Systems Microbiology

Weds Sept 20 - Ch 5 & Ch 17 (p 533-555)

Bioenergetics & Metabolic Diversity

- BASIC MODES OF ENERGY GENERATION
- THERMODYNAMICS OF GROWTH - cont’d
- APPLICATIONS of MICROBIAL CHEMOLITHOTROPHY & ANAEROBIC RESP
Phototrophs
(Use Light as Energy Source)

- Photoautotrophs
  (Use CO₂)
- Photoheterotrophs
  (Use Organic Carbon)

Figure by MIT OCW.
Anoxygenic photoautotrophs utilize cyclic photophosphorylation
LOTS OF DIVERSITY IN BACTERIAL ANOXYGENIC PHOTOTROPHS!

Figure by MIT OCW.
The Z Scheme

Photosystem II

Photosystem I

Cyclic electron flow (generates proton motive force)

Noncyclic electron flow (generates proton motive force)

Light

NAD(P)⁺

NAD(P)H

P680*

P700*

P680

P700

Chl a₀

QA

FeS

Fd

Fp

QA

Ph

Qₐ

Qₜ

Q pool

Cyt bf

Pc

H₂O

½ O₂ + 2H

Light

e⁻
HALOARCHAEA
Live in hypersaline habitats

Aerial photograph of haloarchaea changing the colors of their saltwater habitats removed due to copyright restrictions.
Microbial rhodopsins fall into two main functional classes

**Light-driven ion pumps**

**Sensory rhodopsins**

![Diagram of Rhodopsin Functional Diversity](image)
Bacteriorhodopsin and proteorhodopsin

Light driven proton pumps

Cell interior

Cell exterior

Figure by MIT OCW.
Many new bacterial proteorhodopsins discovered in environmental shotgun sequencing.
Images of a hybrid automobile, hydrocarbons, and electricity removed due to copyright restrictions.
Where do organisms get their energy?

- **ALL ORGANISMS**
  - **chemotrophs**
    - **chemolithotrophs**: Oxidize inorganic compounds
  - **phototrophs**: Derive energy from light
    - **chemoorganotrophs**: Oxidize organic compounds
Microbes can eat & breathe just about anything!
Diagrams removed due to copyright restrictions.

Chemolithoautotroph (chemo [chemical], litho [rock], auto[self], troph [feeding])

Energy source: inorganic substrates (H2, NH3, NO2-, H2S, Fe2+)

Carbon source: CO2

e- acceptor: O2(aerobes), or S(some anaerobes), Fe3+, NO3, SO4

Chemolithoautotrophs can be grouped according to the inorganic compounds that they oxidize for energy:

Nitrifiers - Oxidize reduced Nitrogen compounds such as NH4+

Sulfur Oxidizers- Oxidize reduced Sulfur compounds such as H2S, S0, and S2O-

Iron Oxidizers- Oxidize reduced Iron-Fe2+ (ferrous iron)

Hydrogen Oxidizers-Oxidize Hydrogen gas-H2
### METABOLIC DIVERSITY

**CHEMOLITHOAUTOTROPHS - Examples**

Table 4. Groups of bacteria able to use inorganic electron donors for growth ("chemolithoautotrophs").

