

Hydrogen Enhancement of Sediment Microbial Activity and Contaminant Degradation

RTDF Workshop on *In Situ* Technologies
Baltimore, MD
February 18-19, 2004



The Technology

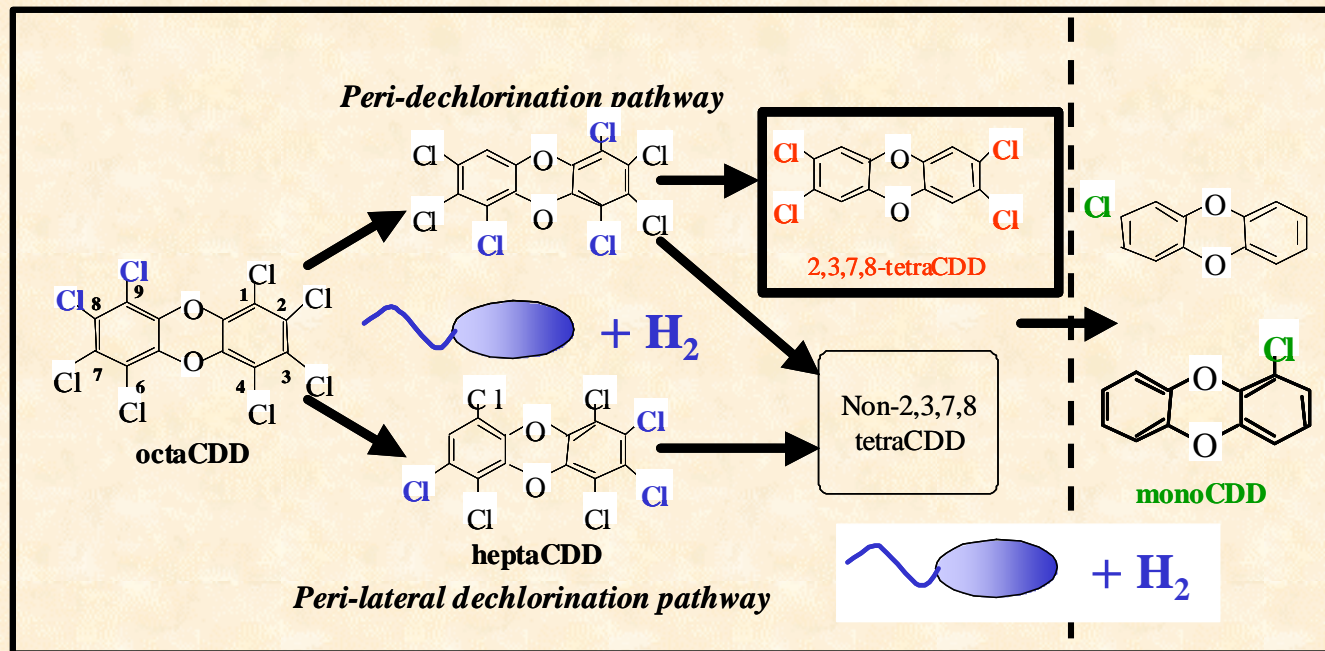
- ◆ Rationale and Hypothesis
- ◆ Approach and Methods
- ◆ Hydrogen-Impacted Microbial Ecology
- ◆ Hydrogen-Enhanced Dechlorination
- ◆ Scientific Challenges
- ◆ Bench-Scale Technology Development
- ◆ Technological Challenges

Rationale

- ◆ Ambient carbon and hydrogen fluxes limit *in situ* microbial activity in reducing sediments
 - To 5-20% of total extractable population
- ◆ Increased hydrogen fluxes enhance total respiratory competence and influence ecological composition
 - Based on redox dyes
- ◆ These combined effects increase dechlorination activity
 - Shown in previous experiments to increase hexachlorobenzene and dioxin dechlorination rates from 2-fold to an order of magnitude
- ◆ Hydrogen gas is cheap and diffuses rapidly in sediments


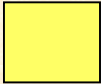

Hypothesis

- ◆ *In situ* amendment with hydrogen can increase metabolic and dechlorination activity
 - Relevant for application to large and complex contaminated areas



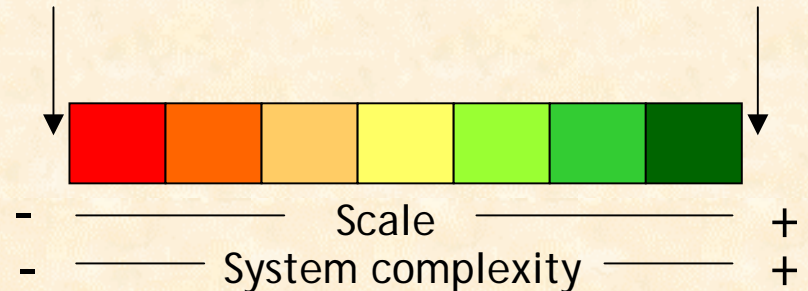
Laboratory Studies

◆ Matrix:

- Cell elution 
- Slurry 
- Column 

Process-level
lab study

Field
application



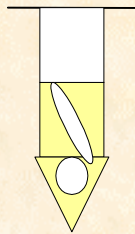
◆ Treatment: H₂ addition, HCB spike

◆ Response:

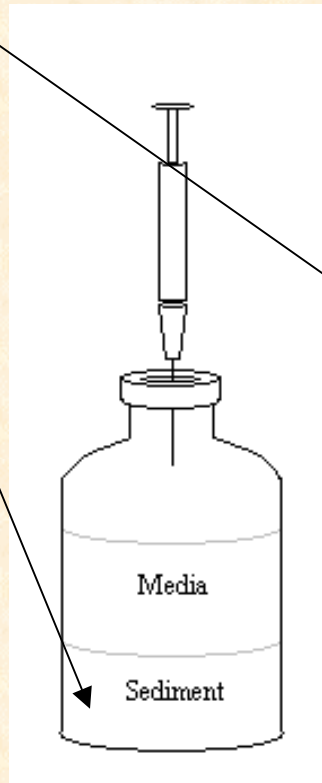
- Microbial activity
- Contaminant degradation

Experimental Matrix

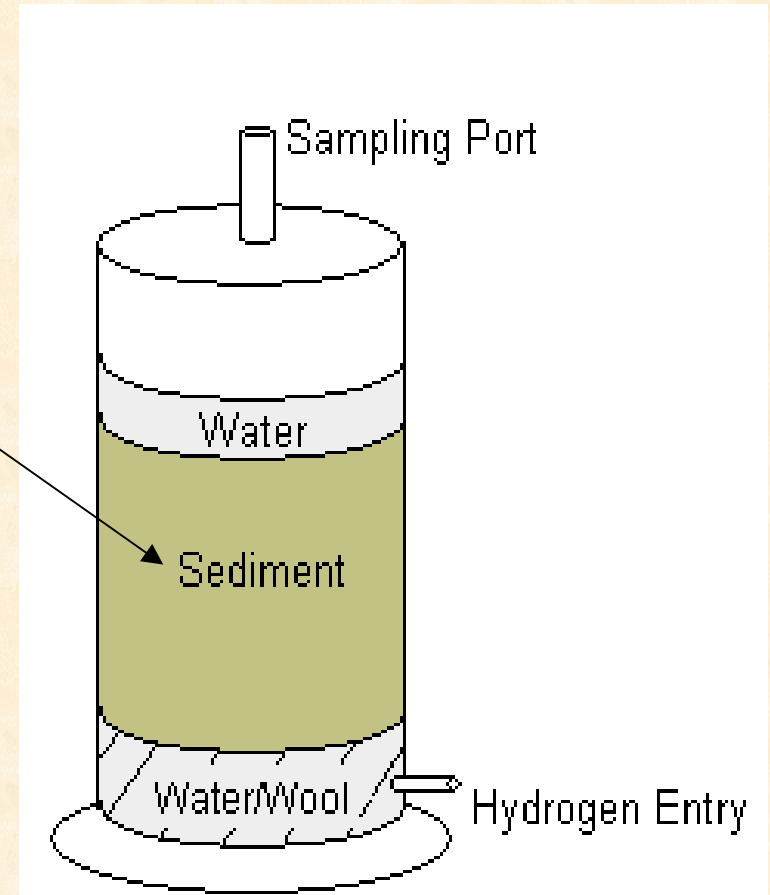
Sediment core



Cell elution



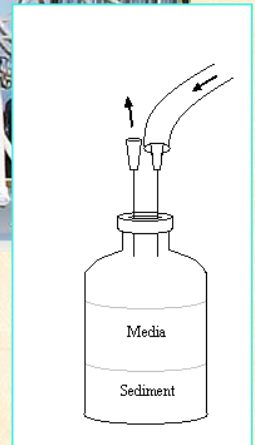
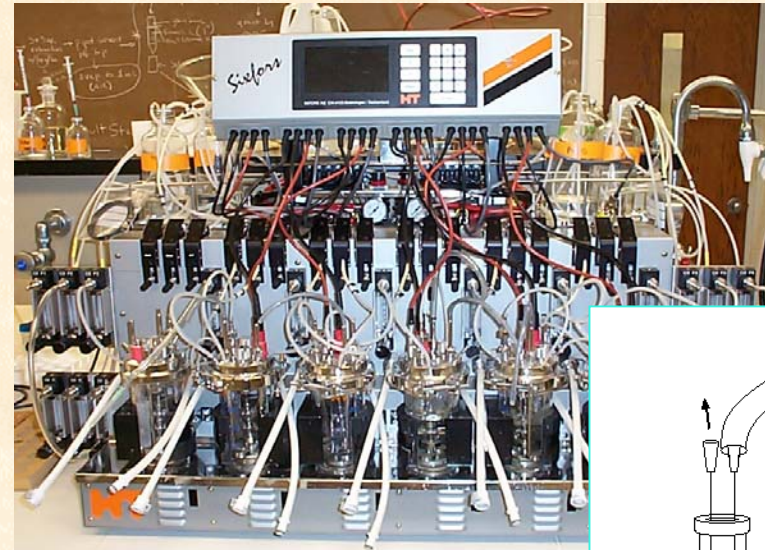
Sediment slurry



