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

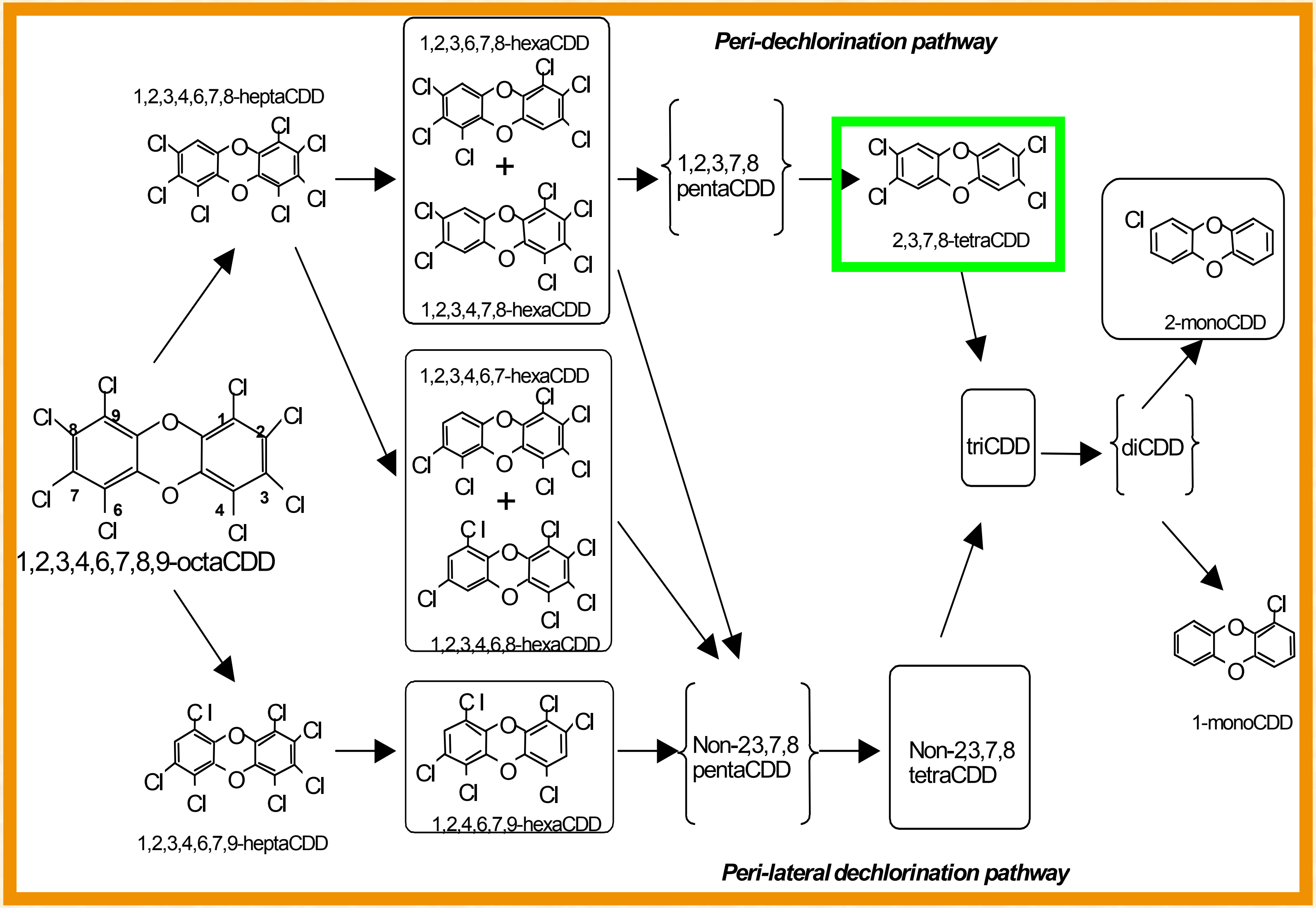
Rationale for Hydrogen-Based Technologies

- 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

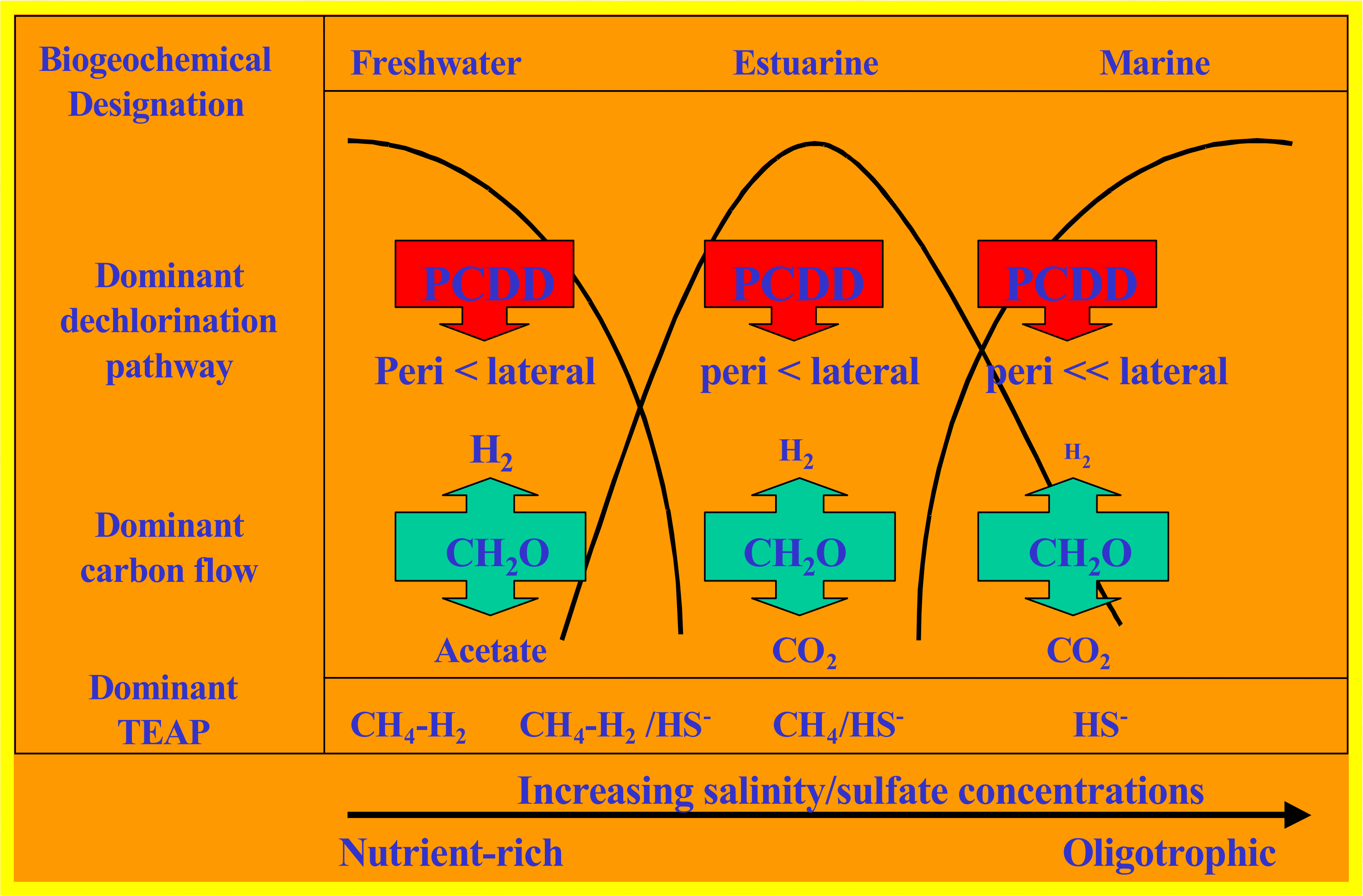
Fundamental Process Understanding: Evidence of Dioxin Dechlorination

