In-Situ Enhancement of Anaerobic Microbial Dechlorination of Polychlorinated Dibenzo-*p*-dioxins and Dibenzofurans in Marine and Estuarine Sediments

Max Häggblom

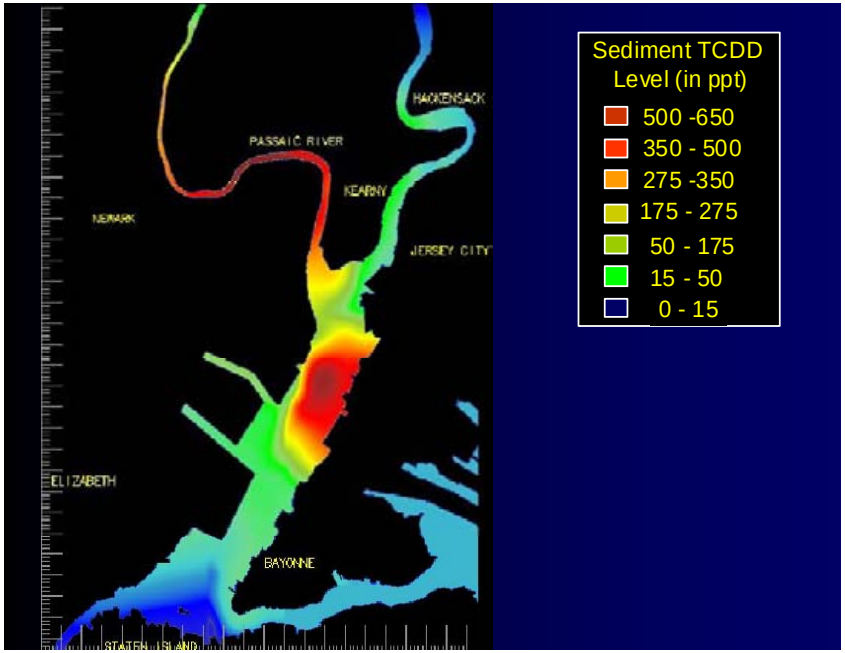
Donna Fennell, Young-Beom Ahn & Lee Kerkhof

Rutgers University
New Brunswick, NJ

Bioremediation of Contaminated Sediments

Development of strategies for *in situ* enhancement of dioxin dehalogenation

- *redox manipulation*
- *electron donor addition*
- *addition of "haloprimers"*

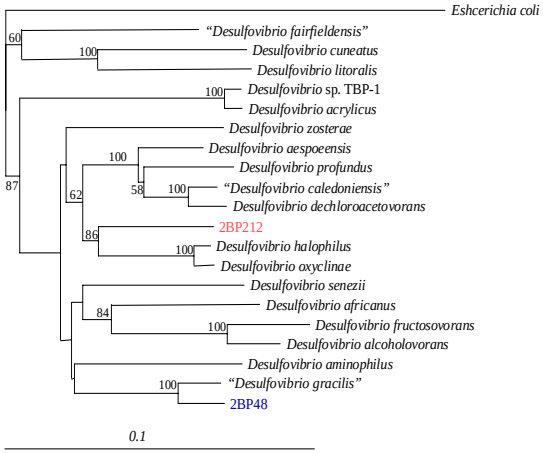


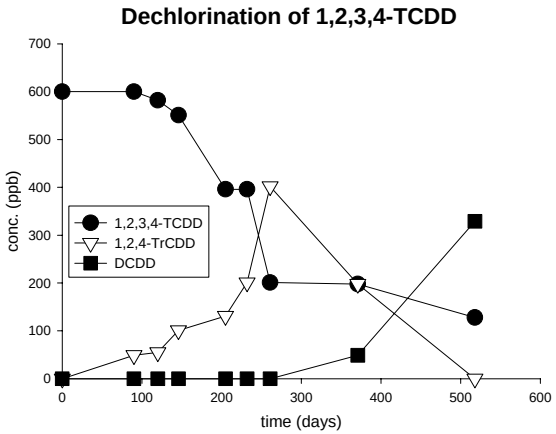
On the search for dehalogenating bacteria

Objectives

- Identify organisms responsible for dechlorination using microbiological and molecular techniques.

- Identify environmental conditions and amendments that enhance and accelerate dechlorination of PCDD/Fs by native microbial populations.





TECHNICAL APPROACH

1. Characterize the PCDD/F-dechlorinating capability of native dehalogenating bacteria in different estuarine and marine sediments.

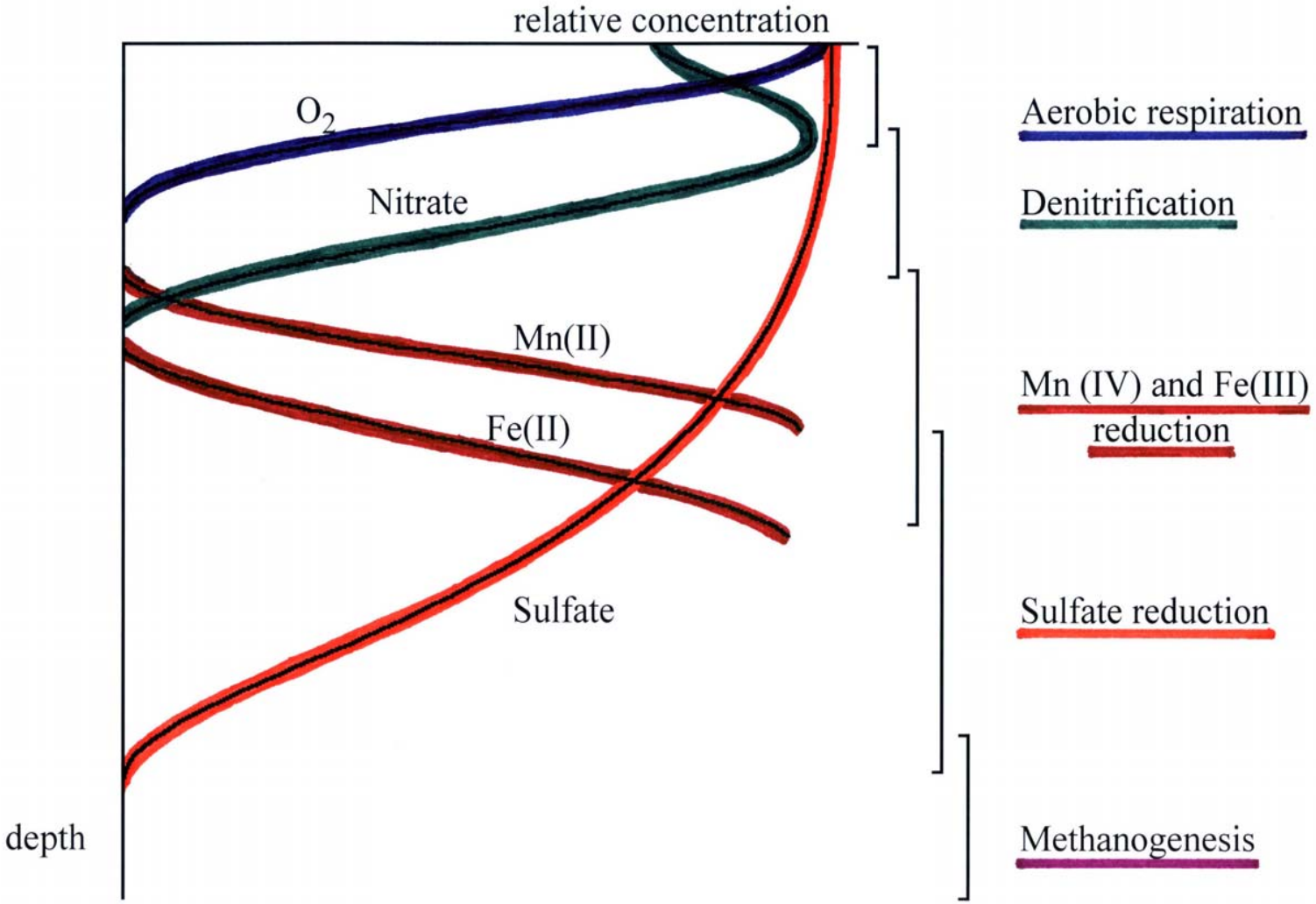
2. Identify specific amendments and their combinations that prime and/or accelerate the dechlorination of PCDD/Fs.