Sediment column

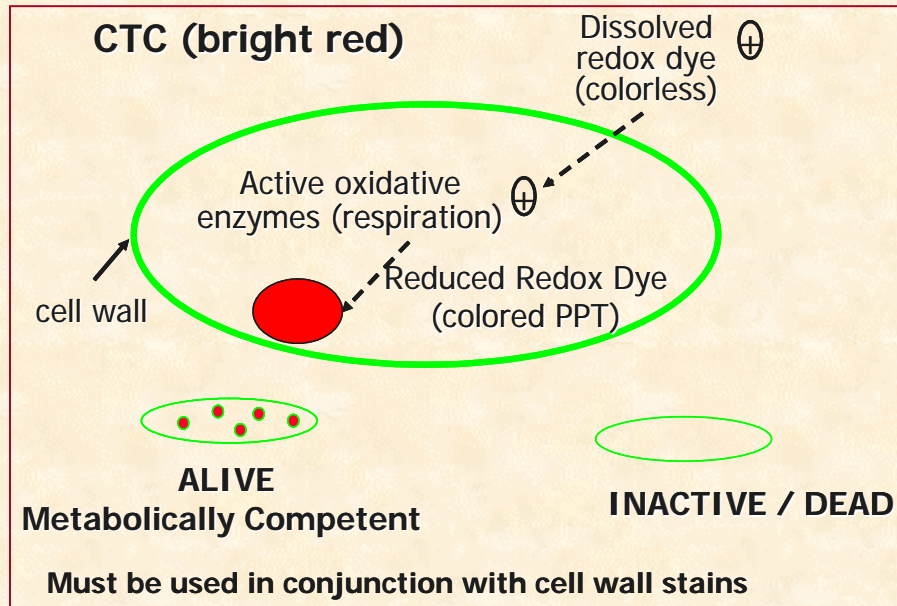
Methods: Hydrogen Enhancement of Elutions and Slurries

- ◆ 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



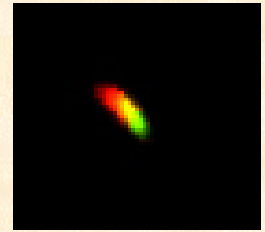
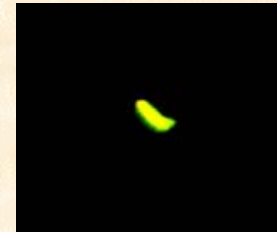
This 6-reactor system is equipped with a H_2/N_2 gas mixing/delivery system, temperature, and pH control.

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



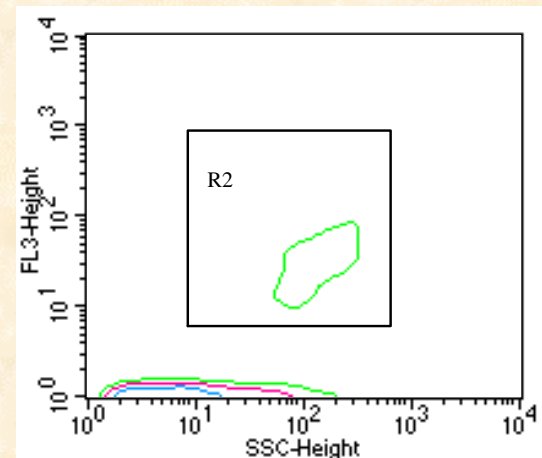
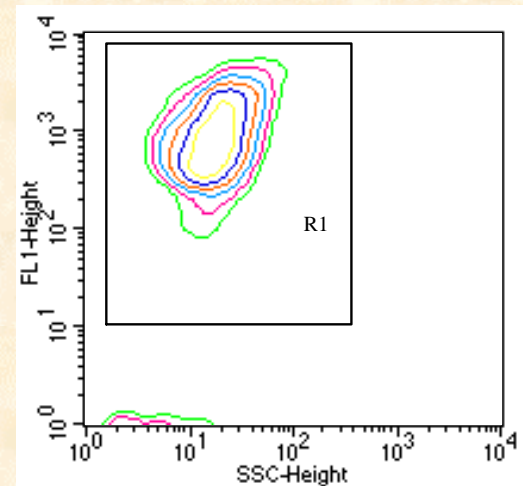
◆ Microscope analysis:

