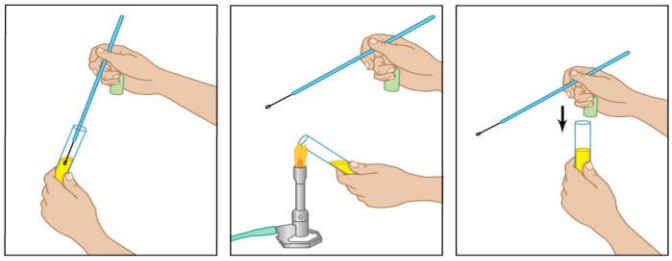
Figure 4.4 Aseptic transfer



(a) Loop is heated until red hot and cooled in air briefly.

(b) Tube is uncapped.

(c) Tip of tube is run through the flame.



(d) Sample is removed on sterile loop for transfer to a sterile medium.

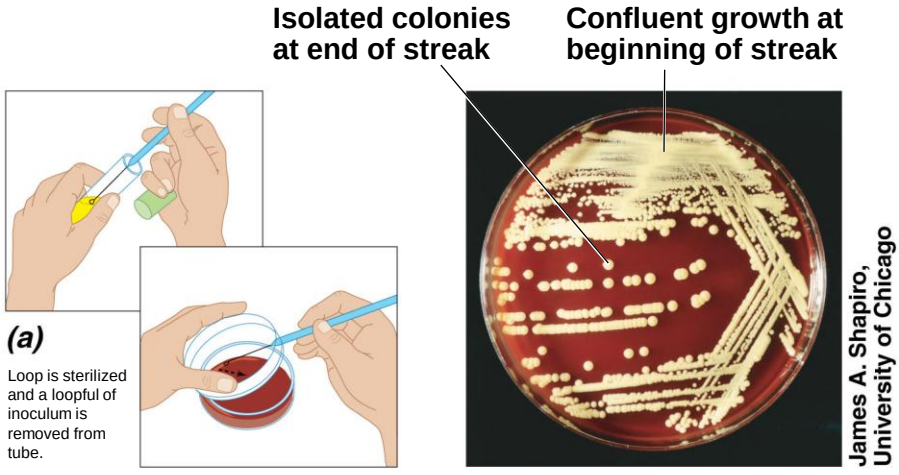
(e) The tube is reflamed

(f) The tube is recapped. Loop is reheated before being taken out of service.

4.3 Laboratory Culture

- Pure culture technique
  - Streak plate (Figure 4.5)
  - Pour plate
  - Spread plate

Figure 4.5 Making a streak plate to obtain pure cultures



(a) Loop is sterilized and a loopful of inoculum is removed from tube.

(b) Streak is made and spread out on a sterile agar plate. Following the initial streak, subsequent streaks are made at angles to it, the loop being resterilized between streaks.

(c) Appearance of a well-streaked plate after incubation, showing colonies of the bacterium *Micrococcus luteus on a* blood agar plate. It is from such well-isolated colonies that pure cultures can usually be obtained.

II. Energetics and Enzymes

- 4.4 Bioenergetics
- 4.5 Catalysis and Enzymes

4.4 Bioenergetics

- Energy is defined in units of kilojoules (kJ), a measure of heat energy
- In any chemical reaction, some energy is lost as heat
- *Free energy (G)*: energy released that is available to do work
- The change in free energy during a reaction is referred to as $\Delta G^{0\prime}$
- $\Delta G^{0\prime}$: under standard conditions; 1 M, pH 7, 25°C, 1 atm

4.4 Bioenergetics

- Reactions with a negative $\Delta G^{0\prime}$ release free energy (*exergonic*)
- Reactions with a positive $\Delta G^{0\prime}$ require energy (*endergonic*)
- To calculate free-energy yield of a reaction, we need to know the *free energy of formation* (G_f^0; the energy released or required during formation of a given molecule from the elements)

4.4 Bioenergetics

- For the reaction A + B ➡ C + D,

  $\Delta G^{0\prime} = G_f^0$ [C+D] - G_f^0[A+B]

- $\Delta G^{0\prime}$ not always a good estimate of actual free-energy changes
- ΔG: free energy that occurs under actual conditions