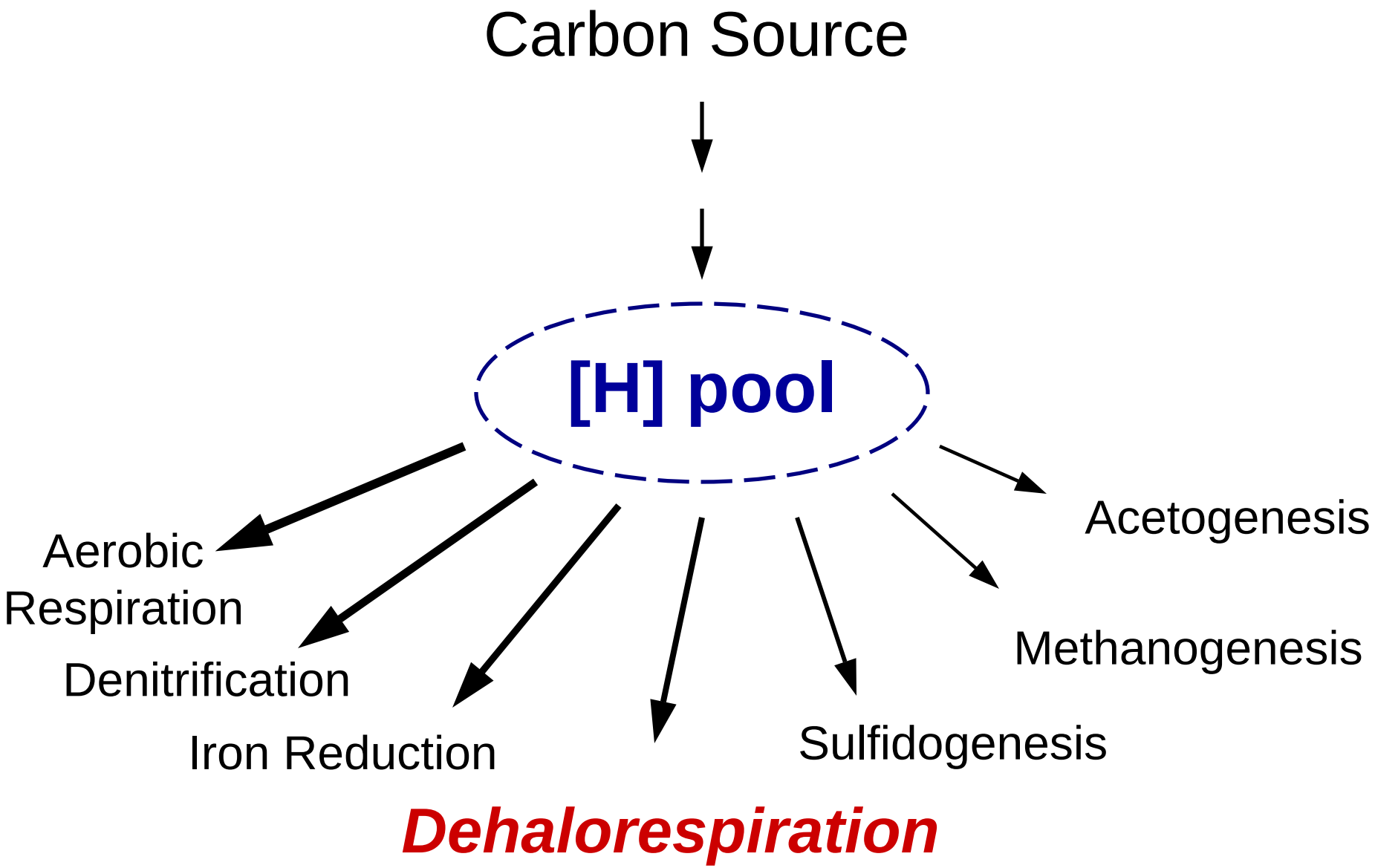
3. Characterize the effect of different competitive terminal electron accepting processes (e.g. sulfidogenesis, iron-reduction, methanogenesis) on the dechlorination / transformation of PCDD/Fs.

4. Identify and characterize specific PCDD/F-dechlorinating bacteria and consortia using complementary molecular techniques.

