Microbial Biogeochemistry

Chemical reactions occurring in the environment mediated by microbial communities

Outline

• Metabolic Classifications.
• Winogradsky columns, Microenvironments.
• Redox Reactions.
• Microbes and Processes in Winogradsky column.
• Competition and Redox cascade
• Winogradsky column biogeochemistry.
• Lab work
Metabolic Classification of Life

Energy Source

- **Light** (Phototrophs)
  - PS I: anaerobic, H₂S
  - PS I+II: aerobic, H₂O

- **Inorganic** (Chemolithotrophs)
  - Aerobic (majority)
  - Anaerobic (few)

- **Chemical** (Chemotrophs)
  - Aerobic respiration
  - Anaerobic respiration
  - Fermentation

Classification

- **Photoautotrophs**
- **Photoheterotrophs**
- **Chemolithoautotrophs**
- **Chemolithoheterotrophs** (or Mixotrophs)
- **Chemoorganoheterotrophs**
- **Chemoorganoheterotrophs** (or Mixotrophs)

Carbon Source

- **CO₂** (Autotrophs)
- **Organic** (Heterotrophs)

Note, organisms that exhibit both autotrophy and heterotrophy are also called mixotrophs.
Winogradsky Column
Microenvironments generated by chemical gradients.

Cyanobacteria
Algae; Bacteria

Sulfur bacteria
Purple nonsulfur bacteria
Purple S bacteria
Green S bacteria
Desulfovibrio
Clostridium

Photoautotrophy: PS I+II
Chemoorganoheterotrophy

Chemolithoautotrophy
Chemolithoheterotrophy
Photoheterotrophy

Photoautotrophy: PS I

Chemoorganoheterotrophy
- sulfate reducers

Chemoorganoheterotrophy
- Fermentation

Conc.
H₂S
O₂
Transport Limitations; Advection

Advevctive transport:

\[ \text{Flux} = uC \equiv \left[ \frac{g}{m^2 s} \right] \]

\[ u: \text{Fluid velocity [m s}^{-1}] \]

\[ \frac{\partial C}{\partial t} = -\frac{\partial}{\partial z} (uC) \]
Transport Limitations; Diffusion

Fickian Diffusion:

\[ Flux = -D \frac{dC}{dz} = \left[ \frac{g}{m^2 s} \right] \]

\[ D: \text{Diffusion Coefficient \([m^2 s^{-1}]\)} \]

\[ \frac{\partial C}{\partial t} = \frac{\partial}{\partial z} \left( D \frac{\partial C}{\partial z} \right) \]

Time=0 Surface: C (C)

Time=0.4 Surface: C (C)

Time=1 Surface: C (C)

Time=2 Surface: C (C)
Transport Limitations; Advection-Diffusion

Transport by advection and diffusion:

\[ \text{Flux} = -D \frac{dC}{dz} + uC = \left[ \frac{g}{m^2 s} \right] \]

Must also account for reactions!

\[ \frac{\partial C}{\partial t} = \frac{\partial}{\partial z} \left( D \frac{\partial C}{\partial z} - uC \right) \]
Redox Reactions

Reduction and Oxidation:

**Half Reactions**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Oxidation</th>
<th>Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>A → B⁺ + e⁻</td>
<td>Oxidation</td>
<td>Reduction</td>
</tr>
<tr>
<td>C + e⁻ → D⁻</td>
<td>Reduction</td>
<td></td>
</tr>
</tbody>
</table>

**Complete Reaction**

A + C → B⁺ + D⁻

Redox Potential, \(E^\circ\)

\[ E_h = E^\circ - \frac{RT}{nF} \ln \frac{\prod_i [Products]_i^{\beta_i}}{\prod_j [Substrates]_j^{\alpha_j}} \]

\[ E^\circ = E^\circ - \frac{2.303RT}{nF} \text{ pH} \]

**Reference Half Reaction:**

\(H_2 \rightarrow 2e^- + 2H^+\)

Units: Volt = J/C

Electron Tower (pH 7)

- 2H⁺ + 2e⁻ → H₂
- CO₂ + 8H⁺ + 8e⁻ → CH₄ + 2H₂O
- SO₄²⁻ + 10H⁺ + 8e⁻ → H₂S + 4H₂O
- 2H⁺ + 2e⁻ → H₂ (pH 0)

Reference cell at pH 0

- \(\frac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_2O\)
- \(NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O\)
- \(NO_3^- + 6H^+ + 5e^- \rightarrow \frac{1}{2}N_2 + 3H_2O\)
- \(Fe^{3+} + e^- \rightarrow Fe^{2+}\)
- \(\frac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_2O\)

\[ \Delta G = -nEF \text{ (Gibbs Free Energy kJ/mol, } E \text{ in volts)} \]

\(F = \text{faraday (96493 Coulombs/mol)}\)

\(R = \text{gas const (8.314 J/K/mol)}\)

\(n = \text{no. of electrons in rxn.} \)

\(m = \text{no. of } H^+ \text{ consumed} \)
Oxidation States and Fermentation

Oxidation states
• Some (many) elements have more than one stable electron configuration.
• Consequently, an element can exist in reduced or oxidized states; e.g., Fe$^{3+}$ or Fe$^{2+}$.