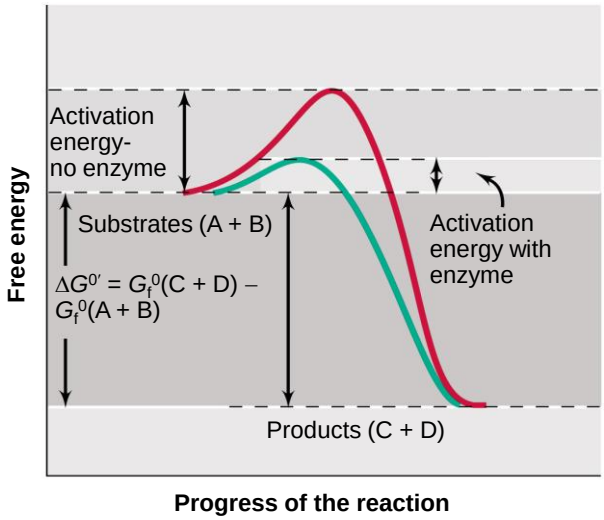
  $\Delta G = \Delta G^{0\prime} + RT \ln k$

  where R (8.29 J/mol/kelvin) and T (K=°C+273.15) are physical constants and k is the equilibrium constant for the reaction in question

4.5 Catalysis and Enzymes

- Free-energy calculations do not provide information on reaction rates
- *Activation energy*: energy required to bring all molecules in a chemical reaction into the reactive state (Figure 4.6)
  - A catalysis is usually required to breach activation energy barrier

Figure 4.6 Activation energy and catalysis



- Even chemical reactions that release energy may not proceed spontaneously, because the reactants must first be activated.
- Once they are activated, the reaction proceeds spontaneously.
- Catalysts such as enzymes lower the required activation energy.

4.5 Catalysis and Enzymes

- *Catalyst*: substance that
  - Lowers the activation energy of a reaction
  - Increases reaction rate
  - Does not affect energetics or equilibrium of a reaction

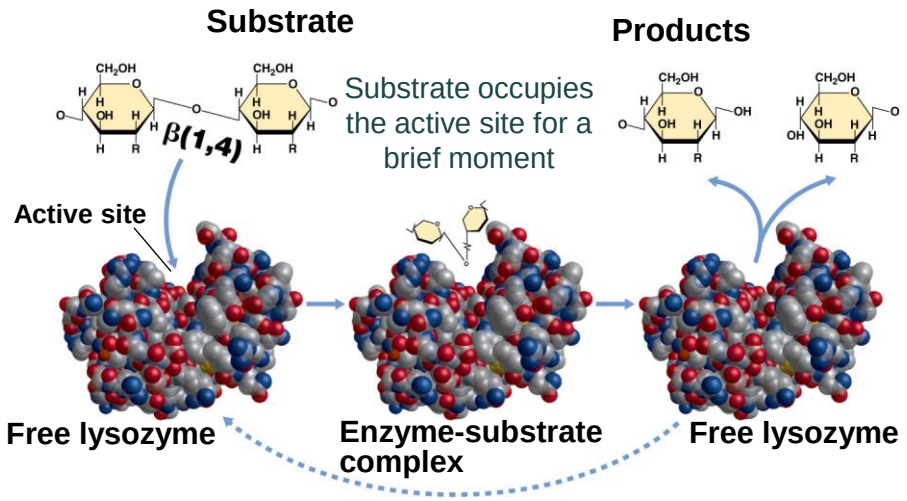
4.5 Catalysis and Enzymes

- *Enzymes*
  - Biological catalysts
  - Typically proteins (some RNAs)
  - Highly specific
  - Generally larger than substrate
  - Typically rely on weak bonds
    - Examples: hydrogen bonds, van der Waals forces, hydrophobic interactions
  - *Active site*: region of enzyme that binds substrate

4.5 Catalysis and Enzymes

- Enzymes (cont'd)
  - Increase the rate of chemical reactions by 10^8 to 10^{20} times the spontaneous rate
  - Enzyme catalysis: E + S ⟷ E – S ⟷ E + P (Figure 4.7)
  - Catalysis dependent on
    - Substrate binding
    - Position of substrate relative to catalytically active amino acids in active site

Figure 4.7 The catalytic cycle of an enzyme



Following binding in the enzyme's active site, strain is placed on the bond, and this favors breakage.