Sediments	*Microorganisms*	*Model DOM*
Passaic River cores Hudson River core	Sediment-eluted mixed communities	Monomers: Catechol, resorcinol, 3,4-dihydroxybenzoic acid Polymers: Polymaleic acid, Aldrich humic
Dioxin Source: Freshwater-spiked Penta- to octaCDD Estuarine-historical residues	Dioxin Source: Freshwater-spiked OCDD Freshwater-hist. residues Estuarine-spiked HpCDD (both isomers separately) Marine-spiked HpCDD	Dioxin Source: Estuarine-spiked HpCDD (both isomers)
Electron donors/primers: Organic acids Hydrogen 2-MonobromoDD	Electron donors/primers: Organic acids (Hydrogen) 2-MonobromoDD	Electron donors: Sulfide Ti-citrate Sediment microorganisms
Electron acceptors: Bicarbonate, Natural (river bottom water)	Electron acceptors: Bicarbonate Sulfate	Electron acceptors: DOM

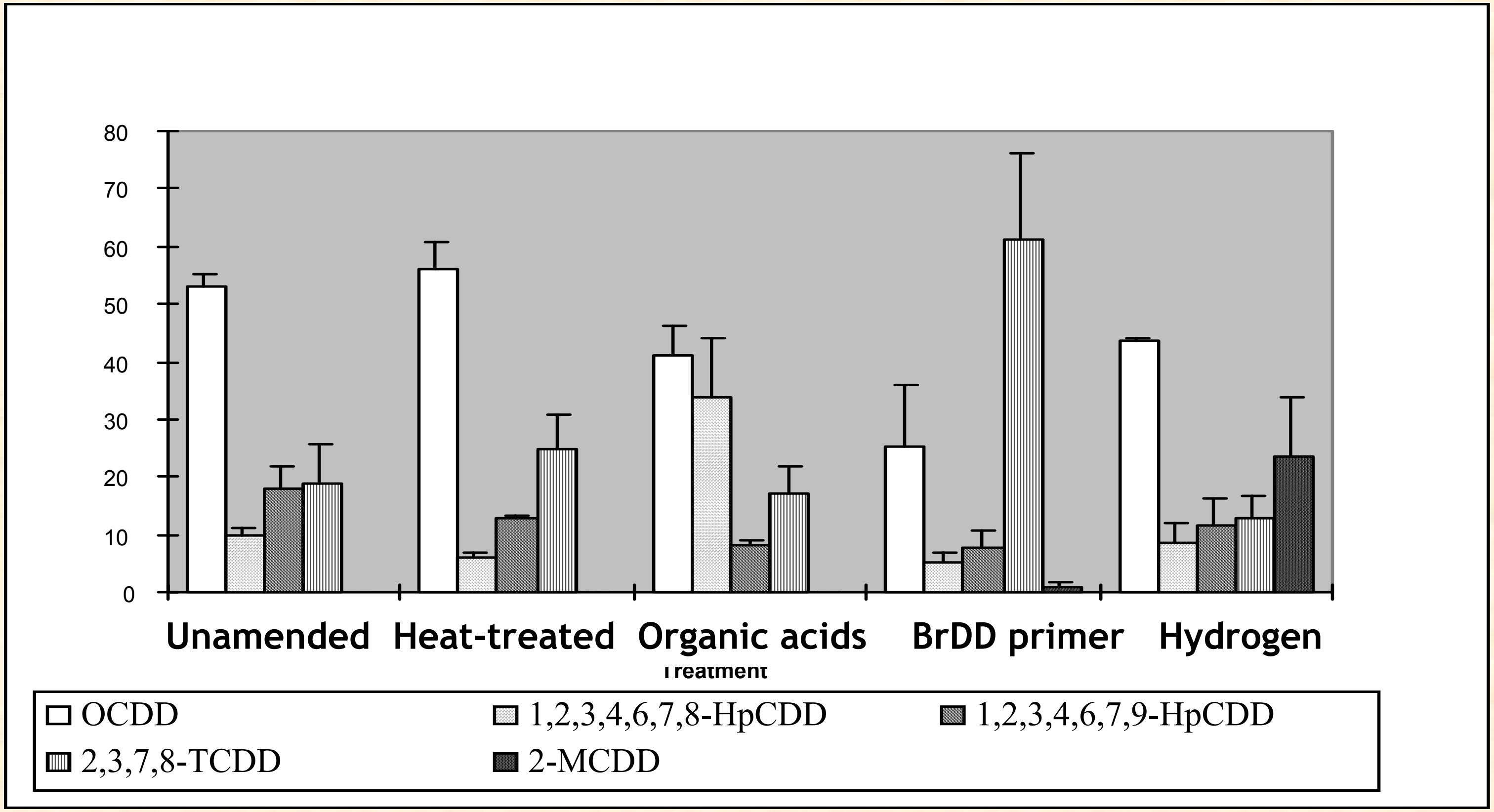
Differentiation of peri (1469)- and lateral (2378)- Dechlorination Pathways