Carbon, Nitrogen and Sulfur have several (assume H: +1; O: -2)

\[
\begin{array}{cccccc}
\text{CH}_4 & -4 & \text{N}_2 & 0 & \text{NH}_3 & -3 \\
\text{CO}_2 & +4 & \text{NO}_3^- & +5 & \text{H}_2\text{S} & -2 \\
\end{array}
\]

\[
\begin{array}{cccccc}
\text{S}_2\text{O}_3^{2-} & +2 & \text{CO}_2 & +4 & \text{NO}_3^- & +5 \\
\text{SO}_4^{2-} & +6 & \end{array}
\]

Fermentation and/or Disproportionation
• Organic carbon present, but no electron acceptors: \(\text{O}_2\), \(\text{NO}_3^-\), \(\text{SO}_2^{2-}\), etc.
• Use organic carbon as both electron acceptor and donor:
\[
\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2 \text{CO}_2 + 2 \text{C}_2\text{H}_6\text{O}
\]
\[
4 \text{S} + 4 \text{H}_2\text{O} \rightarrow 3 \text{H}_2\text{S} + \text{SO}_4^{2-} + 2 \text{H}^+
\]

Autotrophy
\[
6\text{CO}_2 + 24\text{H}^+ + 24\text{e}^- \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{H}_2\text{O}
\]

\[
\text{H}_2\text{S} \rightarrow 2 \text{H}^+ + \text{S} + 2 \text{e}^- \quad \text{PS I or PS II}
\]

\[
\text{H}_2\text{O} \rightarrow 2 \text{H}^+ + \frac{1}{2} \text{O}_2 + 2 \text{e}^- \quad \text{PS I and PS II}
\]

Anoxygenic Photosynthesis

Oxygentic Photosynthesis
Photosystem I Only

Energy production only (cyclic photophosphorylation)

NADPH production only needed to reduce CO₂

These occur in the green and purple sulfur bacteria

(Principles of Modern Microbiology, M. Wheelis)
Photosystem II Only

These occur in the green and purple non-sulfur bacteria

Energy production only

(cyclic photophosphorylation)

NADPH production only needed to reduce CO$_2$

(Principles of Modern Microbiology, M. Wheelis)
Photosystem I+II

These occur in the cyanobacteria, algae and plants.

Energy production only
(cyclic photophosphorylation)

NADPH production only needed to reduce CO$_2$

(Principles of Modern Microbiology, M. Wheelis)
Microbes and Processes in Winogradsky column.

**Aerobic Environment**
- Algae and cyanobacteria (photoautotrophy using PS II)
- Bacteria and eukaryotes respiring (chemoorganoheterotrophy).

**Sulfide oxidizers (or sulfur bacteria):** $\text{H}_2\text{S} + \text{O}_2 \rightarrow \text{S or SO}_4^{2-}$
  - Some use $\text{CO}_2$ (chemolithoautotrophs), others use organic compounds (chemolithoheterotrophs)
  - Examples, *Thiobacillus* sp. And *Beggiatoa* sp.
- Methanotrophs: $\text{CH}_4 + \text{O}_2 \rightarrow \text{CO}_2 + 2\text{H}_2\text{O}$ (chemoorganoheterotrophs)
  - Example, *Ralstonia* sp., *Pseudomonas* sp.

**Anaerobic Environment**

**Fermentors** (chemoorganoheterotrophs)
- Break down cellulose, etc. and ferment sugars into:
  - alcohols acetate
  - organic acids hydrogen
- Many bacterial groups can conduct fermentation, but not all of these have the ability to decompose polymeric compounds such as cellulose.
- Example, *Clostridium* species
Sulfur Compounds
- **Sulfate reducers**: use sulfate, \( \text{SO}_4^{2-} + \text{e}^- \rightarrow \text{S or H}_2\text{S} \), to oxidize organic compounds produced by fermentors. (chemoorganoheterotrophs).
  - Many genera of bacteria. Example, *Desulfovibrio* sp.

- **Phototrophic bacteria**: Use light and \( \text{H}_2\text{S} \) as electron donor (PS I) (photoautotrophs).
  - Examples, purple and green sulfur bacteria.