4.5 Catalysis and Enzymes

- Many enzymes contain small nonprotein molecules that participate in catalysis but are not substrates
- *Prosthetic groups*
  - Bind tightly to enzymes
  - Usually bind covalently and permanently (e.g., heme group in cytochromes)
- *Coenzymes*
  - Loosely bound to enzymes
  - Most are derivatives of vitamins (e.g., NAD^+/NADH)

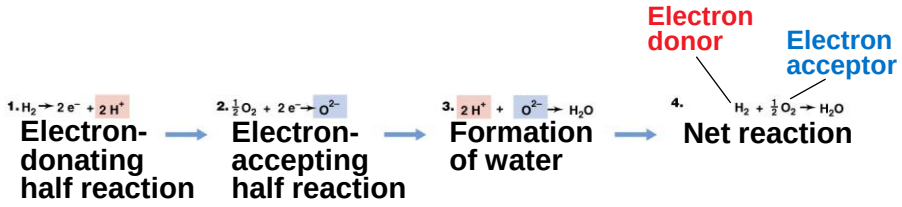
III. Oxidation–Reduction and Energy-Rich Compounds

- 4.6 Electron Donors and Electron Acceptors
- 4.7 Energy-Rich Compounds and Energy Storage

4.6 Electron Donors and Electron Acceptors

- Energy from oxidation–reduction (*redox*) reactions is used in synthesis of energy-rich compounds (e.g., ATP)
- Redox reactions occur in pairs (two *half reactions*; Figure 4.8)
- *Electron donor*: the substance oxidized in a redox reaction
- *Electron acceptor*: the substance reduced in a redox reaction

Figure 4.8 Example of an oxidation–reduction reaction



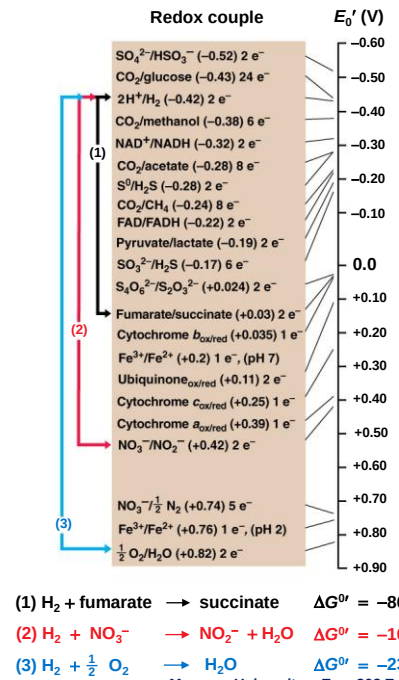
4.6 Electron Donors and Electron Acceptors

- *Reduction potential (E_0')*: tendency to donate electrons
  - Expressed as volts (V)
- Substances can be either electron donors or acceptors under different circumstances (*redox couple*)
- Reduced substance of a redox couple with a more negative E_0' donates electrons to the oxidized substance of a redox couple with a more positive E_0'

4.6 Electron Donors and Electron Acceptors

- The *redox tower* represents the range of possible reduction potentials (Figure 4.9)
- The reduced substance at the top of the tower donates electrons
- The oxidized substance at the bottom of the tower accepts electrons
- The farther the electrons "drop," the greater the amount of energy released