Influence of Sediment Geochemistry on Dioxin Reactivity

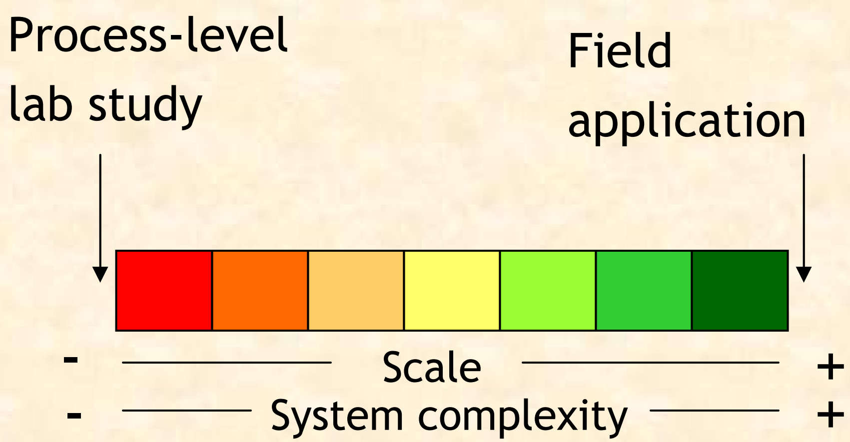


Impact of Hydrogen on Microbial PCDD Dechlorination in Sediments



	Original	Hydrogen	Acids
✓**2378-TCDD (mol%):**	**20**	**12**	**20**
✓**Endpoint:**	**tetra**	**mono**	**tetra**
✓**Rate (pmol TCDD/day):**	**NA**	**28.6**	**-0.4** (net formation)

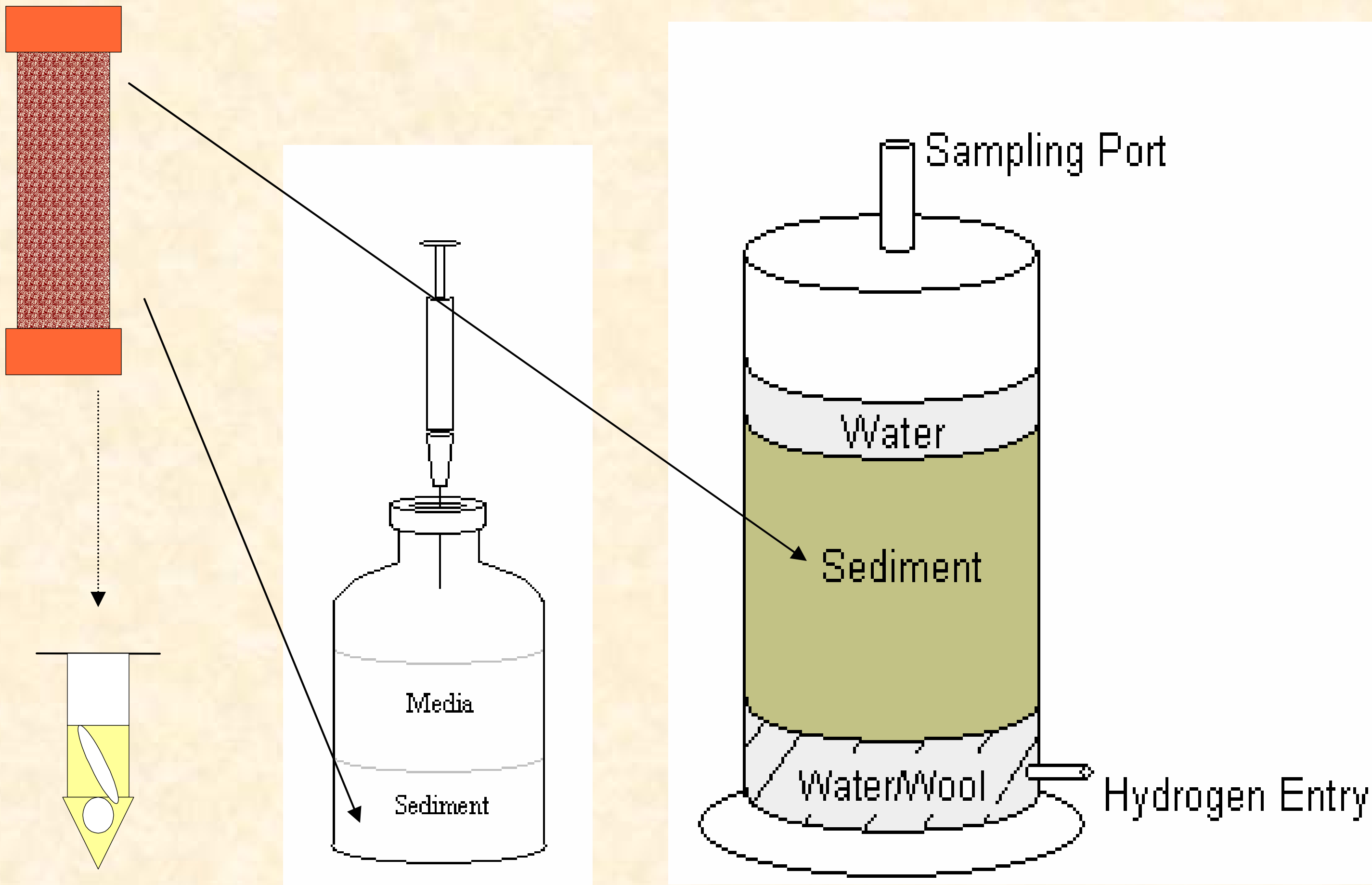
Hydrogen Technology Scaling: Laboratory Studies (EPA-SITE program)