- Green - nonactive cell
- Green/red - active cell



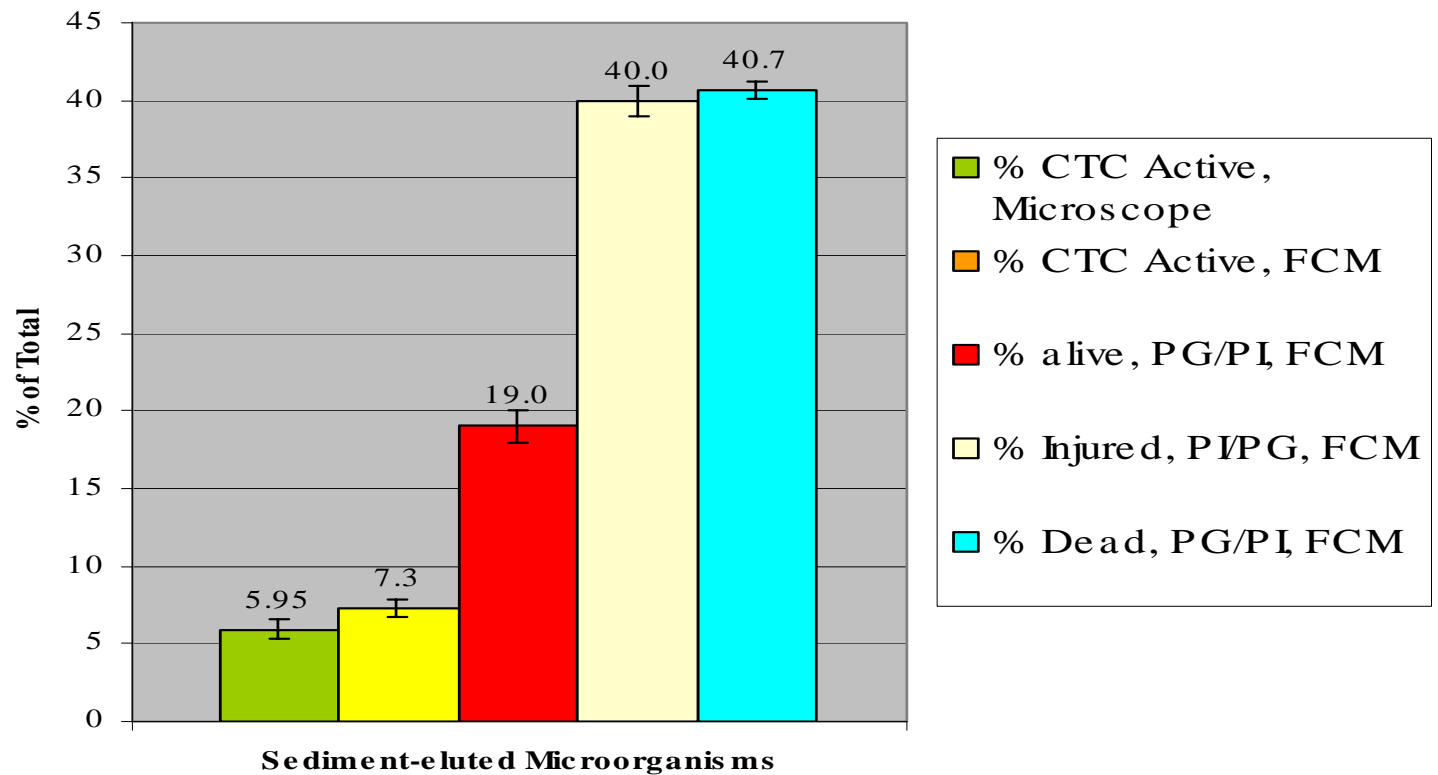
Flow Cytometry

- ◆ 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)