Figure 4.9 The redox tower



4.6 Electron Donors and Electron Acceptors

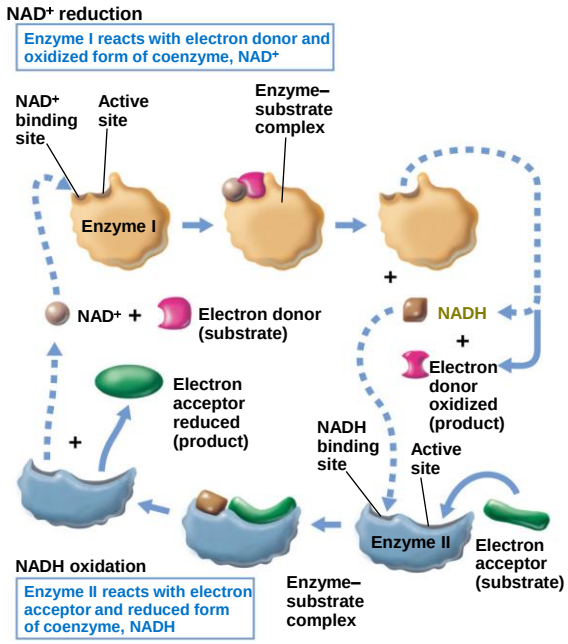
- Redox reactions usually involve reactions between intermediates (*carriers*)
- Electron carriers are divided into two classes
  - Prosthetic groups (attached to enzymes)
  - Coenzymes (diffusible)
    - Examples: NAD^+, $NADP^+$

(NAD^+: nicotinamide adenine dinucleotide)

4.6 Electron Donors and Electron Acceptors

- NAD^+ and NADH facilitate redox reactions without being consumed; they are recycled (Figure 4.11)

Figure 4.11 NAD^+/NADH cycling



4.7 Energy-Rich Compounds and Energy Storage

- Chemical energy released in redox reactions is primarily stored in certain phosphorylated compounds
  - ATP; the prime energy currency
  - Phosphoenolpyruvate
  - Glucose 6-phosphate
- Chemical energy also stored in coenzyme A

4.7 Energy-Rich Compounds and Energy Storage

- Long-term energy storage involves insoluble polymers that can be oxidized to generate ATP
  - Examples in prokaryotes
    - Glycogen
    - Poly-β-hydroxybutyrate and other polyhydroxyalkanoates
    - Elemental sulfur
  - Examples in eukaryotes
    - Starch
    - Lipids (simple fats)

IV. Essentials of Catabolism

- 4.8 Glycolysis
- 4.9 Respiration and Electron Carriers
- 4.10 The Proton Motive Force
- 4.11 The Citric Acid Cycle
- 4.12 Catabolic Diversity

4.8 Glycolysis

- Two reaction series are linked to energy conservation in chemoorganotrophs: fermentation and respiration (Figure 4.13)
- Differ in mechanism of ATP synthesis
  - *Fermentation*: substrate-level phosphorylation; ATP directly synthesized from an energy-rich intermediate
  - *Respiration*: oxidative phosphorylation; ATP produced from proton motive force formed by transport of electrons

4.8 Glycolysis

- Glycolysis
  - Glucose consumed
  - Two ATPs produced
  - Fermentation products generated
    - Some harnessed by humans for consumption

4.9 Respiration and Electron Carriers

- *Aerobic Respiration*
  - Oxidation using O_2 as the terminal electron acceptor
  - Higher ATP yield than fermentations
    - ATP produced at the expense of the proton motive force, which is generated by electron transport

4.9 Respiration and Electron Carriers

- *Electron Transport Systems*
  - Membrane associated
  - Mediate transfer of electrons
  - Conserve some of the energy released during transfer and use it to synthesize ATP
  - Many oxidation–reduction enzymes are involved in electron transport (e.g., NADH dehydrogenases, flavoproteins, iron–sulfur proteins, cytochromes)

4.10 The Proton Motive Force

- Electron transport system oriented in cytoplasmic membrane so that electrons are separated from protons
- Electron carriers arranged in membrane in order of their reduction potential
- The final carrier in the chain donates the electrons and protons to the terminal electron acceptor

