Metabolism: what you know and what might surprise you

**Lecture**

Chapter 5
Enzymes
Aerobic and anaerobic respiration

**Lab**

Streak plate subcultures
Staining: Gram stain
Motility

**Pre-labs**

Review Gram Staining and other assays
Microbial metabolic diversity - how is it possible?

Recipe:
- 500 ml mud from beach at low tide
- 10 g filter paper (cellulose)
- 1 g NH₄Cl
- 1 g KH₂PO₄
- 1 g CaSO₄
- Water
Metabolism is possible through enzymatic diversity
Enzyme structure

Cofactors

Ions of iron, zinc, magnesium and calcium

Coenzymes

Nicotinamide adenine dinucleotide (phosphate) - NAD+/ NADP+ from B vitamin niacin

Flavin adenine dinucleotide- FAD from B vitamin riboflavin
Effects on enzyme activity: temp and pH

Figure 5.6

(a) Optimum temperature curve showing increasing enzyme activity as temperature increases up to an optimum point, beyond which enzymes become denatured.

(b) Optimum pH curve showing increasing enzyme activity as pH increases up to an optimum point.

Active (functional) protein → Denatured protein
Effects on enzyme activity: competitive inhibition

Example:

Sulfa drugs
Effects on enzyme activity: noncompetitive inhibition

Example: Mercury poisoning
Effects on enzyme activity: feedback inhibition

Figure 5.8
Why would it be beneficial to have a fever during a bacterial infection? Why is a fever over 40°C often life threatening?
Metabolism: catabolism and anabolism

anabolism ~ dehydration synthesis ~ condensation

catabolism ~ hydrolysis ~ decomposition

Figure 2.8
Redox reactions - the basis of metabolism

**TABLE 5.1**

<table>
<thead>
<tr>
<th>Oxidation</th>
<th>Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss of electrons (A)</td>
<td>Gain of electrons (B)</td>
</tr>
<tr>
<td>Gain of oxygen</td>
<td>Loss of oxygen</td>
</tr>
<tr>
<td>Loss of hydrogen</td>
<td>Gain of hydrogen</td>
</tr>
<tr>
<td>Loss of energy (liberates energy)</td>
<td>Gain of energy (stores energy in the reduced compound)</td>
</tr>
<tr>
<td>Exothermic; exergonic (gives off heat energy)</td>
<td>Endothermic; endergonic (requires energy, such as heat)</td>
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</table>

**Oxidation**

Transfer

A

B

Oxidized

Reduced

Reduction of B
Redox reactions - the basis of metabolism

**TABLE 5.1**

**Comparison of Oxidation and Reduction**

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Oxidation

Transfer

\[ A \rightarrow B \rightarrow A + B \]

Reduction of B

Oxidized

Reduced

Table 5-1 Microbiology, 6/e
© 2005 John Wiley & Sons
Major electron carriers

- FAD accepts two H⁺/e- → FADH₂
- NAD⁺ accepts one H⁺/e- → NADH
- Cytochromes accept e-
Phosphorylation reactions or
HOW WE MAKE ATP

1. Substrate level phosphorylation

2. Oxidative phosphorylation

3. Photophosphorylation
Phosphorylation reactions or
HOW WE MAKE ATP

1. Substrate level phosphorylation

2. Oxidative phosphorylation

3. Photophosphorylation
1. Substrate level phosphorylation
Phosphorylation reactions or HOW WE MAKE ATP

1. Substrate level phosphorylation

2. Oxidative phosphorylation

3. Photophosphorylation
2. Oxidative Phosphorylation
(Carbohydrate catabolism)

Aerobic respiration
Anaerobic respiration

Fermentation
- Alcohol ferm
- Lactic acid ferm
- Mixed acid ferm
- Butanediol ferm
- Butylic/butyric acid
- Etc.
Let’s review: aerobic respiration

Steps:

1. Glycolysis
   1a. Pentose phosphate pathway
   1b. Entner-Doudoroff pathway
2. Transition/preparatory step
3. Krebs Cycle/ TCA
4. Electron transport chain (ETC)

Total energy output:
How does the ETC make so much ATP?
What is a terminal electron acceptor?