- **Matrix:**
  - Cell elution
  - Slurry
  - Column
- **Treatment:** H_2 addition, HCB spike
- **Response:**
  - Microbial activity
  - Contaminant degradation

Experimental Matrix (marine harbor sediment)

Sediment core



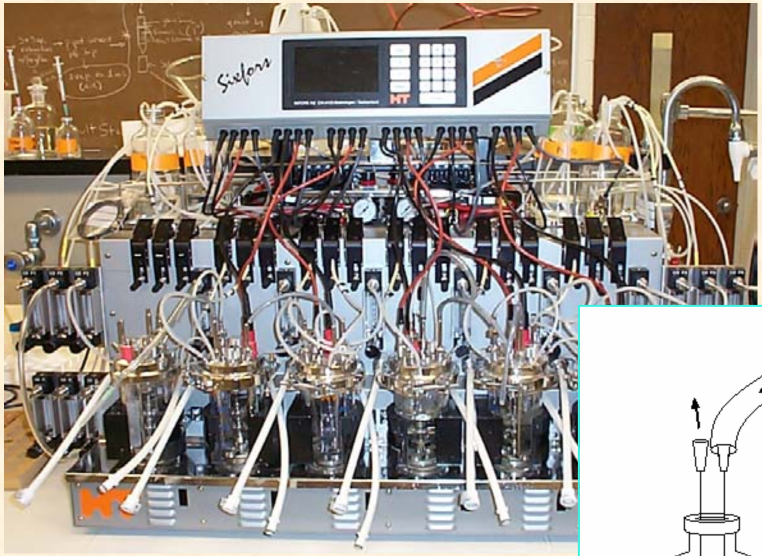
Cell elution

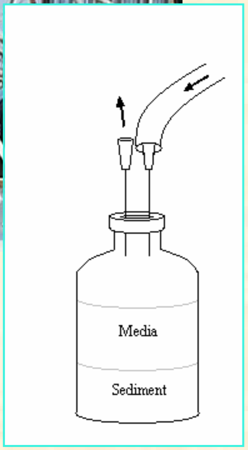
Sediment slurry

Sediment column

Methods: Hydrogen Enhancement of Elutions and Slurries

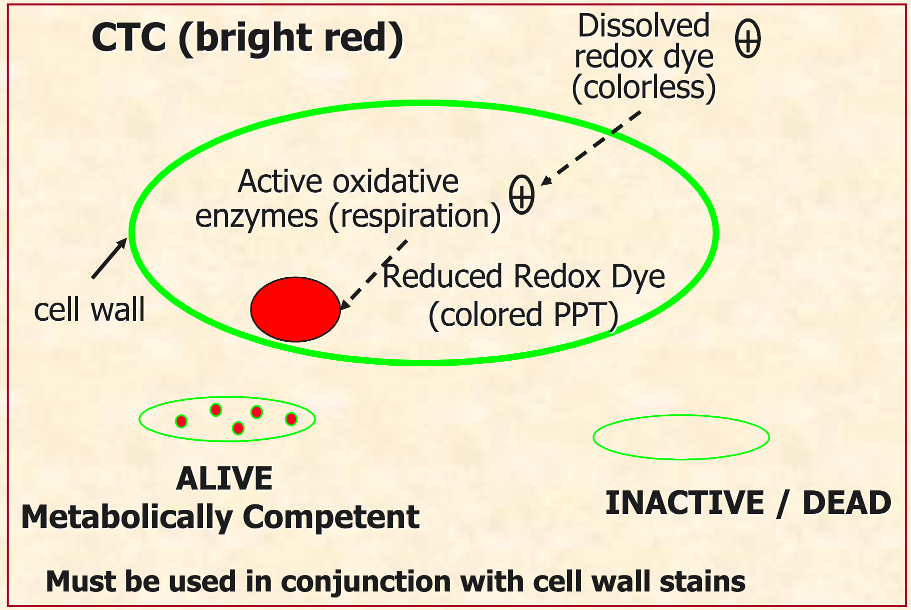
- Sediment-eluted microorganisms are dispensed in the SIXFORS system in sulfate-rich estuarine media.
- The reactors are amended with varying H_2 fluxes to prime cells.
  - Sparged with H_2/N_2 mix including up to 1% H_2
- Organic acid cocktail added at t=0: 10 mg/L benzoic + 15 mg/L butyric + 75 mg/L acetic



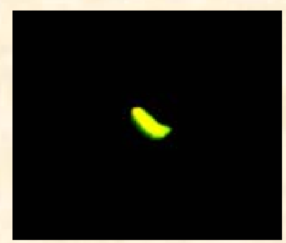


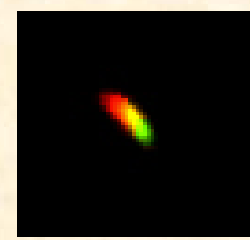
This 6-reactor system is equipped with a H_2/N_2 gas mixing/delivery system, temperature, and pH control.