5. Produce site conceptual models and bioprocess models to describe the effects of different amendment strategies.

Reductive processes in estuarine sediments



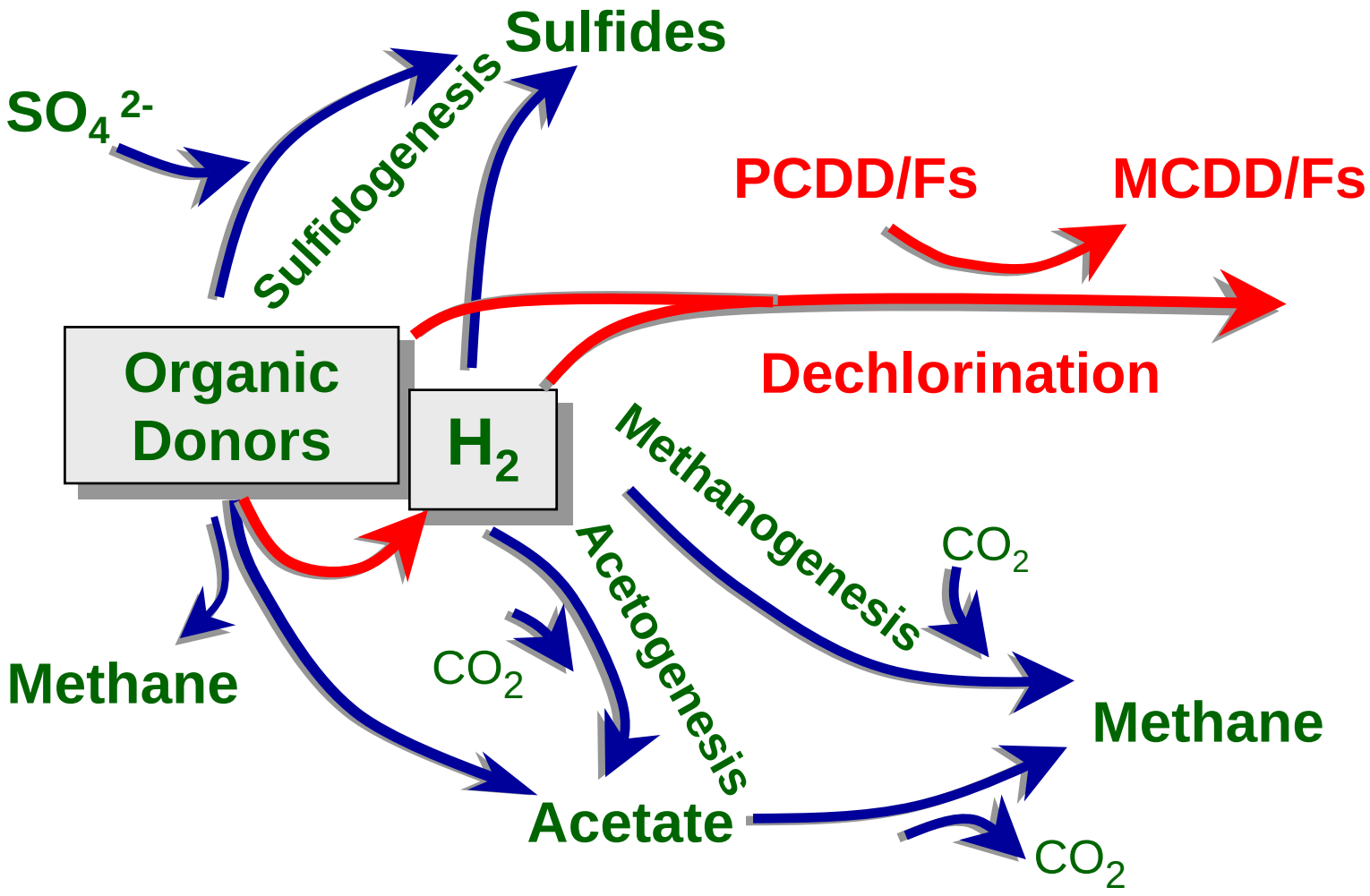

Carbon Source
[H] pool
Aerobic Respiration
Denitrification
Iron Reduction
Acetogenesis
Methanogenesis
Sulfidogenesis
Dehalorespiration

Affinity for H_2 determines predominant electron accepting processes

Electron Acceptor Process	Hydrogen Concentration (nM)
Aerobic (O_2) respiration	<0.1
Denitrification	<0.1
Iron(III) reduction	0.2 - 0.6
Dehalorespiration	< 0.31
Sulfate reduction	1 - 4
Methanogenesis	>5
Acetogenesis	>336

Bottom Line: Dehalorespiring bacteria can out-compete methanogens for hydrogen

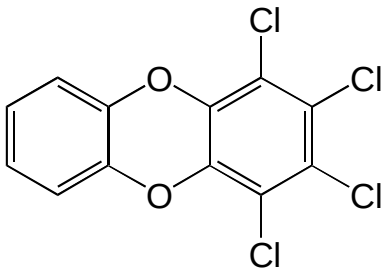
Dechlorination in anaerobic food-webs

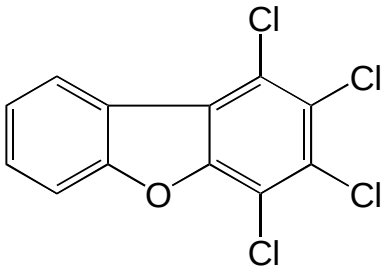


Competing electron flow pathways in anaerobic sediments

Establishment of Anaerobic Enrichments

1,2,3,4-TCDD and 1,2,3,4-TCDF as model compounds



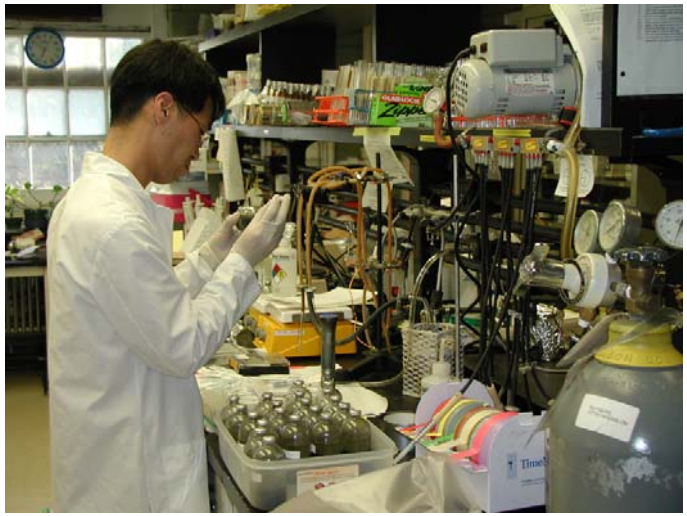


No added electron acceptor | 20 mM Sulfate | 200 mM Fe(III)

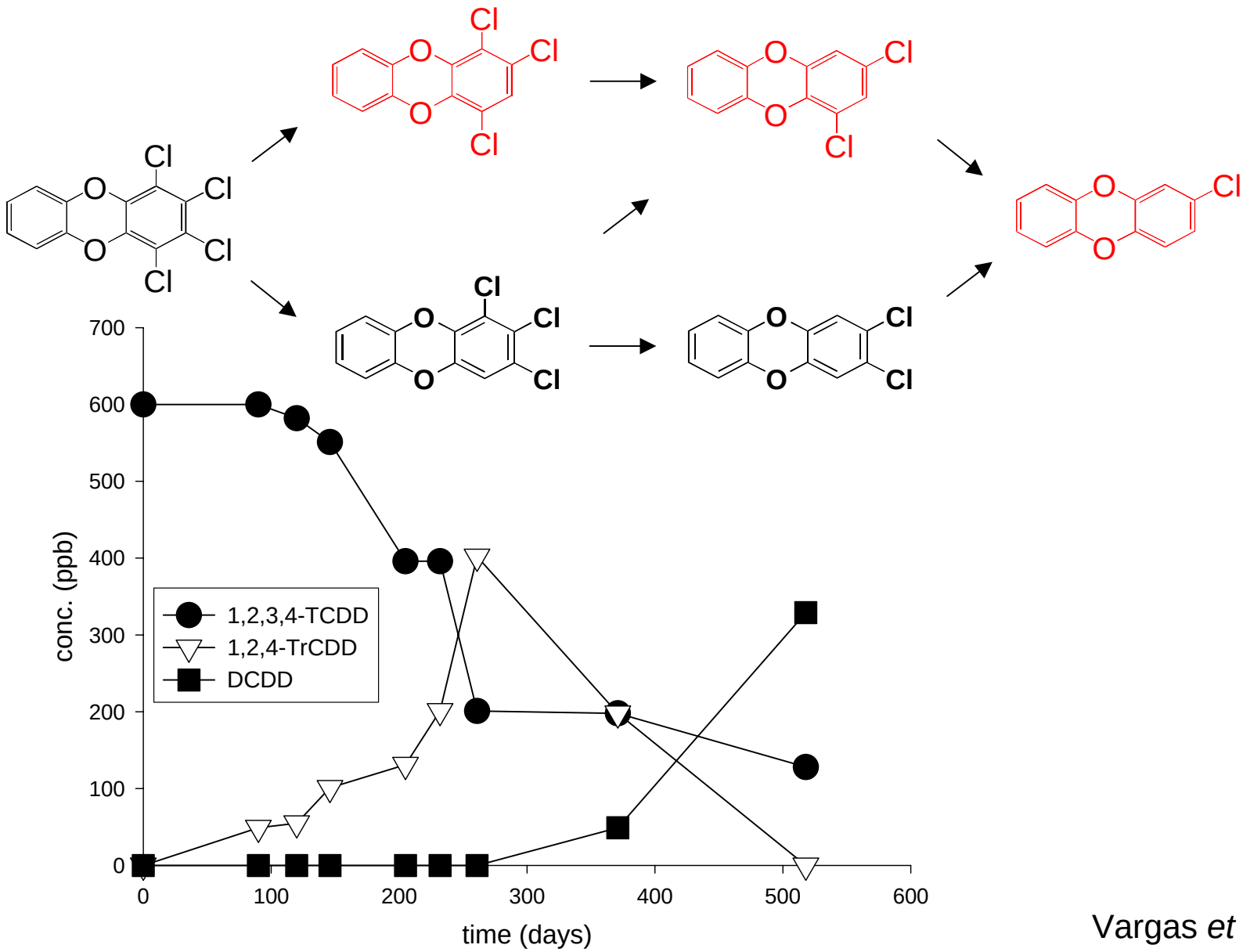
- Assess dechlorination potential in different sediments
- Develop dechlorinating enrichment cultures for elucidating microbial processes and dechlorination pathways