<table>
<thead>
<tr>
<th>Bacterial group</th>
<th>Typical species</th>
<th>Metabolic process</th>
<th>Electron donor</th>
<th>Electron acceptor</th>
<th>Carbon source</th>
<th>Prod</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen-oxidizing bacteria</td>
<td><em>Alcaligenes eutrophus</em></td>
<td>H₂ Oxidation</td>
<td>H₂</td>
<td>O₂</td>
<td>CO₂</td>
<td>H₂O</td>
</tr>
<tr>
<td>Carbon monoxide-oxidizing bacteria</td>
<td><em>Pseudomonas carboxydovorans</em></td>
<td>CO oxidation</td>
<td>CO</td>
<td>O₂</td>
<td>CO₂</td>
<td>CO₂</td>
</tr>
<tr>
<td>Ammonium-oxidizing bacteria</td>
<td><em>Nitrosomonas europaea</em></td>
<td>Ammonium oxidation</td>
<td>NH₄⁺</td>
<td>O₂</td>
<td>CO₂</td>
<td>NO₂⁻</td>
</tr>
<tr>
<td>Nitrite-oxidizing bacteria</td>
<td><em>Nitrobacter winogradskyi</em></td>
<td>Nitrite oxidation</td>
<td>NO₂⁻</td>
<td>O₂</td>
<td>CO₂</td>
<td>NO₃⁻</td>
</tr>
<tr>
<td>Sulfur-oxidizing bacteria</td>
<td><em>Thiobacillus thiooxidans</em></td>
<td>Sulfur oxidation</td>
<td>S, S₂O₃²⁻</td>
<td>O₂</td>
<td>CO₂</td>
<td>SO₄²⁻</td>
</tr>
<tr>
<td>Iron-oxidizing bacteria</td>
<td><em>Thiobacillus ferrooxidans</em></td>
<td>Iron oxidation</td>
<td>Fe²⁺</td>
<td>O₂</td>
<td>CO₂</td>
<td>Fe³⁺</td>
</tr>
<tr>
<td>Methanogenic bacteria</td>
<td><em>Methanobacterium thermoautotrophicum</em></td>
<td>Methanogenesis</td>
<td>H₂</td>
<td>CO₂</td>
<td>CO₂</td>
<td>CH₄</td>
</tr>
<tr>
<td>Acetogenic bacteria</td>
<td><em>Acetobacterium woodii</em></td>
<td>Acetogenesis</td>
<td>H₂</td>
<td>CO₂</td>
<td>CO₂</td>
<td>CH₃⁻ COO⁻</td>
</tr>
</tbody>
</table>
CHEMOLITHOTROPHIC AMMONIA OXIDATION - AEROBIC

\[ \text{NH}_4^+ + \frac{3}{2}\text{O}_2 \rightarrow \text{NO}_2^- + \text{H}_2\text{O} + 2\text{H}^+ \]

Figure by MIT OCW.
Anammox means "anaerobic ammonium oxidation". Anammox is both a new low-cost method of N-removal in wastewater treatment, and a spectacular microbial way of life - *woo - woo*!

Courtesy of the Department of Microbiology at Radboud University Nijmegen. Used with permission.
\[ \Delta E_o = E_o \text{ (electron acceptor)} - E_o \text{ (electron donor)} = 1233 \text{ mV} \]

\[ \Delta G_o = -nF \Delta E_o = -(3) \ (96.5 \text{ kJ/Vmol})(1.233V) = - 357 \text{ kJ/mol} \]

Broda predicted, based solely on the thermodynamics, that such microorganisms should exist. (And also the fact that if a bioenergetically favorable niche exists, a microbe will evolve to fill it!).

About a decade later, the 'bugs' were discovered in bioreactors started from waste water treatment plants.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nitrification $\text{NH}_4^+ + \text{O}_2 \rightarrow \text{NO}_2^-$</th>
<th>Anammox $\text{NH}_4^+ + \text{NO}_2^- \rightarrow \text{N}_2$</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free energy</td>
<td>−275</td>
<td>−357</td>
<td>kJ/mol</td>
</tr>
<tr>
<td>Biomass yield</td>
<td>0.08</td>
<td>0.07</td>
<td>mol/mol C</td>
</tr>
<tr>
<td>Aerobic rate</td>
<td>200–600</td>
<td>0</td>
<td>nmol/min/mg protein</td>
</tr>
<tr>
<td>Anaerobic rate</td>
<td>2</td>
<td>60</td>
<td>nmol/min/mg protein</td>
</tr>
<tr>
<td>Growth rate</td>
<td>0.04</td>
<td>0.003</td>
<td>/h</td>
</tr>
<tr>
<td>Doubling time</td>
<td>0.73</td>
<td>10.6</td>
<td>days</td>
</tr>
<tr>
<td>$K_s \text{NH}_4^+$</td>
<td>5–2600</td>
<td>5</td>
<td>µM</td>
</tr>
<tr>
<td>$K_s \text{NO}_2^-$</td>
<td>N/A</td>
<td>&lt;5</td>
<td>µM</td>
</tr>
<tr>
<td>$K_s \text{O}_2$</td>
<td>10–50</td>
<td>N/A</td>
<td>µM</td>
</tr>
</tbody>
</table>
Diagram removed due to copyright restrictions.
Partial nitrification/Anammox® is a new method for nitrogen removal from wastewater. It targets wastewater streams (or gases) high in ammonium (>0.2 g/l) and low in organic carbon (C:N ratio lower than 0.15). The two processes proceed as follows:

- **(partial nitrification)**
  \[ 2NH_4^+ + 1.5O_2 = NH_4^+ + NO_2^- + H_2O + 2H^+ \]

- **(anammox)**
  \[ NH_4^+ + NO_2^- = N_2 + 2H_2O \]

- **(total)**
  \[ 2NH_4^+ + 1.5O_2 = N_2 + 3H_2O + 2H^+ \]

(the produced acid is balanced by the counter-ion of ammonium, usually bicarbonate or sulfide)

**Compared to conventional nitrification/denitrification, this method saves 100% of the carbon source, & 50% of the required oxygen. This leads to a reduction of operational costs of 90%, a decrease in CO₂ emissions of more than 100% (the process actually consumes CO₂), and a decrease in energy demand.**
Anaerobic ammonium oxidation by anammox bacteria in the Black Sea

Graphs removed due to copyright restrictions.
**ANAEROBIC RESPIRATION** = Dumping your electrons on something other than oxygen

### Table 2. Physiological groups of bacteria able to grow under anaerobic conditions using external electron acceptors for electron transport (“erobic respiration”).

<table>
<thead>
<tr>
<th>Bacterial group</th>
<th>Typical species</th>
<th>Metabolic process</th>
<th>Electron acceptor</th>
<th>Reduction products(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denitrifiers</td>
<td><em>Pseudomonas denitrificans</em></td>
<td>Nitrate respiration NO$_3^-$</td>
<td>N$_2$, N$_2$O, NO$_2^-$</td>
<td></td>
</tr>
<tr>
<td>Sulfate reducers</td>
<td><em>Desulfovibrio vulgaris</em></td>
<td>Sulfate respiration SO$_4^{2-}$</td>
<td>S$^{2-}$</td>
<td></td>
</tr>
<tr>
<td>Sulfur reducers</td>
<td><em>Desulfuromonas acetoxidans</em></td>
<td>Sulfur respiration S$^{0}$</td>
<td>S$^{2-}$</td>
<td></td>
</tr>
<tr>
<td>Methanogenic bacteria</td>
<td><em>Methanobacterium thermoautotrophicum</em></td>
<td>Carbonate respiration CO$_2$</td>
<td>CH$_4$</td>
<td></td>
</tr>
<tr>
<td>Acetogenic bacteria</td>
<td><em>Acetobacterium woodii</em></td>
<td>Carbonate respiration CO$_2$</td>
<td>CH$_3$—COOH</td>
<td></td>
</tr>
<tr>
<td>Succinogenic bacteria</td>
<td><em>Wolinella succinogenes</em></td>
<td>Fumarate respiration Fumarate</td>
<td>Succinate</td>
<td></td>
</tr>
<tr>
<td>Iron reducers</td>
<td><em>Pseudomonas GS-15</em></td>
<td>Iron respiration Fe$^{3+}$</td>
<td>Fe$^{2+}$</td>
<td></td>
</tr>
</tbody>
</table>
Denitrification = Use of NO$_3^-$ as terminal electron acceptor, that results in complete conversion to N$_2$ gas.
Image of geobacter growing on iron oxides removed due to copyright restrictions.
Microbial redox interactions with uranium: an environmental perspective

Organic Matter Degradation In Anaerobic Environments
Diagram removed due to copyright restrictions.

Fig. 1. The distribution of terminal electron-accepting processes (TEAPs) found with depth in aquatic and marine sediments.
Energetic explains order: not all e-acceptors are equal!