Methanogens and Acetogens
- **Methanogens**: \( \text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O} \) (chemolithoautotrophs)
  - Example: *Methanobacterium* (Archaea)

- **Acetogens**: \( 2\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_3\text{COOH} + 2\text{H}_2\text{O} \) (chemolithoautotrophs)
  - Example: *Homoacetogens*
Other possible microbes

Aerobic Environments

Hydrogen

- Hydrogen oxidizers: \( \text{H}_2 + \frac{1}{2}\text{O}_2 \rightarrow \text{H}_2\text{O} \) (both chemolithoheterotrophs and chemolithoautotrophs). However, it is unlikely that \( \text{H}_2 \) will make it to the aerobic interface (it will be used in the anaerobic environment first).
  - Example, *Ralstonia eutrophus*

Iron

- Iron oxidizers: \( \text{Fe}^{2+} + \text{H}^+ + \frac{1}{4}\text{O}_2 \rightarrow \text{Fe}^{3+} + \frac{1}{2}\text{H}_2\text{O} \) (chemolithoautotrophs)
  - Occurs only at low pH (~2)
  - Example: *Thiobacillus ferrooxidans*

Ammonium

- Nitrifiers:
  \[
  \text{NH}_3 + 1\frac{1}{2} \text{O}_2 \rightarrow \text{NO}_2^- + \text{H}^+ + \text{H}_2\text{O} \\
  \text{NO}_2^- + \frac{1}{2} \text{O}_2 \rightarrow \text{NO}_3^- 
  \]
  - Example: *Nitrosomonas* and *Nitrobacter*, respectively. Both chemolithoautotrophs.

Anaerobic Environments

Nitrate

- Denitrifiers: \( \text{NO}_3^- + 6\text{H}^+ + 5\text{e}^- \rightarrow \frac{1}{2}\text{N}_2 + 3\text{H}_2\text{O} \)
  - Reaction combined with oxidation of organic matter.

Iron

- Iron reducers: Many organisms can utilize \( \text{Fe}^{3+} \) as electron acceptor.
**Chemical Potential Exploitation**

**H₂S oxidation by NO₃⁻**
- Schulz et al. 1999: *Thiomargarita namibiensis*

**Anammox**
- Strous et al. 1999: Planctomycete
  - NH₄⁺ + NO₂⁻ = N₂ + 2H₂O

**CH₄ oxidation by SO₄²⁻**
- Boetius et al. 2000:

**CH₄ oxidation by NO₃⁻** (Raghoebarsing et al. 2006)
  - 5CH₄ + 8NO₃⁻ + 8H⁺ → 5CO₂ + 4N₂ + 14H₂O
Competition and Redox cascade

How do the chemical gradients arise in the Winogradsky column, or in natural environments?

Bacteria that are able to use the most energetic reactions in their surrounding environment will dominate that microenvironment. Transport combined with the microbial sources and sinks will determine the resulting chemical gradients. Chemical gradients can be transient as substrates are exhausted or products become toxic. This leads to succession.

Energetics are governed by the redox potentials of the possible reactions:

- Electron acceptors: $O_2 > NO_3^- > Mn^{4+} > Fe^{3+} > SO_4^{2-} > CO_2 >$ Fermentation
Winogradsky column biogeochemistry

With $\text{SO}_4^{2-}$:

- $\text{CO}_2 \rightarrow \text{CH}_2\text{O} + \text{O}_2$
- $\text{CH}_2\text{O} + \text{O}_2 \rightarrow \text{CO}_2$

- $\text{SO}_4, \text{S}$
- $\text{FeS} \leftarrow \text{H}_2\text{S}$
- Light
- $\text{Organics, H}_2, \text{Acetate}$
- $\text{Sugars}$
- $\text{Cellulose}$

Without $\text{SO}_4^{2-}$:

- $\text{CO}_2 \rightarrow \text{CH}_2\text{O} + \text{O}_2$
- $\text{CH}_2\text{O} + \text{O}_2 \rightarrow \text{CO}_2$

- $\text{CO}_2, \text{H}_2, \text{Acetate}$
- $\text{Organics}$
- $\text{Sugars}$
- $\text{Cellulose}$

Concentration:

- $\text{H}_2\text{S}$
- $\text{CH}_4$
- $\text{O}_2$
Laboratory Work

**Tuesday:** Measure hydrogen sulfide profiles in columns using spectrometer assay.

**Thursday:** Measure methane profiles in columns using gas chromatogram.
Winogradsky Column from 1999 Class
Microbial Fuel Cells (Possible Project?)

Electrons flow from the biofilm 1 on the anode 2 through the circuit board 3 to the cathode 4 where they are picked up by oxygen molecules 5 which form H2O.

Positive ions A flow through the sand layer B to complete the circuit while odors C from the bacteria are neutralized.

Aerobic Chamber (O2 dissolved in H2O)

CATHODE

H2O

H2O

Ion-permeable sand barrier

Bio-film

ANODE

Anaerobic Chamber (bacteria in organic "fuel")