Anaerobic microcosms:
Spike with PCDD/F
+ H donor
+ halogenated “primer”

10-25% v/v sediment inoculum
alternate electron acceptors

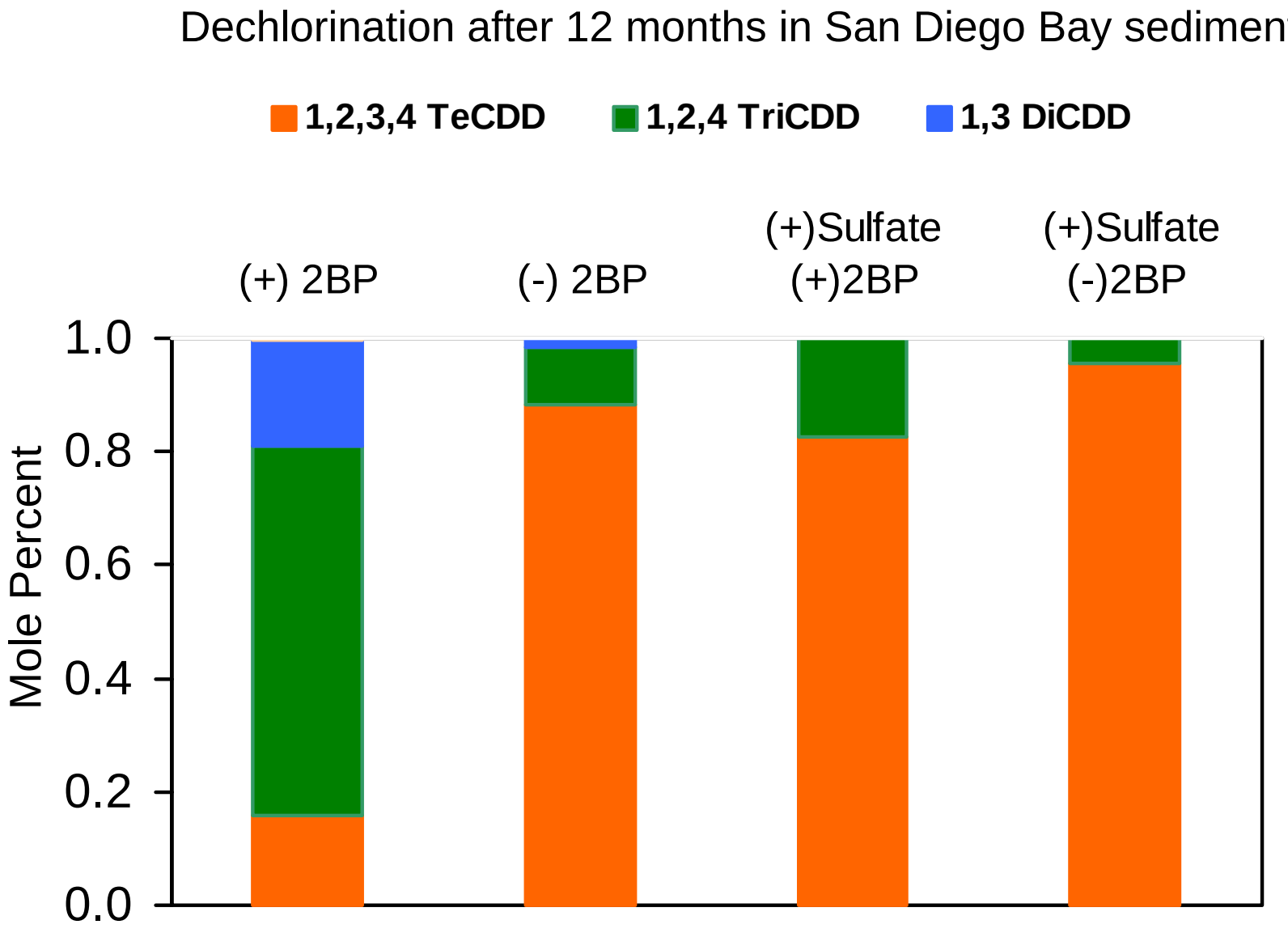


Dechlorination of Tetrachloro-*p*-dioxin

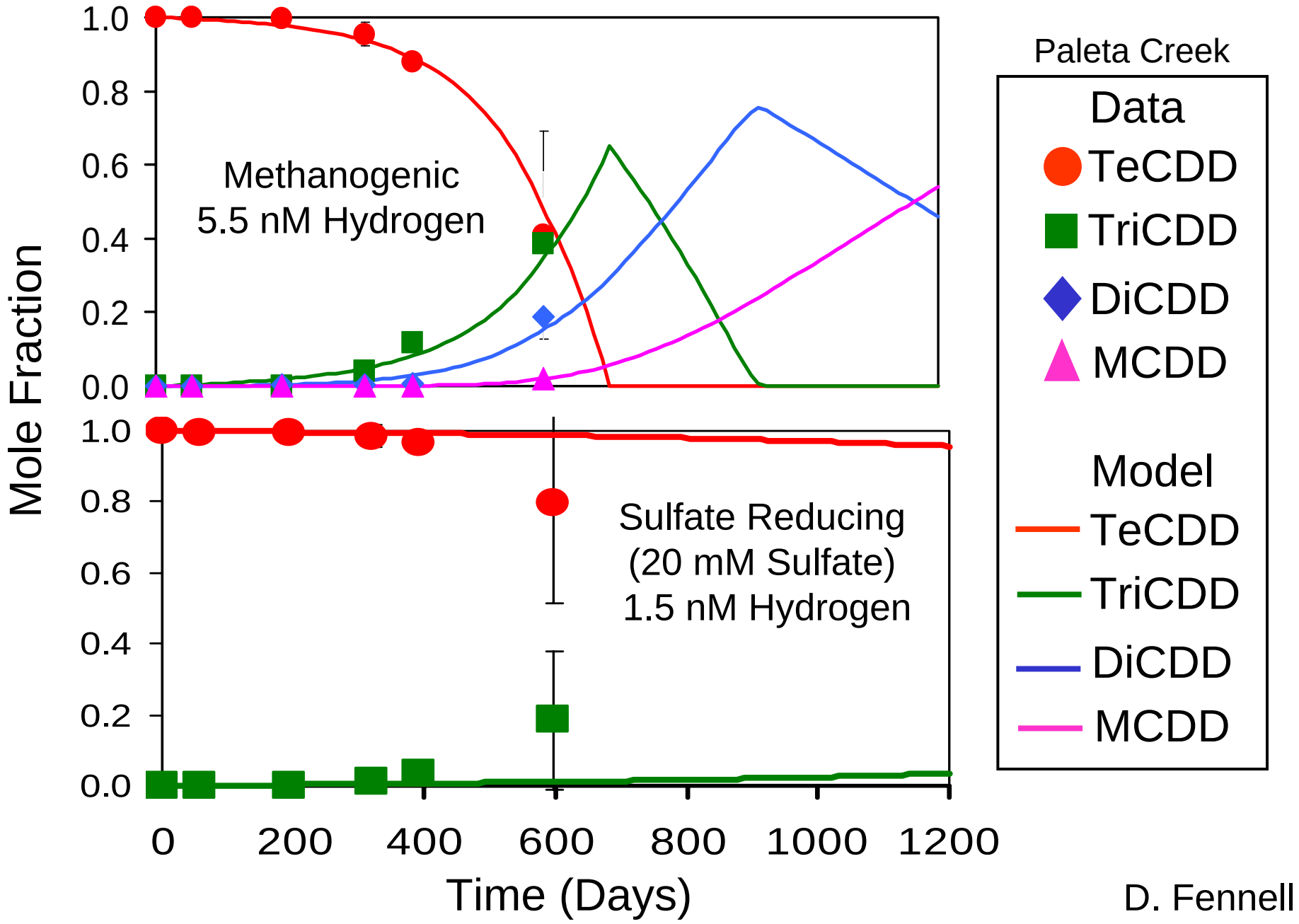


Vargas *et al*