<table>
<thead>
<tr>
<th>E- acceptor</th>
<th>$\Delta G^o'$ (using glucose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen</td>
<td>-3190 kJ/mol</td>
</tr>
<tr>
<td>NO3-</td>
<td>-3030</td>
</tr>
<tr>
<td>Mn (IV)</td>
<td>-3090</td>
</tr>
<tr>
<td>Fe(III)</td>
<td>-1410</td>
</tr>
<tr>
<td>Sulfate</td>
<td>-380</td>
</tr>
<tr>
<td>CO2</td>
<td>-350</td>
</tr>
</tbody>
</table>

From Nealson and Saffarini 1994
Generalized pathway for the anaerobic oxidation of organic matter to carbon dioxide with Fe$^{3+}$ oxide serving as an electron acceptor in temperate, freshwater and sedimentary environments. The process is mediated by a consortium of fermentative microorganisms and Geobacter species.
Microbial redox interactions with uranium: an environmental perspective

The distribution of terminal electron-accepting processes (TEAPs) observed within anaerobic portions of aquifers contaminated with organic compounds.
Images removed due to copyright restrictions.
Anaerobic respiration to “clean up” of uranium pollution

Soluble= mobile
Insoluble, immobile

Acetate + U (VI) $\rightarrow$ U (IV)$_s$ + CO$_2$

CH$_3$C00- + 4 U(VI) $\rightarrow$ U (IV)$_s$ + 2HCO$_3$- + 9H+

Carried out by *Geobacter*

Example of “bioremediation”

Diagram removed due to copyright restrictions.

Fig. 5. Stimulated U(VI) reduction in aquifers upon the steady addition of low concentrations of a suitable electron donor such as acetate. Fe(III) and U(VI) reduction are stimulated upon the depletion of O₂, NO₃⁻ and Mn(IV) as electron acceptors.
Diagram removed due to copyright restrictions.
Diagram and photograph of a sediment microbial fuel cell removed due to copyright restrictions.

Figure 3 | A sediment microbial fuel cell. a | A schematic of a sediment microbial fuel cell. Organisms in the family Geobacteraceae can oxidize acetate and other fermentation products, and transfer the electrons to graphite electrodes in the sediment. These electrons flow to the cathode in the overlying aerobic water where they react with oxygen. b | An actual sediment fuel cell before deployment.
Correspondence between pilus current and applied voltage demonstrating the linear, ohmic, response characteristic of a true conductor.

Extracellular electron transfer via microbial nanowires.

Schematic of the electronic connection of the AFM tip in a conducting probe atomic force microscope (CP-AFM). HOPG, highly oriented pyrolytic graphite.
Table 1. Standard reduction potential ($E_0'$) values (at 25°C and pH 7)

Since e^- are being added to the reactants on the left sides of the equations, these reactions are showing reduction reactions.