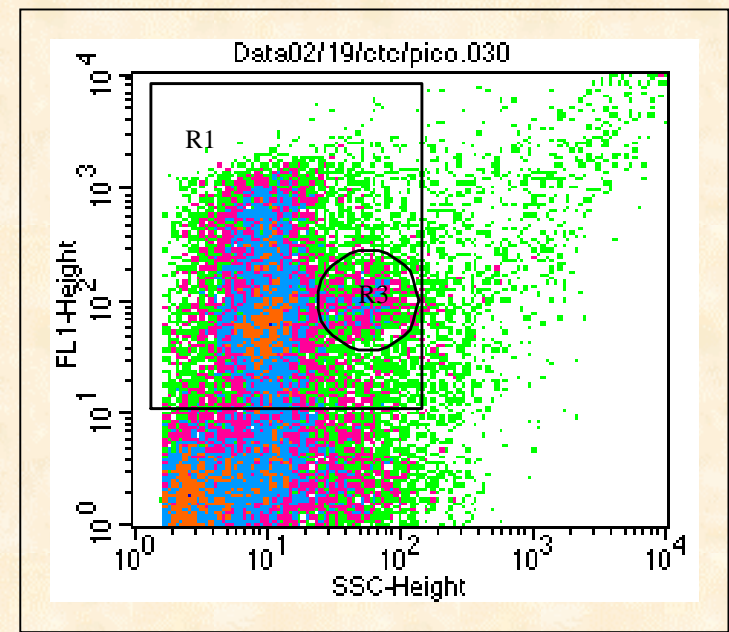
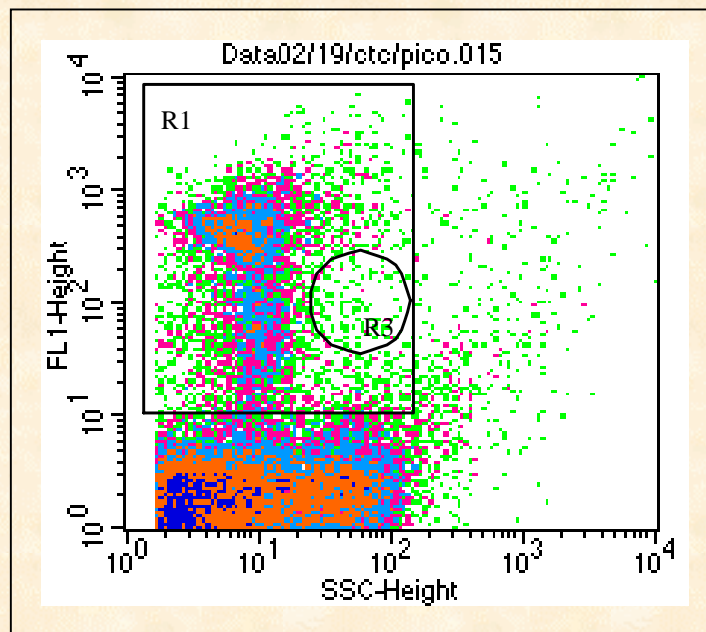
Multi-Parametric Microbial Characterization (Passaic River, NJ)

PG: PicoGreen (general nucleic acid stain)
PI: Propidium iodide (membrane permeability)

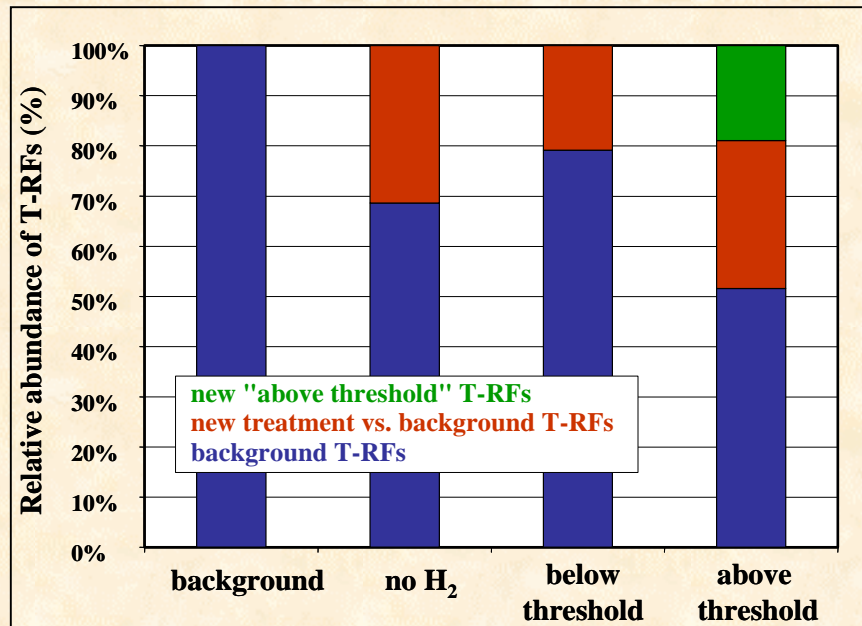


Ecological Response to Hydrogen: Flow Cytometry Analysis (Passaic R.)