Effect of Sulfate on Dechlorination



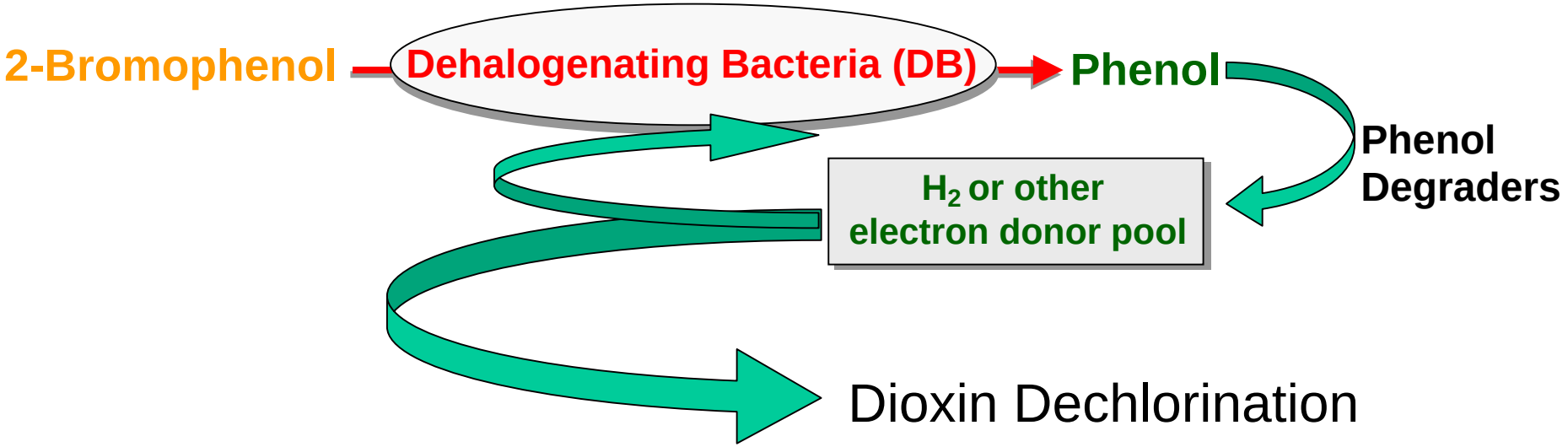
Model Simulation

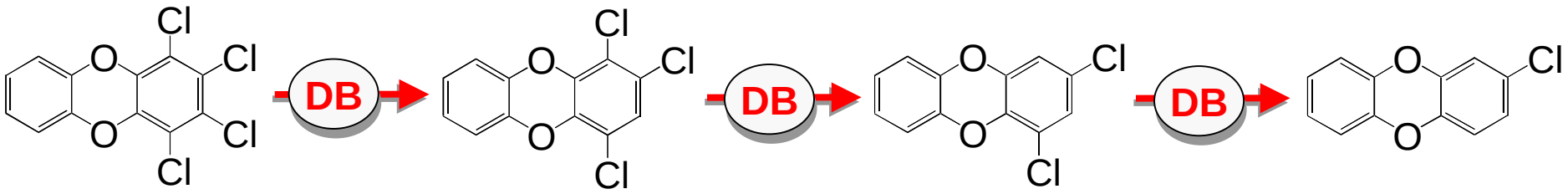


D. Fennell

“PRIMING” DECHLORINATION

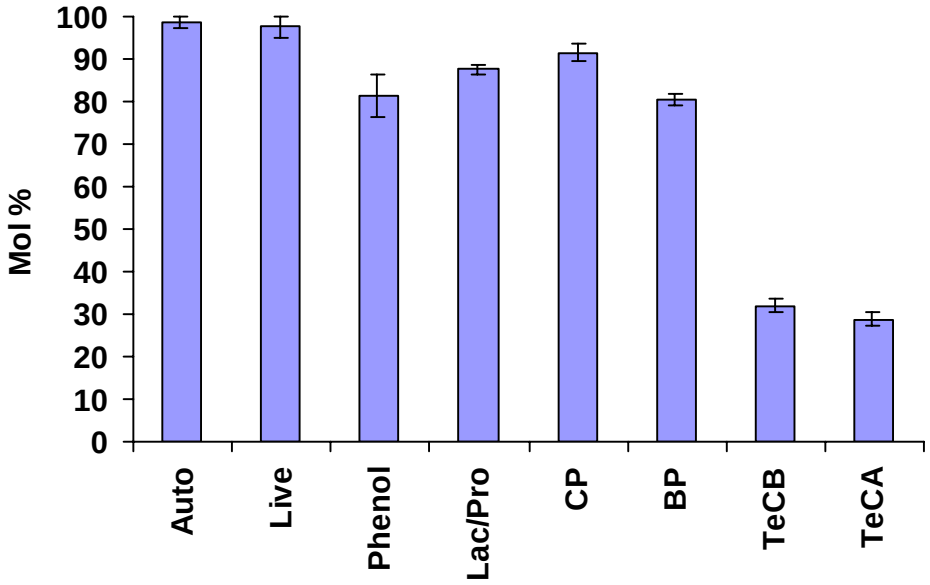
Proposed Roles of Halogenated Primers in PCDD Dechlorination