Microbial Metabolic Response to Hydrogen: Redox dye (CTC) measurements



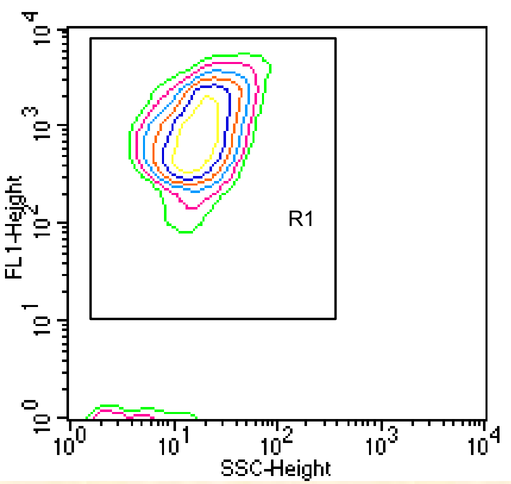
- Microscope analysis:
  - Green – nonactive cell
  - Green/red – active cell

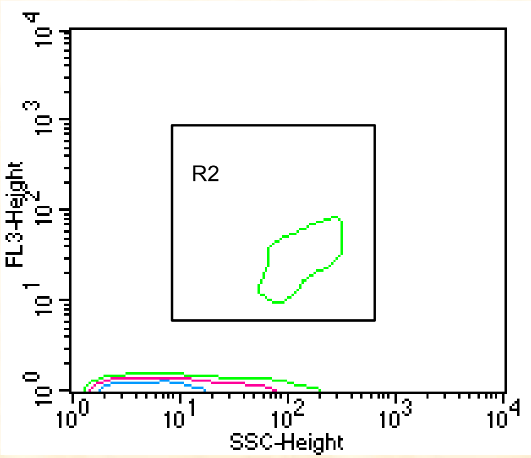




Flow Cytometry: Cell number and activity quantification

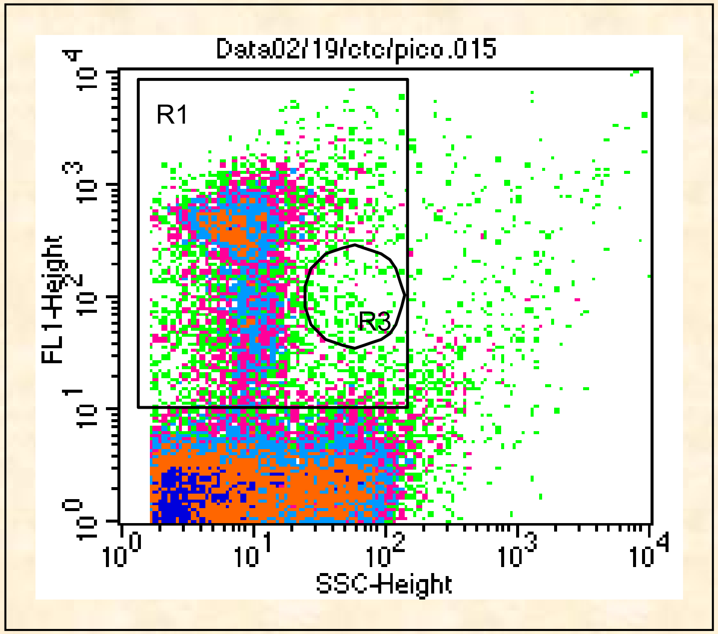
- Automates cell counting
  - Density with green fluorescence (FL1) gives total cells
  - Density with red fluorescence (FL3) gives active cells
  - About 5% of cells typically CTC active (Marine Harbor sample)

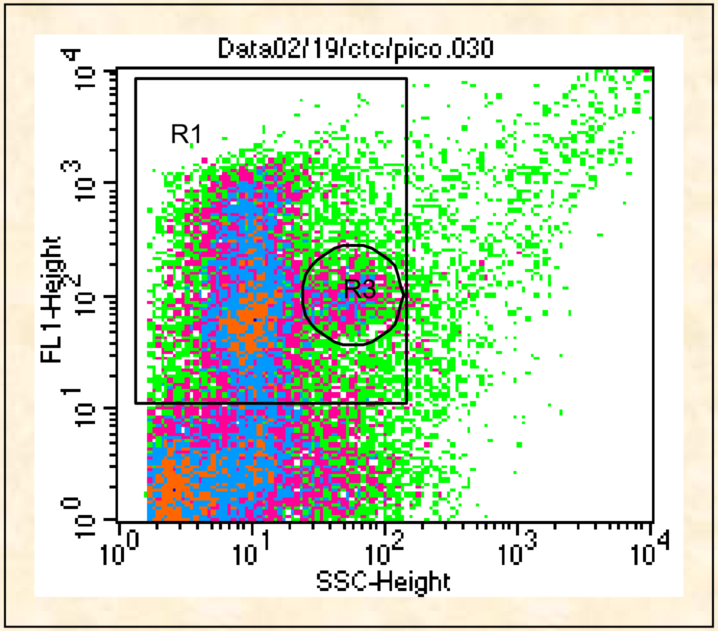




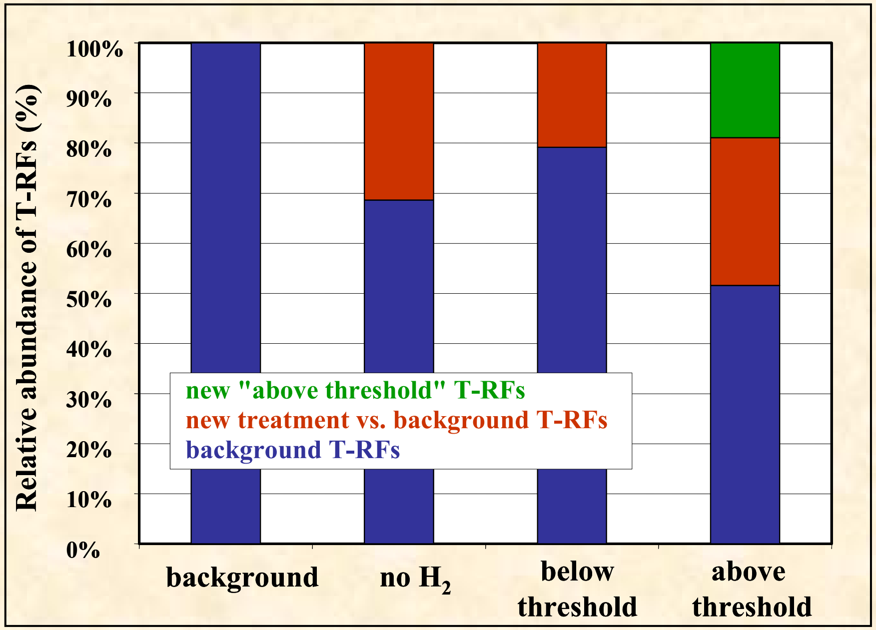
Ecological Response to Hydrogen: Flow Cytometry Analysis (Passaic R.)