- 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)



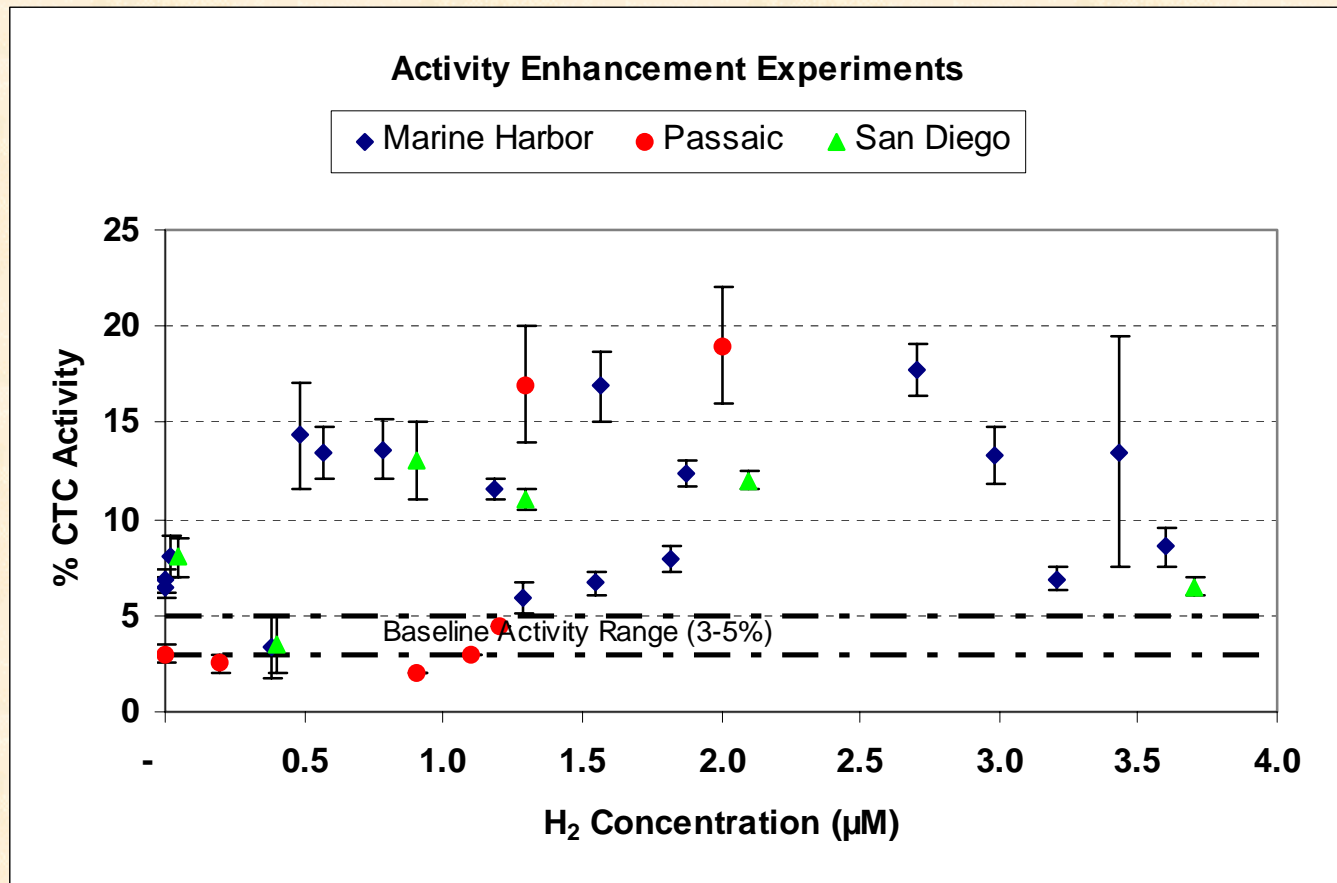
- ◆ Amendments of microbial elutions with nitrogen gas (no H₂) and H₂ fluxes not impacting CTC activity result in 20-30% emerging T-RFs
- ◆ Amendments above threshold of CTC activity result in emergence of 20% distinct RFs
- ◆ No populations (out of a total of 74 T-RFs) could be identified using *MspI*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

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

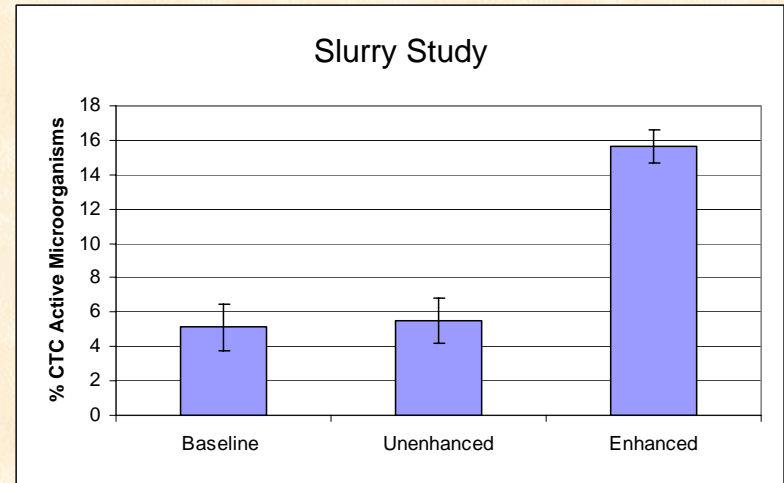
Activity Enhancement for Three Sites - Cell Elutions