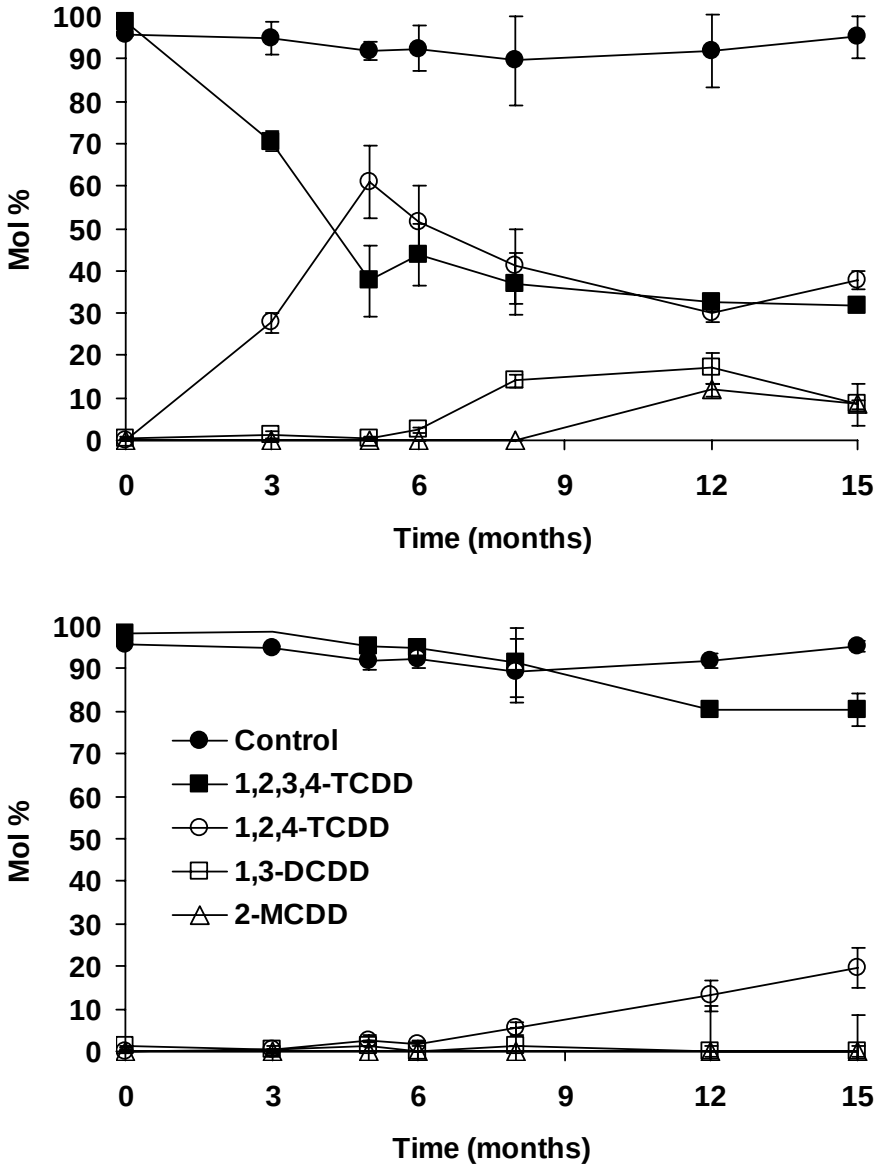
Priming Dechlorination in Sediments

+TeCB

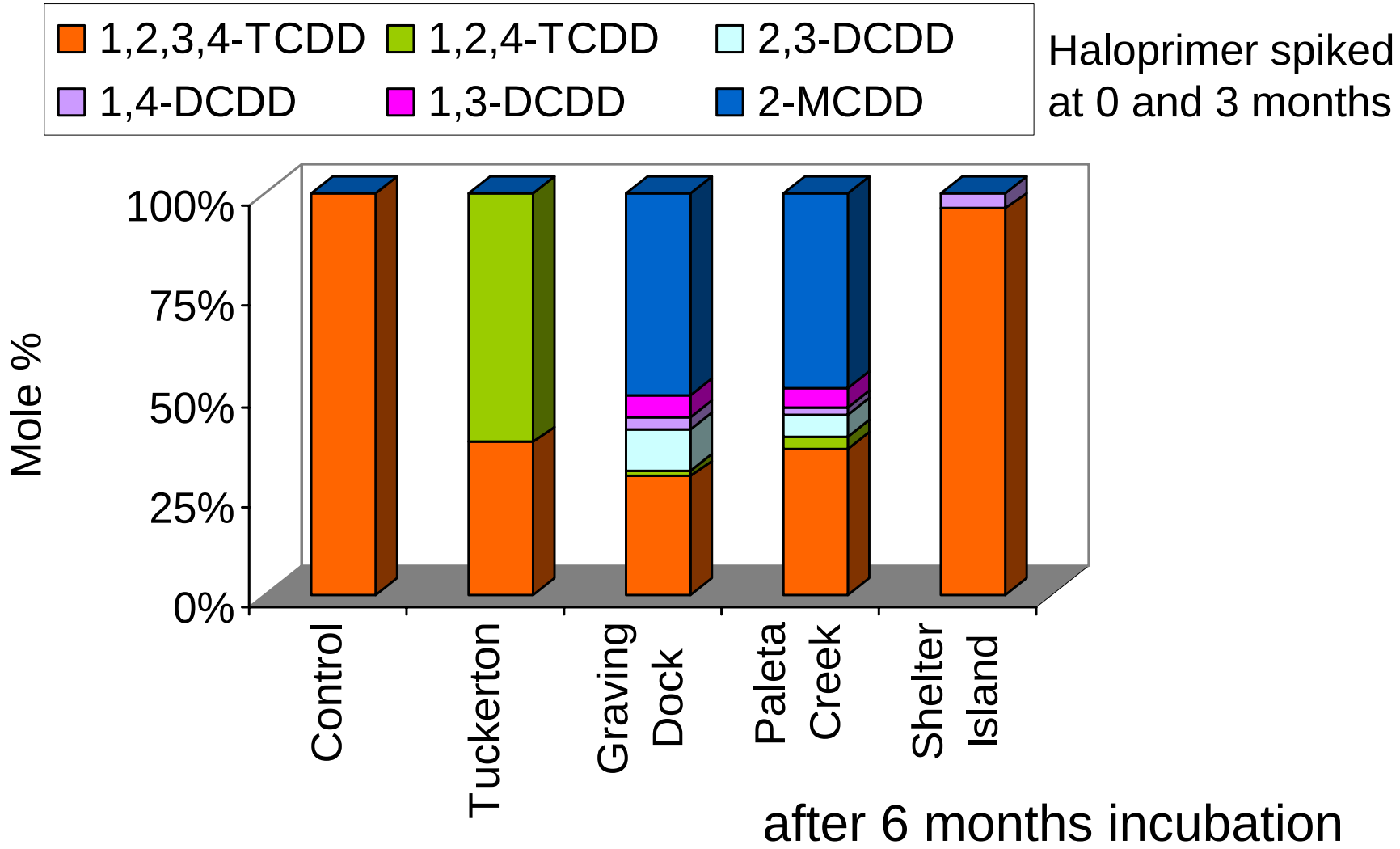


1,2,3,4-TeCDD remaining in Paleta Creek sediment slurries under different e-donor and co-amendment conditions after 15 months

