Hydrogen Enhancement of Sediment Microbial Activity and Contaminant Degradation

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The Technology

- Hypothesis
- Rationale
- Approach and Methods
- Hydrogen-Impacted Microbial Ecology
- Hydrogen-Enhanced Dechlorination
- Scientific Challenges
- Bench-Scale Technology Development
- Technological Challenges
Hypotheses for Hydrogen-Based Enhancement

- *In situ* amendment with hydrogen can increase metabolic and dechlorination activity.
- The technology is scalable.
- The technology can be cost-effectively applied to large and complex contaminated areas.
Rationale for Hydrogen-Based Technologies

- Ambient carbon and hydrogen fluxes limit *in situ* microbial activity in reducing soils and sediments
  - 5-20% of total extractable population
- Increased hydrogen fluxes enhance total respiratory competence and influence ecological composition
  - 15-80% of total extractable population
- Hydrogen gas is cheap and diffuses rapidly in sediments
## Fundamental Process Understanding: Evidence of Dioxin Dechlorination

<table>
<thead>
<tr>
<th><strong>Sediments</strong></th>
<th><strong>Microorganisms</strong></th>
<th><strong>Model DOM</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Passaic River cores</td>
<td>Sediment-eluted mixed communities</td>
<td><strong>Monomers:</strong></td>
</tr>
<tr>
<td>Hudson River core</td>
<td></td>
<td>Catechol, resorcinol, 3,4-dihydroxybenzoic acid</td>
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<td></td>
<td></td>
<td><strong>Polymers:</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Polymaleic acid, Aldrich humic</td>
</tr>
<tr>
<td><strong>Dioxin Source:</strong></td>
<td><strong>Dioxin Source:</strong></td>
<td><strong>Dioxin Source:</strong></td>
</tr>
<tr>
<td>Freshwater-spiked Penta- to octaCDD</td>
<td>Freshwater-spiked OCDD</td>
<td>Estuarine-spiked HpCDD (both isomers)</td>
</tr>
<tr>
<td>Estuarine-historical residues</td>
<td>Freshwater-hist. residues</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Estuarine-spiked HpCDD</td>
<td></td>
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<tr>
<td></td>
<td>(both isomers separately)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Marine-spiked HpCDD</td>
<td></td>
</tr>
<tr>
<td><strong>Electron donors/primers:</strong></td>
<td><strong>Electron donors/primers:</strong></td>
<td><strong>Electron donors:</strong></td>
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<tr>
<td>Organic acids</td>
<td>Organic acids</td>
<td>Sulfide</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>(Hydrogen)</td>
<td>Ti-citrate</td>
</tr>
<tr>
<td>2-MonobromoDD</td>
<td>2-MonobromoDD</td>
<td>Sediment microorganisms</td>
</tr>
<tr>
<td><strong>Electron acceptors:</strong></td>
<td><strong>Electron acceptors:</strong></td>
<td><strong>Electron acceptors:</strong></td>
</tr>
<tr>
<td>Bicarbonate, Natural (river bottom water)</td>
<td>Bicarbonate</td>
<td>DOM</td>
</tr>
<tr>
<td></td>
<td>Sulfate</td>
<td></td>
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<td></td>
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<td></td>
<td><strong>Electron acceptors:</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Model DOM</td>
<td></td>
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</tbody>
</table>
Differentiation of peri (1469)- and lateral (2378)- Dechlorination Pathways
Influence of Sediment Geochemistry on Dioxin Reactivity

<table>
<thead>
<tr>
<th>Biogeochemical Designation</th>
<th>Freshwater</th>
<th>Estuarine</th>
<th>Marine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominant dechlorination pathway</td>
<td>PCDD</td>
<td>PCDD</td>
<td>PCDD</td>
</tr>
<tr>
<td>Peri &lt; lateral</td>
<td>peri &lt; lateral</td>
<td>peri &lt;&lt; lateral</td>
<td></td>
</tr>
<tr>
<td>Dominant carbon flow</td>
<td>H₂</td>
<td>H₂</td>
<td>H₂</td>
</tr>
<tr>
<td>Acetate</td>
<td>CO₂</td>
<td>CO₂</td>
<td></td>
</tr>
<tr>
<td>CH₂O</td>
<td>CH₂O</td>
<td>CH₂O</td>
<td></td>
</tr>
<tr>
<td>CH₄-H₂</td>
<td>CH₄-H₂/HS⁻</td>
<td>CH₄/HS⁻</td>
<td></td>
</tr>
<tr>
<td>Nutrient-rich</td>
<td>Oligotrophic</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Increasing salinity/sulfate concentrations
Impact of Hydrogen on Microbial PCDD Dechlorination in Sediments

- **2378-TCDD (mol%):**
  - Original: 20
  - Hydrogen: 12
  - Acids: 20
- **Endpoint:**
  - Original: tetra
  - Hydrogen: mono
  - Acids: tetra
- **Rate (pmol TCDD/day):**
  - Original: NA
  - Hydrogen: 28.6
  - Acids: -0.4 (net formation)
Hydrogen Technology Scaling: Laboratory Studies (EPA-SITE program)

- **Matrix:**
  - Cell elution
  - Slurry
  - Column

- **Treatment:** $\text{H}_2$ addition, HCB spike

- **Response:**
  - Microbial activity
  - Contaminant degradation
Experimental Matrix (marine harbor sediment)
Methods: Hydrogen Enhancement of Elutions and Slurries

- Sediment-eluted microorganisms are dispensed in the SIXFORS system in sulfate-rich estuarine media.