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

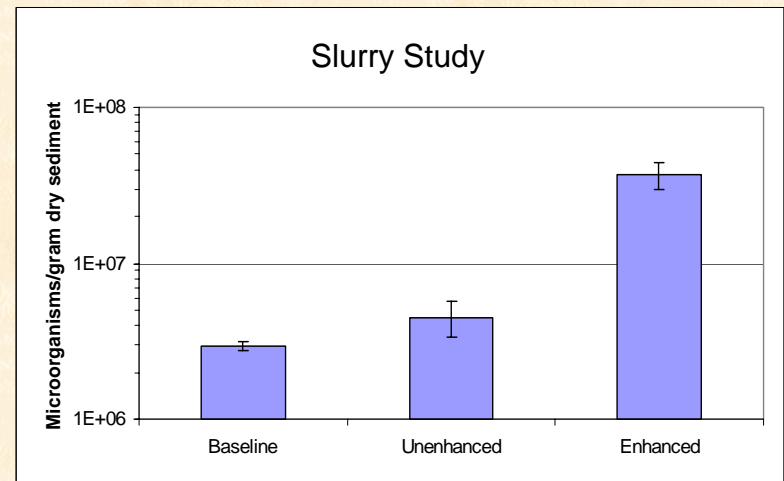


Activity Results - Slurry Study (Marine Harbor)