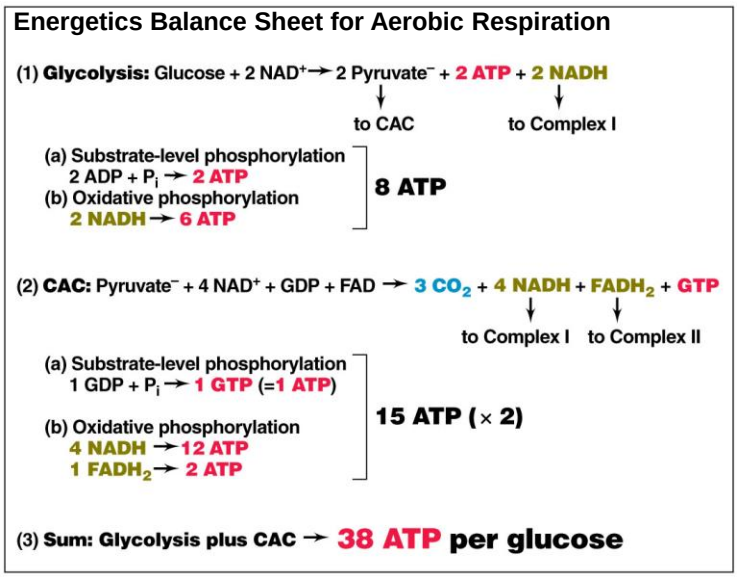
4.10 The Proton Motive Force

- During electron transfer, several protons are released on outside of the membrane
  - Protons originate from NADH and the dissociation of water
- Results in generation of pH gradient and an electrochemical potential across the membrane (the *proton motive force*)
  - The inside becomes electrically negative and alkaline
  - The outside becomes electrically positive and acidic

4.11 The Citric Acid Cycle

- *Citric acid cycle (CAC)*: pathway through which pyruvate is completely oxidized to CO_2
  - Initial steps (glucose to pyruvate) same as glycolysis
  - Per glucose molecule, 6 CO_2 molecules released and NADH and FADH generated
  - Plays a key role in catabolism and biosynthesis
- Energetics advantage to aerobic respiration

Figure 4.21b The citric acid cycle

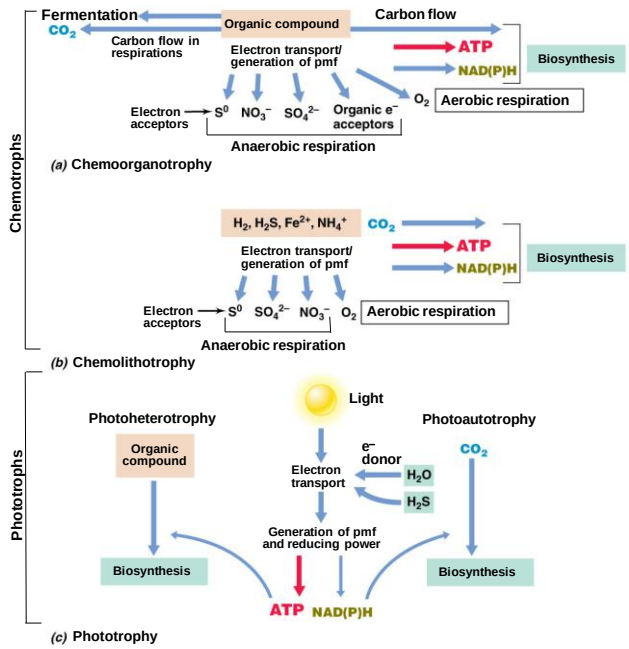


(b)

4.12 Catabolic Diversity

- Microorganisms demonstrate a wide range of mechanisms for generating energy (Figure 4.22)
  - Fermentation
  - Aerobic respiration
  - Anaerobic respiration
  - Chemolithotrophy
  - Phototrophy

Figure 4.22 Catabolic diversity



Chemoorganotrophs differ from chemolithotrophs in two important ways:

1. The nature of the electron donor (organic vs. inorganic compounds, respectively) and
2. The nature of the source of cellular carbon (organic compounds vs. CO_2 respectively).

However, note the importance of electron transport driving proton motive force formation in all forms of respiration and in photosynthesis.