- **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**





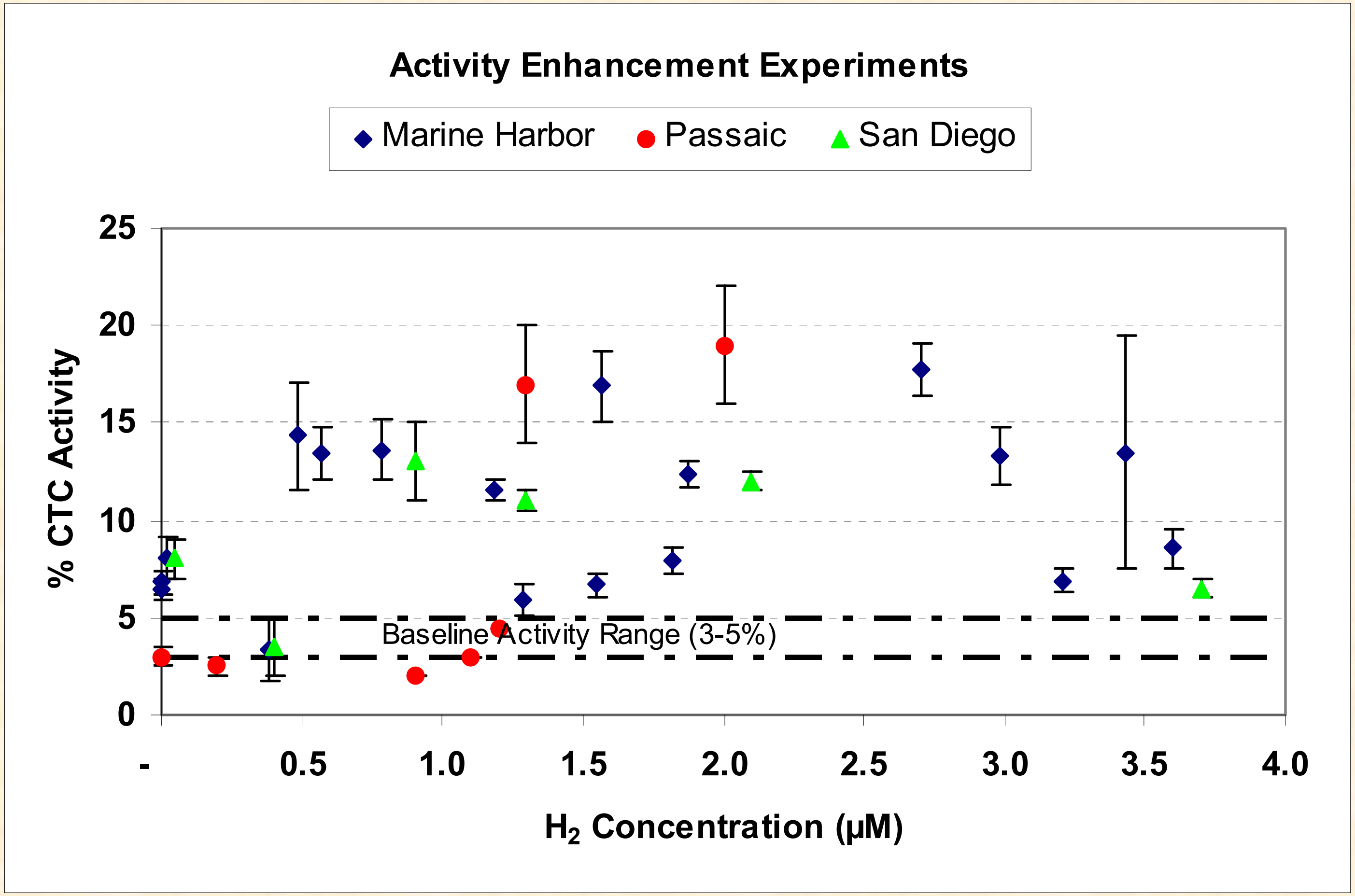
Ecological Response to Hydrogen: T-RFLP Analysis (Passaic River)



- 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

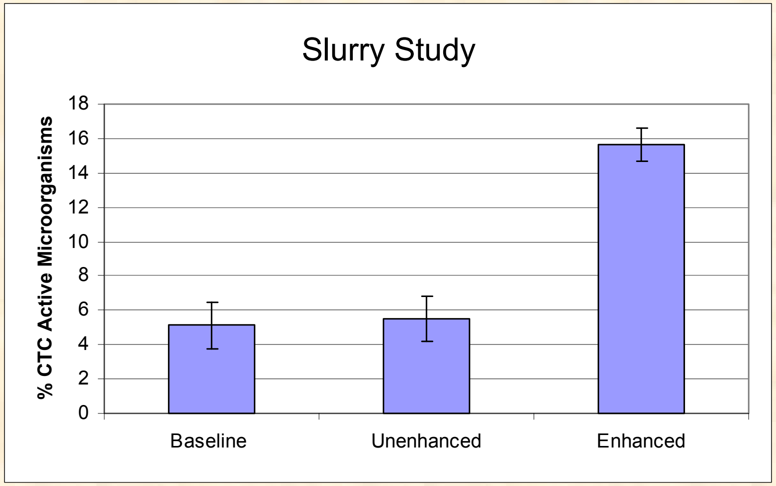
Activity Enhancement for Three Sites - Based on Cell Elutions