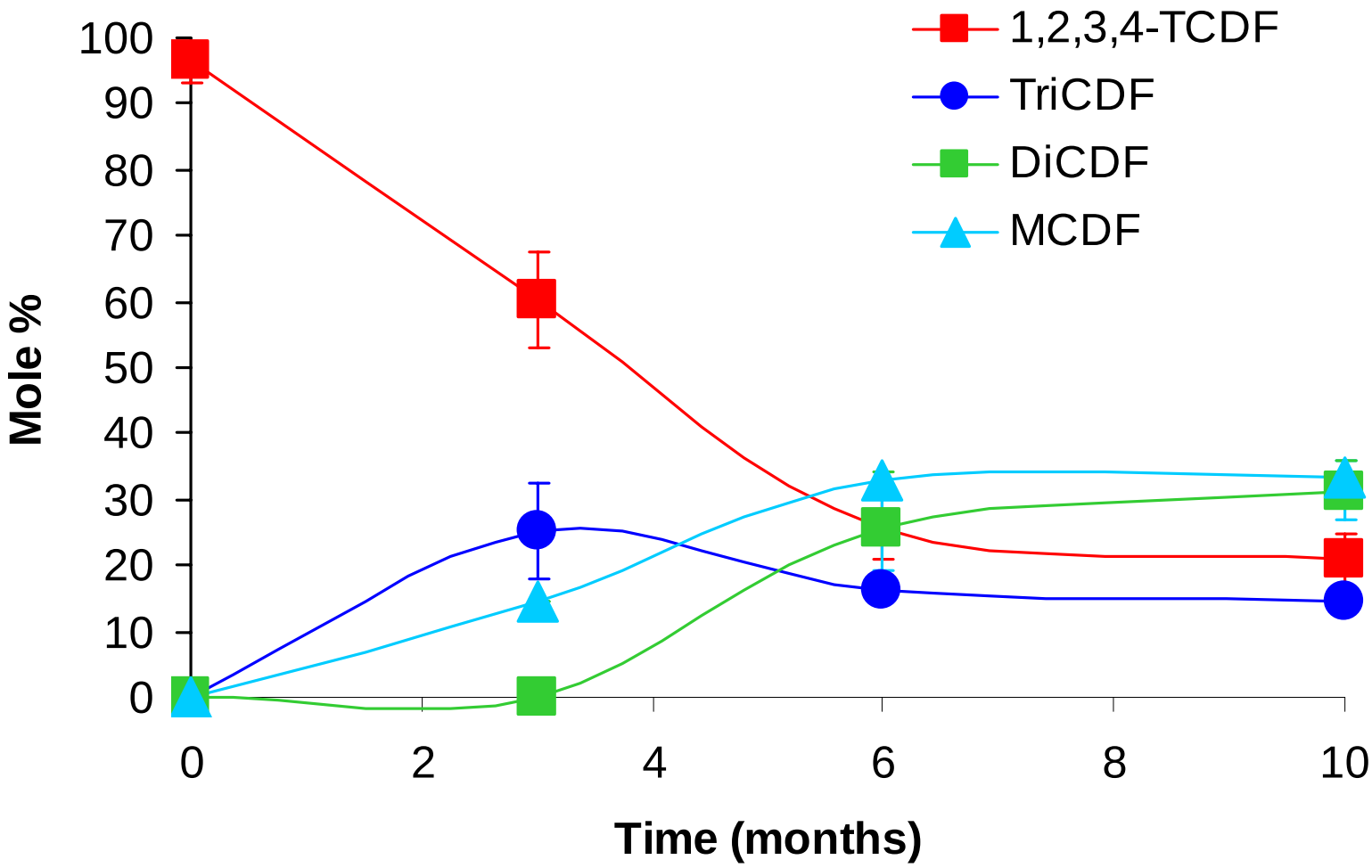
+BP control



Dechlorination of TCDD primed by TeCB at several sites

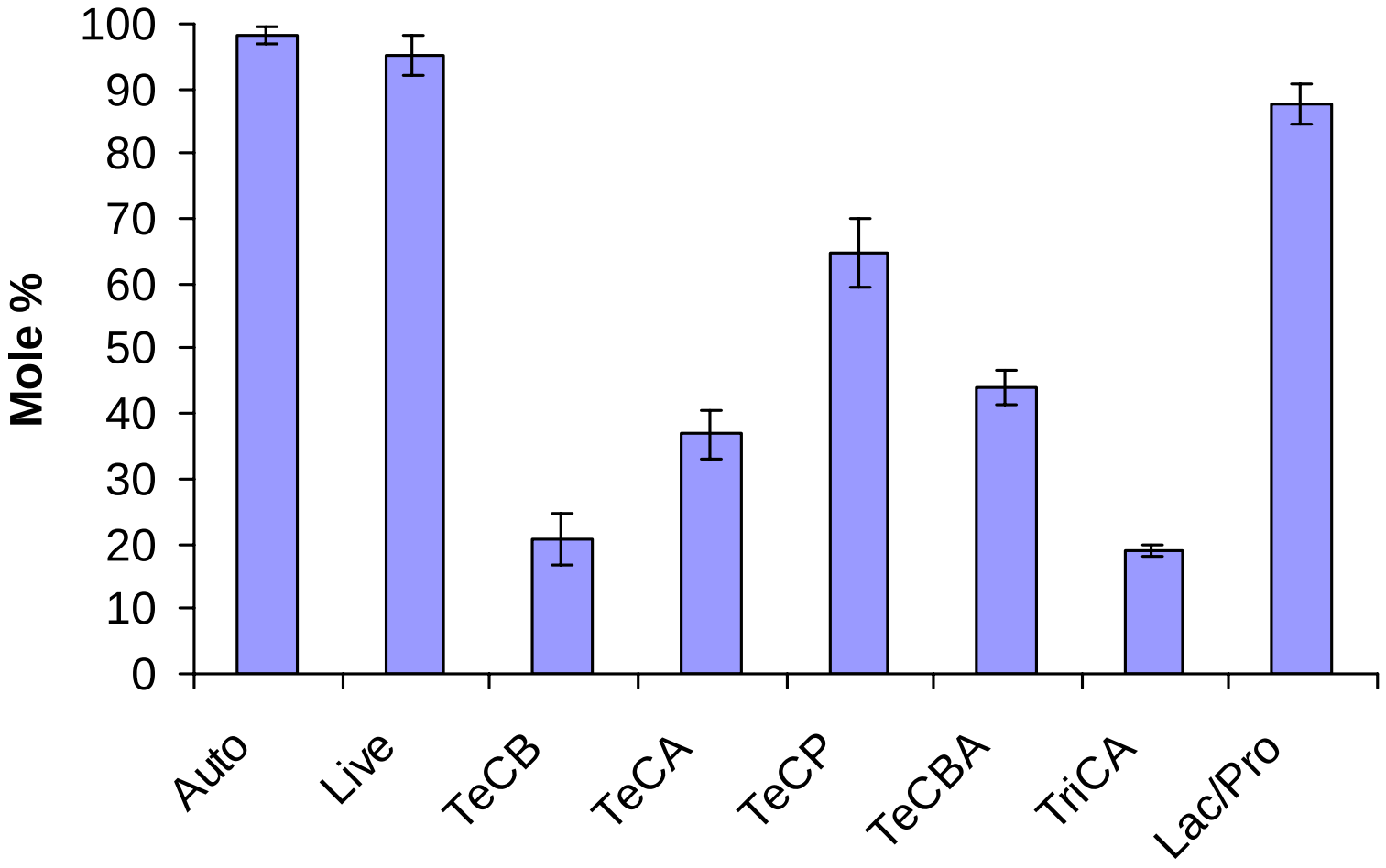


Dehalogenation of 1,2,3,4-TCDF



Paleta Creek microcosm, primed with TeCB

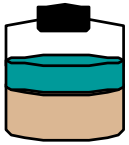
Effect of haloprimers on TCDF dechlorination



Paleta Creek sediments
Dechlorination of TCDF after 10 months incubation

Culture and Analysis Protocol

Environmental sample or microcosm



Cellular lipids

community changes

DNA

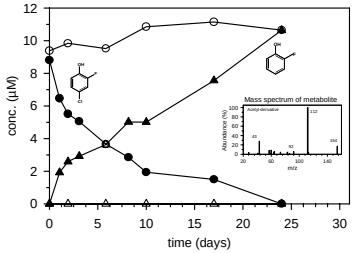