4.12 Catabolic Diversity

- *Anaerobic Respiration*
  - The use of electron acceptors other than oxygen
    - Examples include nitrate (NO_3^-), ferric iron (Fe^{3+}), sulfate (SO_4^{2-}), carbonate (CO_3^{2-}), certain organic compounds
  - Less energy released compared to aerobic respiration
  - Dependent on electron transport, generation of a proton motive force, and ATPase activity

4.12 Catabolic Diversity

- *Chemolithotrophy*
  - Uses inorganic chemicals as electron donors
    - Examples include hydrogen sulfide (H_2S), hydrogen gas (H_2), ferrous iron (Fe^{2+}), ammonia (NH_3)
  - Typically aerobic
  - Begins with oxidation of inorganic electron donor
  - Uses electron transport chain and proton motive force
  - Autotrophic; uses CO_2 as carbon source

4.12 Catabolic Diversity

- *Phototrophy*: uses light as energy source
  - *Photophosphorylation*: light-mediated ATP synthesis
  - *Photoautotrophs*: use ATP for assimilation of CO_2 for biosynthesis
  - *Photoheterotrophs*: use ATP for assimilation of organic carbon for biosynthesis

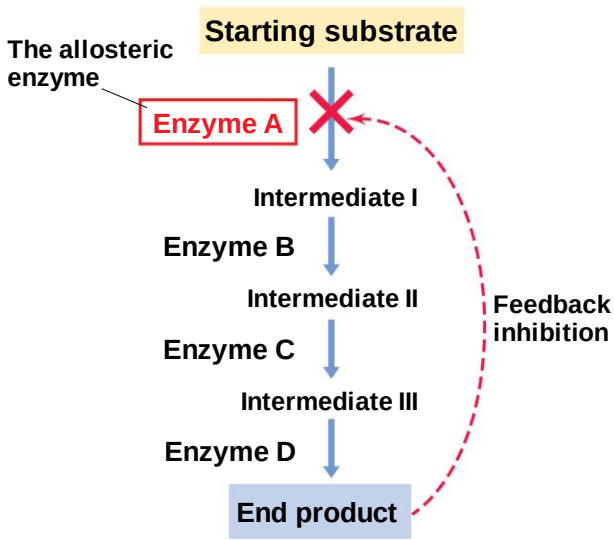
Regulating the Activity of Biosynthetic Enzymes

- Two major modes of enzyme regulation
  - *Amount*
    - Regulation at the gene level
  - *Activity*
    - Temporary inactivation of the protein through changes in enzyme structure

Regulating the Activity of Biosynthetic Enzymes

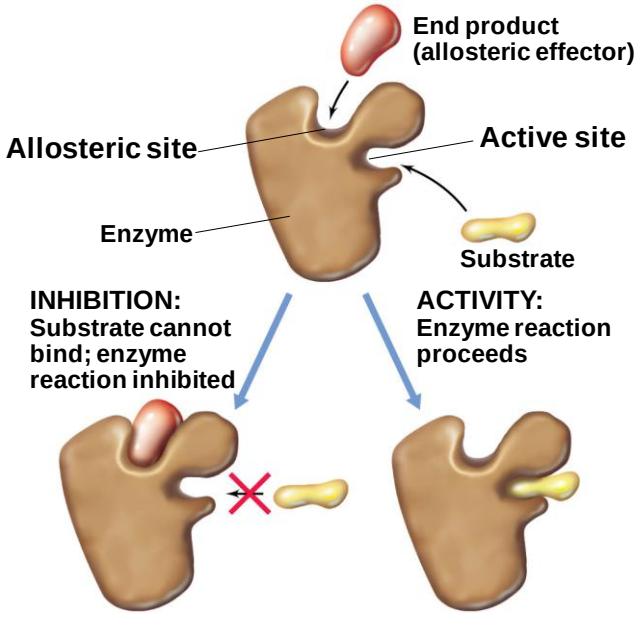
- *Feedback Inhibition*: mechanism for turning off the reactions in a biosynthetic pathway (Figure 4.28)
  - End product of the pathway binds to the first enzyme in the pathway, thus inhibiting its activity
  - The inhibited enzyme is an *allosteric* enzyme (Figure 4.29)
    - Two binding sites: active and allosteric
  - Reversible reaction

Figure 4.28 Feedback inhibition of enzyme activity



The activity of the first enzyme of the pathway is inhibited by the end product, thus shutting off the production of the three intermediates and the end product

Figure 4.29 The mechanism of allosteric inhibition by the end product of a pathway



- When the end product binds at the allosteric site, conformation of the enzyme is so altered that the substrate can no longer bind to the active site.
- However, inhibition is reversible, and end product limitation will once again activate the enzyme.