- 1.0 – 3.5 µM H_2 increases CTC activity ~ 3-fold

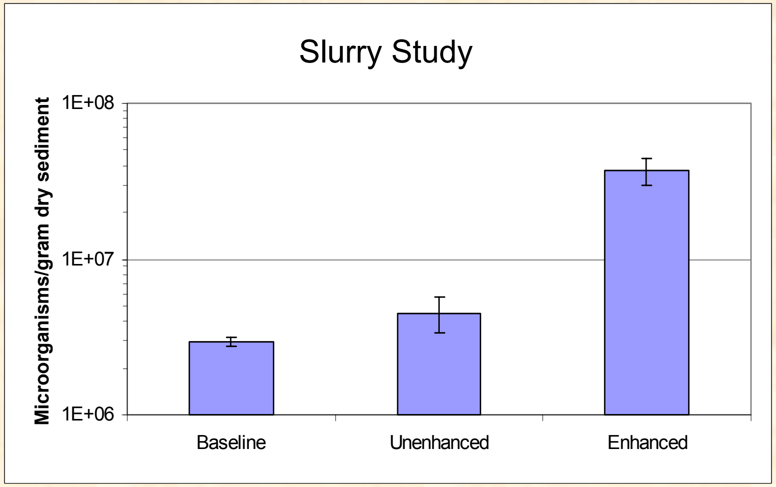


Activity Results - Slurry Study (Marine Harbor)

- CTC activity increased about 3 fold

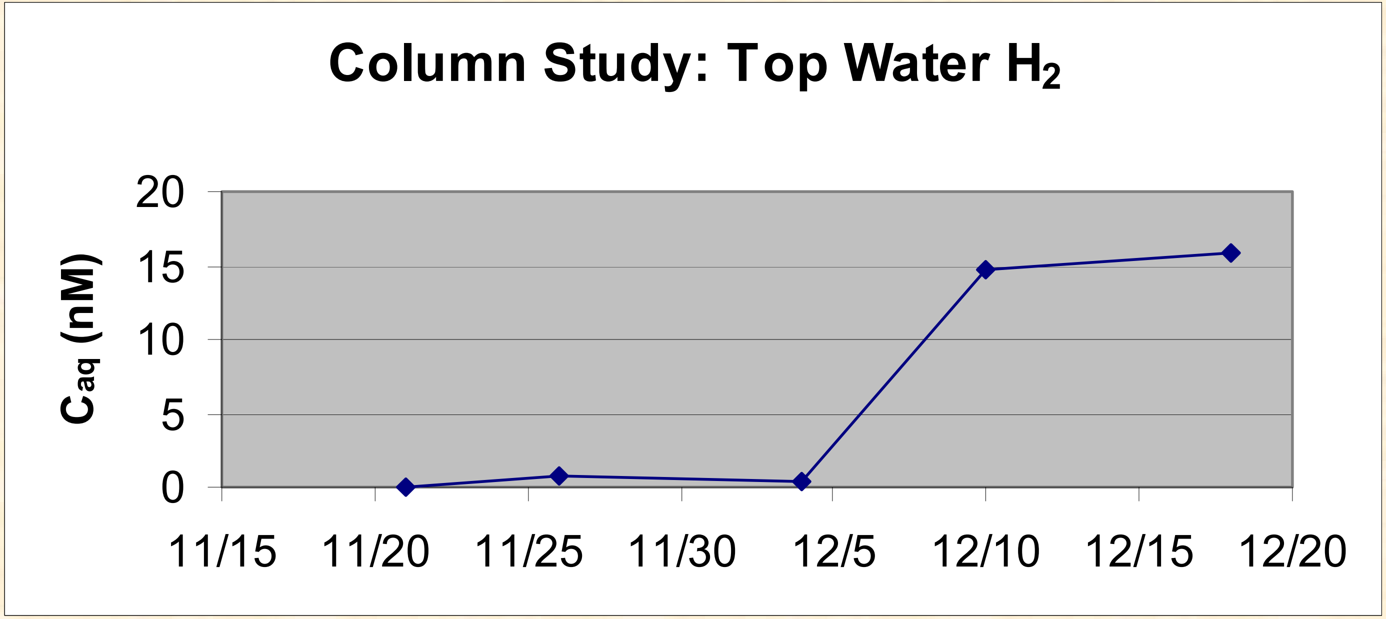


- Cells counts increased about 8 fold



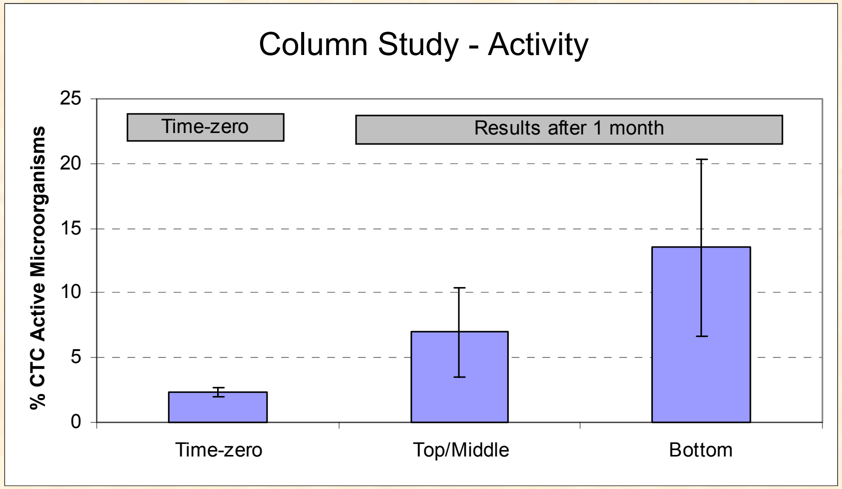
H_2 Amendment, Column Study (Marine Harbor)