<table>
<thead>
<tr>
<th>Half-Reaction</th>
<th>$E_0'$ (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{O}_2 + 2 \text{H}^+ + 2 \text{e}^-$ → H$_2$O</td>
<td>+0.816</td>
</tr>
<tr>
<td>Fe$^{3+}$ + e^- → Fe$^{2+}$</td>
<td>+0.771</td>
</tr>
<tr>
<td>NO$_3^-$ + 6 H$^+$ + 6 e^- → _N$_2$ + 3 H$_2$O</td>
<td>+0.75</td>
</tr>
<tr>
<td>NO$_3^-$ + 2 H$^+$ + 2 e^- → NO$_2^-$ + H$_2$O</td>
<td>+0.421</td>
</tr>
<tr>
<td>NO$_3^-$ + 10 H$^+$ + 8 e^- → NH$_4^+$ + 3 H$_2$O</td>
<td>+0.36</td>
</tr>
<tr>
<td>NO$_2^-$ + 8 H$^+$ + 6 e^- → NH$_4^+$ + 2 H$_2$O</td>
<td>+0.34</td>
</tr>
<tr>
<td>CH$_3$OH + 2 H$^+$ + 2 e^- → CH$_4$ + H$_2$O</td>
<td>+0.17</td>
</tr>
<tr>
<td>fumarate + 2 H$^+$ + 2 e^- → succinate</td>
<td>+0.031</td>
</tr>
<tr>
<td>2 H$^+$ + 2 e^- → H$_2$ (pH 0)</td>
<td>+0.00</td>
</tr>
<tr>
<td>oxaloacetate + 2 H$^+$ + 2 e^- → malate</td>
<td>-0.166</td>
</tr>
<tr>
<td>CH$_3$O + 2 H$^+$ + 2 e^- → CH$_3$OH</td>
<td>-0.18</td>
</tr>
<tr>
<td>pyruvate + 2 H$^+$ + 2 e^- → lactate</td>
<td>-0.185</td>
</tr>
<tr>
<td>acetaldehyde + 2 H$^+$ + 2 e^- → ethanol</td>
<td>-0.197</td>
</tr>
<tr>
<td>SO$_4^{2-}$ + 8 H$^+$ + 6 e^- → S + 4 H$_2$O</td>
<td>-0.20</td>
</tr>
<tr>
<td>SO$_3^{2-}$ + 10 H$^+$ + 8 e^- → H$_2$S + 4 H$_2$O</td>
<td>-0.21</td>
</tr>
<tr>
<td>FAD + 2 H$^+$ + 2 e^- → FADH$_2$</td>
<td>-0.219</td>
</tr>
<tr>
<td>CO$_2$ + 8 H$^+$ + 8 e^- → CH$_4$ + 2 H$_2$O</td>
<td>-0.24</td>
</tr>
<tr>
<td>S + 2 H$^+$ + 2 e^- → H$_2$S</td>
<td>-0.243</td>
</tr>
<tr>
<td>N$_2$ + 8 H$^+$ + 6 e^- → 2 NH$_4^+$</td>
<td>-0.28</td>
</tr>
<tr>
<td>NAD$^+$ + H$^+$ + 2 e^- → NADH</td>
<td>-0.320</td>
</tr>
<tr>
<td>NADP$^+$ + H$^+$ + 2 e^- → NADPH</td>
<td>-0.324</td>
</tr>
<tr>
<td>2 H$^+$ + 2 e^- → H$_2$ (pH 7)</td>
<td>-0.414</td>
</tr>
<tr>
<td>CO$_2$ + 4 H$^+$ + 4 e^- → 1/6 glucose + H$_2$O</td>
<td>-0.43</td>
</tr>
</tbody>
</table>
| Fe$^{2+}$ + 2 e^- → Fe                     | -0.85      

'Good' electron acceptors

Electrons moving this way $\Delta G^0 < 0$

Electrons moving this way $\Delta G^0 > 0$

'Good' electron donors
Photograph removed due to copyright restrictions


METHANE FLUX

SO$_4^{2-}$ Diffusion

Seawater

Sea Floor

Sulfate Reduction Zone

Figure by MIT OCW.
**ANAEROBIC METHANE OXIDATION**

**Geochemical Observations:**

\[
\text{CH}_4 + \text{SO}_4^{2-} \rightarrow \text{HCO}_3^- + \text{HS}^- + \text{H}_2\text{O}
\]

**Microbiologically ???**

\[
\text{CH}_4 + 2\text{H}_2\text{O} \rightarrow \text{CO}_2 + 4\text{H}_2 \quad \text{“Reverse Methanogenesis”} \quad \Delta G'_o = +131 \text{ kJ/mol}
\]

\[
\text{HSO}_4^- + 4\text{H}_2 \rightarrow \text{HS}^- + 4\text{H}_2\text{O} \quad \text{Sulfate reduction} \quad \Delta G'_o = -156 \text{ kJ/mol}
\]

\[
\text{CH}_4 + \text{HSO}_4^{2-} \rightarrow \text{CO}_2 + \text{HS}^- + 2\text{H}_2\text{O} \quad \Delta G'_o = -25 \text{ kJ/mol}
\]