- The reactors are amended with varying H₂ fluxes to prime cells.
  - Sparged with H₂/N₂ mix including up to 1% H₂

- Organic acid cocktail added at t=0: 10 mg/L benzoic + 15 mg/L butyric + 75 mg/L acetic

This 6-reactor system is equipped with a H₂/N₂ gas mixing/delivery system, temperature, and pH control.
Microbial Metabolic Response to Hydrogen: Redox dye (CTC) measurements

- **Microscope analysis:**
  - Green - nonactive cell
  - Green/red - active cell
Flow Cytometry: Cell number and activity quantification

- Automates cell counting
  - Density with green fluorescence (FL1) gives total cells
  - Density with red fluorescence (FL3) gives active cells
  - About 5% of cells typically CTC active (Marine Harbor sample)
Ecological Response to Hydrogen: Flow Cytometry Analysis (Passaic R.)

- Microbial population density (measured using PicoGreen™): R1 = total eluted bacteria; R3 = bacteria present at elevated hydrogen concentrations (above CTC enhancement threshold)
- R3 represents less than 10% of total cell density, but is 80% CTC active
- Microbial community was analyzed using T-RFLP
Ecological Response to Hydrogen: T-RFLP Analysis (Passaic River)

- Amendments of microbial elutions with nitrogen gas (no H2) and H2 fluxes not impacting CTC activity result in 20-30% emerging T-RFs
- Amendments above threshold of CTC activity result in emergence of 20% distinct RFs
- No populations (out of a total of 74 T-RFs) could be identified using MspI
- Cross-referencing and multi-database search using three restriction enzymes is underway
Activity Enhancement for Three Sites - Based on Cell Elutions

- 1.0 - 3.5 µM H₂ increases CTC activity ~ 3-fold

![Activity Enhancement Experiments Graph]

- Marine Harbor
- Passaic
- San Diego
Activity Results - Slurry Study (Marine Harbor)

- CTC activity increased about 3 fold
- Cells counts increased about 8 fold
H₂ Amendment, Column Study (Marine Harbor)

- **Porewater H₂ limited by diffusion**
  - Leading edge advanced ~ 0.5’/month
  - Annual zone of influence up to ~6 feet
CTC activity increased about 4-fold in bottom layer

Cell counts increased about 3-fold in bottom layer
**HCB Results - Cell Elution Study (Marine Harbor)**

- **H₂ treatment increased HCB degradation rate by ~ 50%**

<table>
<thead>
<tr>
<th>Hydrogen Amendment</th>
<th>Degradation Rate (1/hr)</th>
<th>Initial HCB Concentration (ppb)</th>
<th>HCB Concentration after 48 hrs (ppb)</th>
<th>Change in HCB (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Below threshold (&lt; 0.5 µM H₂)</td>
<td>0.0135</td>
<td>5.6 (std dev 0.17)</td>
<td>3.2 (std dev 0.01)</td>
<td>43%</td>
</tr>
<tr>
<td>Above threshold (0.6 µM H₂)</td>
<td>0.0201</td>
<td>8.7 (std dev 0.09)</td>
<td>3.2 (std dev 0.05)</td>
<td>63%</td>
</tr>
<tr>
<td>Above threshold (1.8 µM H₂)</td>
<td>0.0214</td>
<td>6.8 (std dev 0.12)</td>
<td>2.5 (std dev 0.05)</td>
<td>63%</td>
</tr>
</tbody>
</table>
HCB Results: Slurry and Column Studies (Marine Harbor)

- **Slurry (at 2 months)**
  - Treatment effects not yet statistically significant
  - Two future sampling events

- **Column (at 1 month)**
  - Treatment effects not yet statistically significant
  - Two more columns
Scientific Challenges

◆ Better understanding of hydrogen diffusion in sediment, including spatial distribution
◆ Development of correlation between hydrogen enhancement, ecological response and dechlorination activity
◆ Temporal effect:
  - Amendment to CTC activity increase
  - CTC to dechlorination activity increase
  - Pulsed vs. continuous amendment
  - Limiting ratios of carbon to hydrogen
  - Impact of bioavailability on long term activity
Future Steps for Technology Development

- Translate/scale effects on spiked HCB to effects on target contaminants

- Key issues for introducing H₂ in field:
  - As dissolved H₂?
  - To what depth?
  - How to minimize resuspension?
  - Spacing of injection points?

- What’s next?
  - Slurry and column studies to completion
  - Bench studies of H₂ injection grid (H2-GRID) to refine design parameters
  - Scale-up cost analysis
  - Design and conduct field pilot
Acknowledgements

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