◆ CTC activity increased about 3 fold

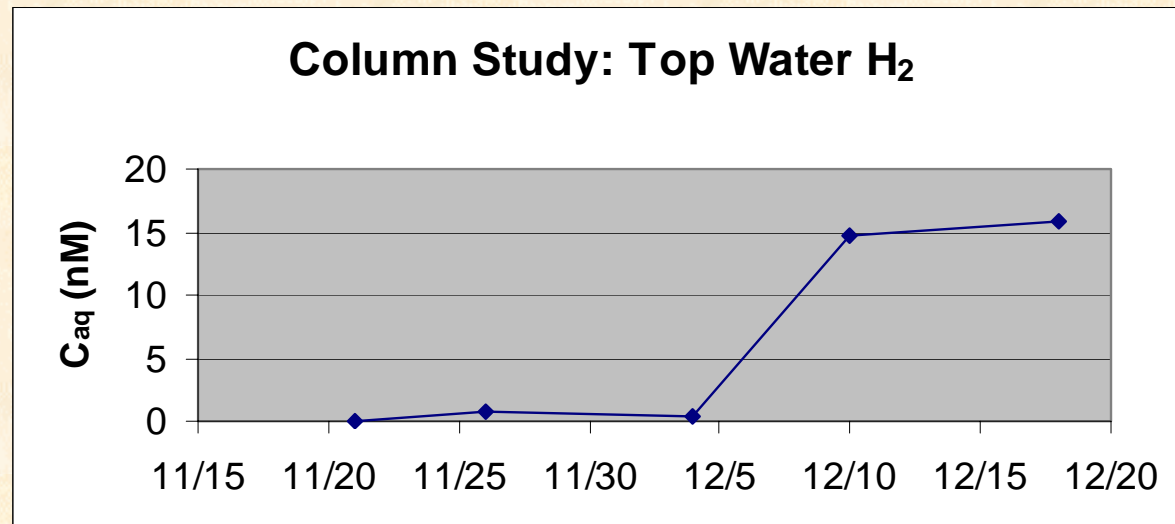


◆ Cells counts increased about 8 fold



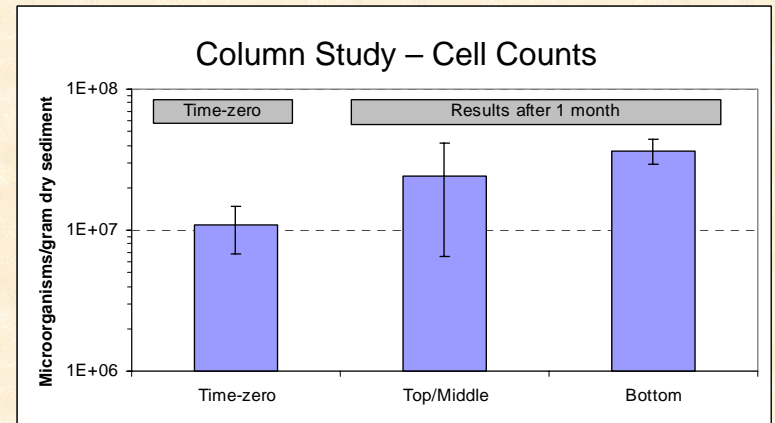
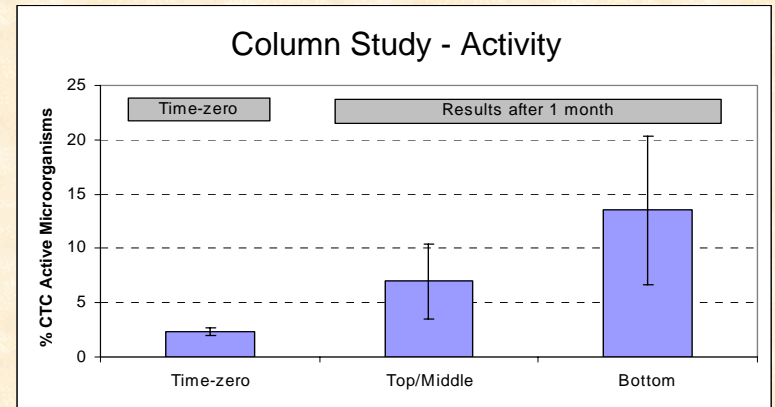
H₂ Amendment, Column Study (Marine Harbor)

- ◆ Porewater H₂ limited by diffusion
 - Leading edge advanced ~ 0.5' /month
 - Annual zone of influence up to ~6 feet



Activity Results - Column Study (Marine Harbor)

- ◆ CTC activity increased about 4-fold in bottom layer
- ◆ Cell counts increased about 3-fold in bottom layer



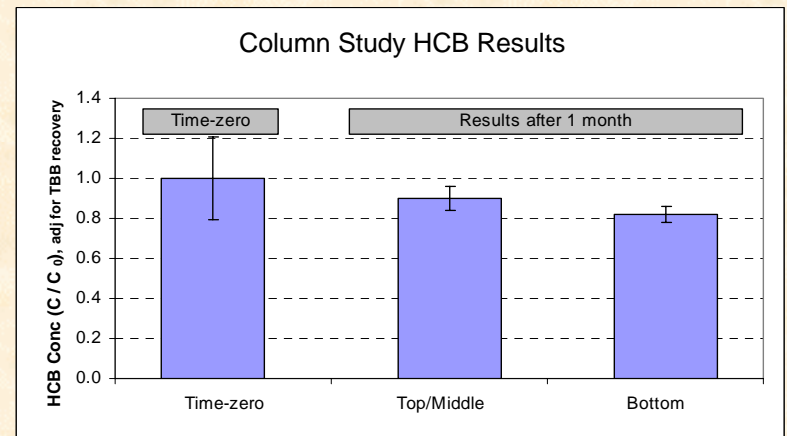
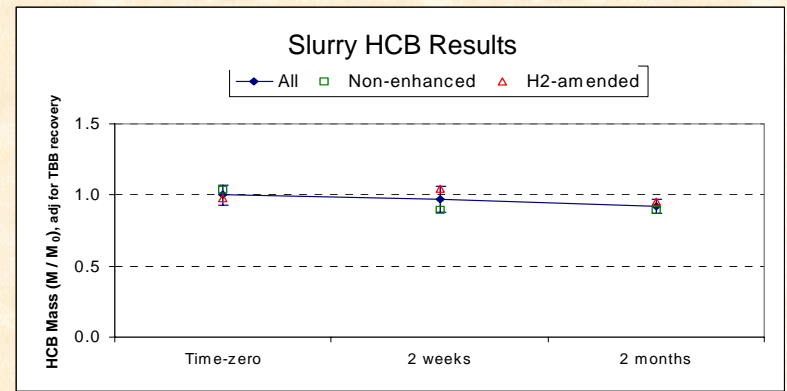
HCB Results - Cell Elution Study (Marine Harbor)

- ◆ H₂ 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 ($< 0.5 \mu\text{M H}_2$)	0.0135 95% confidence interval: 0.0082 – 0.0188	5.6 (std dev 0.17)	3.2 (std dev 0.01)	43%
Above threshold ($0.6 \mu\text{M H}_2$)	0.0201 95% confidence interval: 0.0176 – 0.0225	8.7 (std dev 0.09)	3.2 (std dev 0.05)	63%
Above threshold ($1.8 \mu\text{M H}_2$)	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



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 to refine design parameters
 - Scale-up cost analysis
 - Design and conduct field pilot

Acknowledgements

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