- Porewater H_2 limited by diffusion
  - Leading edge advanced ~ 0.5'/month
  - Annual zone of influence up to ~6 feet

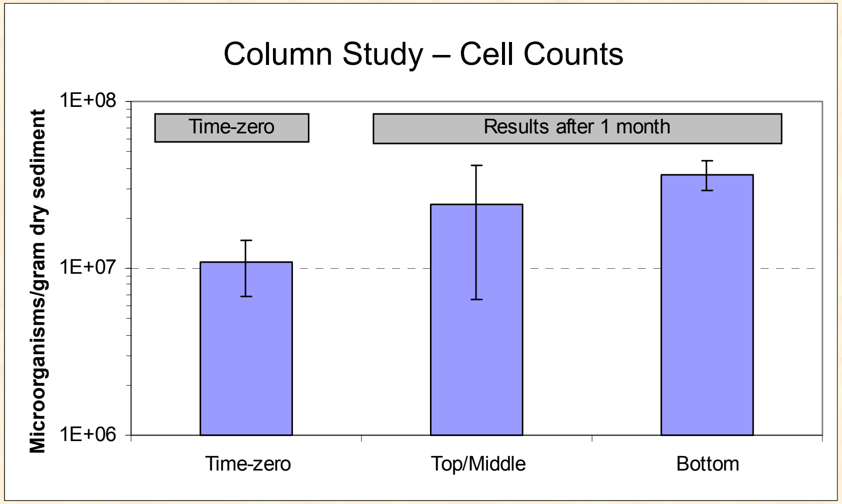


Activity Results - Column Study (Marine Harbor)

- CTC activity increased about 4-fold in bottom layer



- Cell counts increased about 3-fold in bottom layer



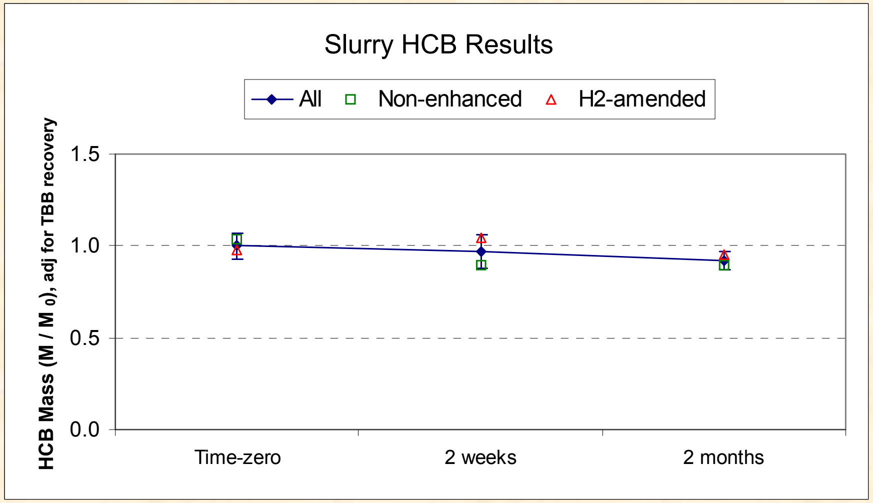
HCB Results - Cell Elution Study (Marine Harbor)

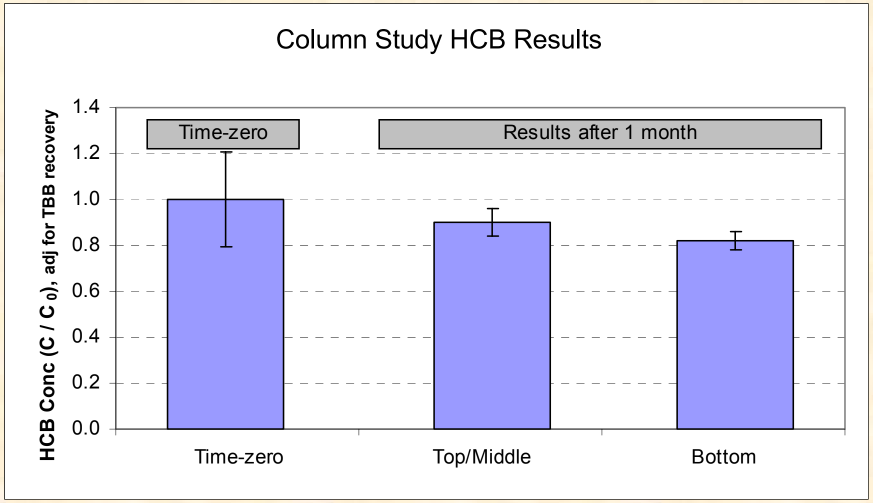
- H_2 treatment increased HCB degradation rate by ~ 50%

Hydrogen Amendment	Degradation Rate (1/hr)	Initial HCB Concentration (ppb)	HCB Concentration after 48 hrs (ppb)	Change in HCB (%)
Below threshold ($< 0.5\ \mu M\ H_2$)	0.0135 95% confidence interval: 0.0082 – 0.0188	5.6 (std dev 0.17)	3.2 (std dev 0.01)	43%
Above threshold ($0.6\ \mu M\ H_2$)	0.0201 95% confidence interval: 0.0176 – 0.0225	8.7 (std dev 0.09)	3.2 (std dev 0.05)	63%
Above threshold ($1.8\ \mu M\ H_2$)	0.0214 95% confidence interval: 0.0199 – 0.0228	6.8 (std dev 0.12)	2.5 (std dev 0.05)	63%

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 H_2 in field:
  - As dissolved H_2?
  - To what depth?
  - How to minimize resuspension?
  - Spacing of injection points?
- What's next?
  - Slurry and column studies to completion
  - Bench studies of H_2 injection grid (H2-GRID) to refine design parameters
  - Scale-up cost analysis
  - Design and conduct field pilot

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