In aerobic respiration = oxygen

In anaerobic respiration = no oxygen

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<tr>
<th>Bacteria</th>
<th>Electron acceptor</th>
<th>Products</th>
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<tr>
<td><em>Pseudomonas</em>, <em>Bacillus</em></td>
<td>NO$_3^-$</td>
<td>NO$_2^-$, N$_2$ + H$_2$O</td>
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<tr>
<td><em>Desulfovibrio</em></td>
<td>SO$_4^{2-}$</td>
<td>H$_2$S + H$_2$O</td>
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<tr>
<td>methanogens</td>
<td>CO$_3^{2-}$</td>
<td>CH$_4$ + H$_2$O</td>
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Anaerobic respiration

Steps:

1. Glycolysis
   1a. Pentose phosphate pathway
   1b. Entner-Doudoroff pathway

2. Intermediate step

3. Krebs Cycle/ TCA

4. Electron transport chain (ETC)

Total energy output:
Independent Study

1. Review the light dependent and light independent reactions of photosynthesis (see Figure 5.25 and 5.26).

***Print out and bring **APO-2: A Metabolism Case Study** for next class.
More cool microbial metabolism

**Lecture**
- Continue Chapter 5
- Fermentation
- Photophosphorylation
- Microbial metabolic diversity
- APO 2: Case study in fermentation

**Lab**
- Acid fast, spore and capsule stains
- Pre-labs
  - Using the Spectrophotometer and Exam Review
Let’s review: aerobic respiration

Steps:

1. Glycolysis
   - 2 substrate level ATP
   - 2 NADH
   1a. Pentose phosphate pathway
   1b. Entner-Doudoroff pathway

2. Transition/preparatory step
   - 2CO₂
   - 2 NADH

3. Krebs Cycle/ TCA
   - 2 substrate level ATP
   - 4 CO₂
   - 6 NADH
   - 2 FADH₂

4. Electron transport chain (ETC)
   - 34 ATP

Total energy output:

- 38 ATP (prokaryotes)
- 36 ATP (eukaryotes)
What is a terminal electron acceptor?

In **aerobic respiration** = oxygen

In **anaerobic respiration** = no oxygen

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Anaerobic respiration

Steps:
1. Glycolysis
   - 2 substrate level ATP
   - 2 NADH
1a. Pentose phosphate pathway
1b. Entner-Doudoroff pathway
2. Intermediate step
   - 2CO₂
   - 2 NADH
3. Krebs Cycle/ TCA
   - partially utilized
4. Electron transport chain (ETC)
   - partially utilized

Total energy output:
Varied, between 2-38 ATP
Varieties of fermentation

Steps:

1. Glycolysis
   - 2 substrate level ATP
   - 2 NADH

2. Fermentative pathway
   - **Lactic acid fermentation
     - Homolactic
     - OR
     - Heterolactic
   - **Alcoholic fermentation

Additional fermentation pathways
Fermentative microbes

See Figure 5.18 and Table 5.4
# Comparison of catabolic efficiency

<table>
<thead>
<tr>
<th>Energy-Producing Process</th>
<th>Growth Conditions</th>
<th>Final Hydrogen (Electron) Acceptor</th>
<th>Type of Phosphorylation Used to Generate ATP</th>
<th>ATP Molecules Produced per Glucose Molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aerobic Respiration</strong></td>
<td>Aerobic</td>
<td>Molecular oxygen (O₂)</td>
<td>Substrate-level and oxidative</td>
<td>36 (eukaryotes) 38 (prokaryotes)</td>
</tr>
<tr>
<td><strong>Anaerobic Respiration</strong></td>
<td>Anaerobic</td>
<td>Usually an inorganic substance (such as NO₃⁻, SO₄²⁻, or CO₃²⁻) but not molecular oxygen (O₂)</td>
<td>Substrate-level and oxidative</td>
<td>Variable (fewer than 38 but more than 2)</td>
</tr>
<tr>
<td><strong>Fermentation</strong></td>
<td>Aerobic or anaerobic</td>
<td>An organic molecule</td>
<td>Substrate-level</td>
<td>2</td>
</tr>
</tbody>
</table>

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Reminder: other organic molecules can be used for ATP production
What good are alternative metabolisms to us?

Pt. Loma Wastewater Treatment Plant

How does it happen?

(a) PRIMARY TREATMENT
1. Sewage is screened, skimmed, and ground.
2. Solid matter settles out.

(b) SECONDARY TREATMENT (biological oxidation)
3. Primary effluent undergoes aeration; microorganisms oxidize organic matter.
   - Trickling filter (see Figure 27.21)

(c) DISINFECTION AND RELEASE
4. Effluent is disinfected by chlorination and released.
   - Chlorinator

(d) SLUDGE DIGESTION
5. Remaining sludge is digested anaerobically, producing methane.
6. Sludge effluent is dried.
7. Sludge is removed and disposed of in landfill or agricultural land.

KEY
- Physical processes
- Microbial processes
- Chemical processes

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Phosphorylation reactions or HOW WE MAKE ATP

1. Substrate level phosphorylation

2. Oxidative phosphorylation

3. Photophosphorylation
3. Photophosphorylation

Photo reactions of photosynthesis
Photo reactions: cyclic and non-cyclic photophosphorylation

Cyclic outcomes

e- thru ETC produce ATP
e- recycle back to chlorophyll

Non-cyclic outcomes

e- thru ETC produce ATP
Terminal acceptor is NADP+
Photoysis recycles e- to chlorophyll:
\[ H_2O \rightarrow 2H^+ + \frac{1}{2}O_2 + 2e^- \]
What is the ATP and NADPH used for?

**Calvin-Benson cycle**

Input:
- 3 CO₂

3 ATP → 3 ADP

3 ATP → Input

Output:
- 1 Glycerol 3-phosphate
- 1 Glycerol 3-phosphate

Glycerol 3-phosphate → Glucose and other sugars

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Synthesis reactions
## Varieties of photosynthesis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Eukaryotes</th>
<th>Cyanobacteria</th>
<th>Green Bacteria</th>
<th>Prokaryotes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substance That Reduces CO₂</td>
<td>H atoms of H₂O</td>
<td>H atoms of H₂O</td>
<td>Sulfur, sulfur compounds, H₂ gas</td>
<td>Sulfur, sulfur compounds, H₂ gas</td>
</tr>
<tr>
<td>Oxygen Production</td>
<td>Oxygenic</td>
<td>Oxygenic (and anoxic)</td>
<td>Anoxic</td>
<td>Anoxic</td>
</tr>
<tr>
<td>Type of Chlorophyll</td>
<td>Chlorophyll a</td>
<td>Chlorophyll a</td>
<td>Bacteriochlorophyll a</td>
<td>Bacteriochlorophyll a or b</td>
</tr>
<tr>
<td>Site of Photosynthesis</td>
<td>Chloroplasts with thylakoids</td>
<td>Thylakoids</td>
<td>Chlorosomes</td>
<td>Chromatophores</td>
</tr>
<tr>
<td>Environment</td>
<td>Aerobic</td>
<td>Aerobic (and anaerobic)</td>
<td>Anaerobic</td>
<td>Anaerobic</td>
</tr>
</tbody>
</table>
Example of anoxygenic photosynthesis

Green sulfur bacteria
Chlorobiaceae

Anoxygenic photosynthesis

$H_2S + CO_2 \xrightleftharpoons[\text{hv}]{\text{light}} SO_4^{2-} + C_{org}$

- requires reduced sulfur
- requires light
- strictly anaerobic

Biomarkers of Chlorobiaceae

- chlorobactane
- isorenieratane

Summons et al., 1987; JJ Brocks et al. 2005
Nutritional classification of organisms

**ALL ORGANISMS**

**Energy source**

Chemical

- **Chemotrophs**
  - **Carbon source**
    - Organic compounds
      - **Chemoheterotrophs**
        - **Final electron acceptor**
          - **O₂**
            - All animals, most fungi, protozoa, bacteria
          - **Not O₂**
            - Organic compound: *Streptococcus*, for example
            - Inorganic compound: Electron transport chain: *Clostridium*, for example
  - **Chemooxytrophs**
    - **Hydrogen-, sulfur-, iron-, nitrogen-, and carbon monoxide-oxidizing bacteria**

- **CO₂**
  - **Chemoautotrophs**
    - **Green nonsulfur bacteria, purple nonsulfur bacteria**

Light

- **Phototrophs**
  - **Carbon source**
    - Organic compounds
      - **Photoheterotrophs**
        - **Use H₂O to reduce CO₂?**
          - **Yes**
            - Oxygenic photosynthesis (plants, algae, cyanobacteria)
          - **No**
            - Anoxic oxygenic photosynthetic bacteria (green and purple bacteria)
    - **Photoautotrophs**
      - **CO₂**
Independent Study

1. Test yourself on the energy and carbon needs of microbes. Use the blank flowchart in the following slide and fill in the appropriate nutritional categories. Once you have done this, use the flowchart to answer question #2.

2. Determine carbon source, energy course, and type of metabolism (i.e. aerobic or anaerobic respiration, fermentation, oxygenic or oxygenic photosynthesis) for the following organisms:

   a. *Pseudomonas*, an aerobic chemoheterotroph
   b. *Clostridium*, an anaerobic chemoheterotroph
   c. *Spirulina*, an oxygenic photoautotroph
   d. *Ectothiorhodopsin*, an anoxygenic photoautotroph
   e. *Nitrosomonas*, a nitrogen oxidizing chemoautotroph

3. Study for Exam 1
All organisms

Energy source

Carbon source

Final electron acceptor

Use \( H_2O \) to reduce \( CO_2 \)?