presence/absence

RNA

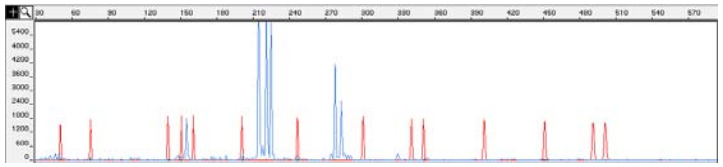
active population

Extract PCR

Analysis of activity

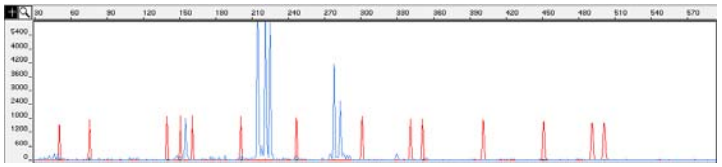


Extract RT-PCR

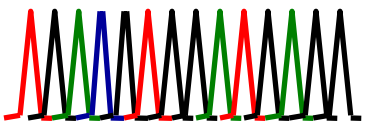


TRFLP analysis of PCR products

TRFLP analysis of RT-PCR products

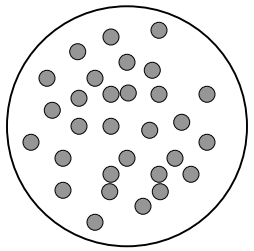


T G A C G T G G A T G A G G

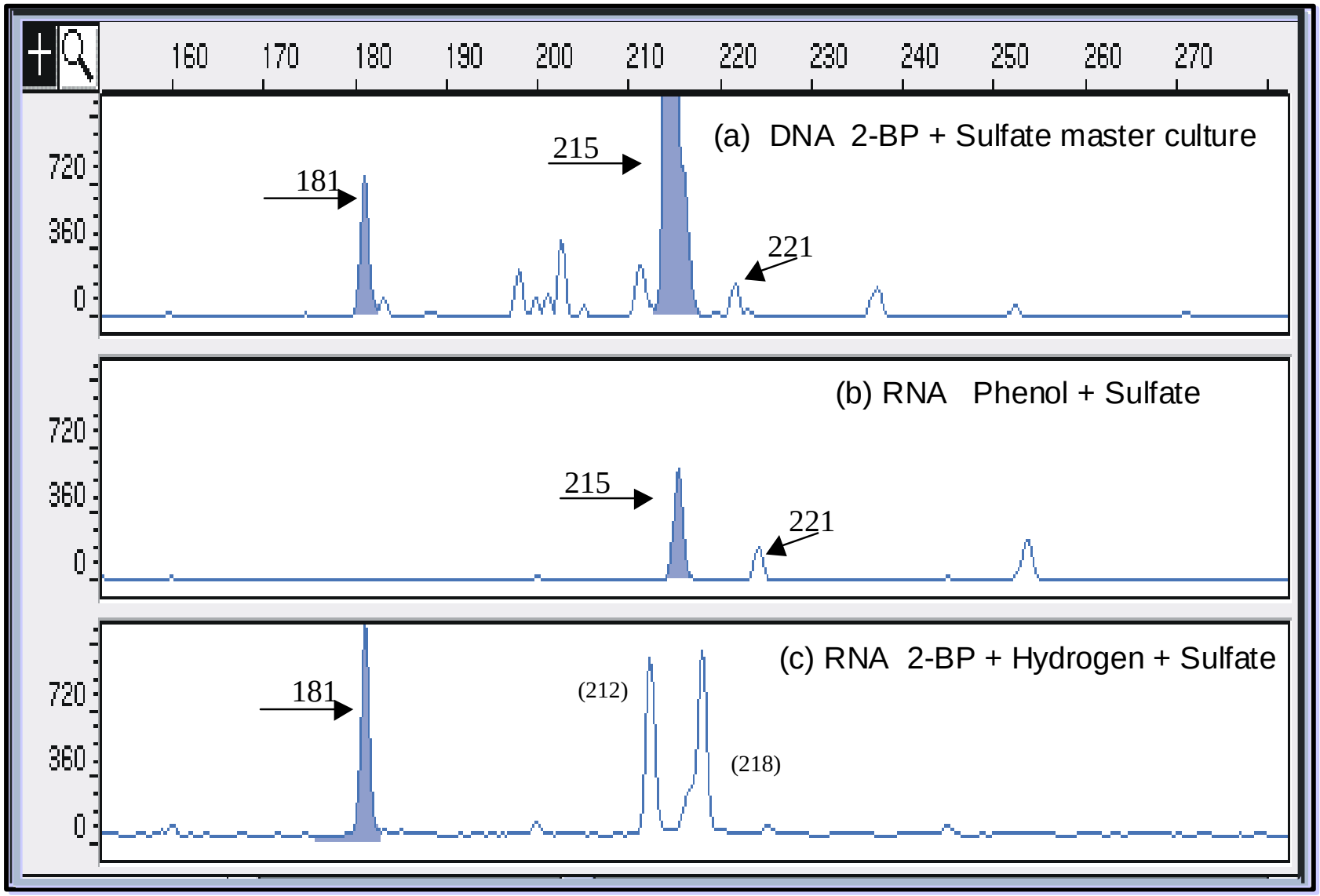


clone, sequence, and analyze phylogenetically

isolate, characterize physiologically, engineer into systems

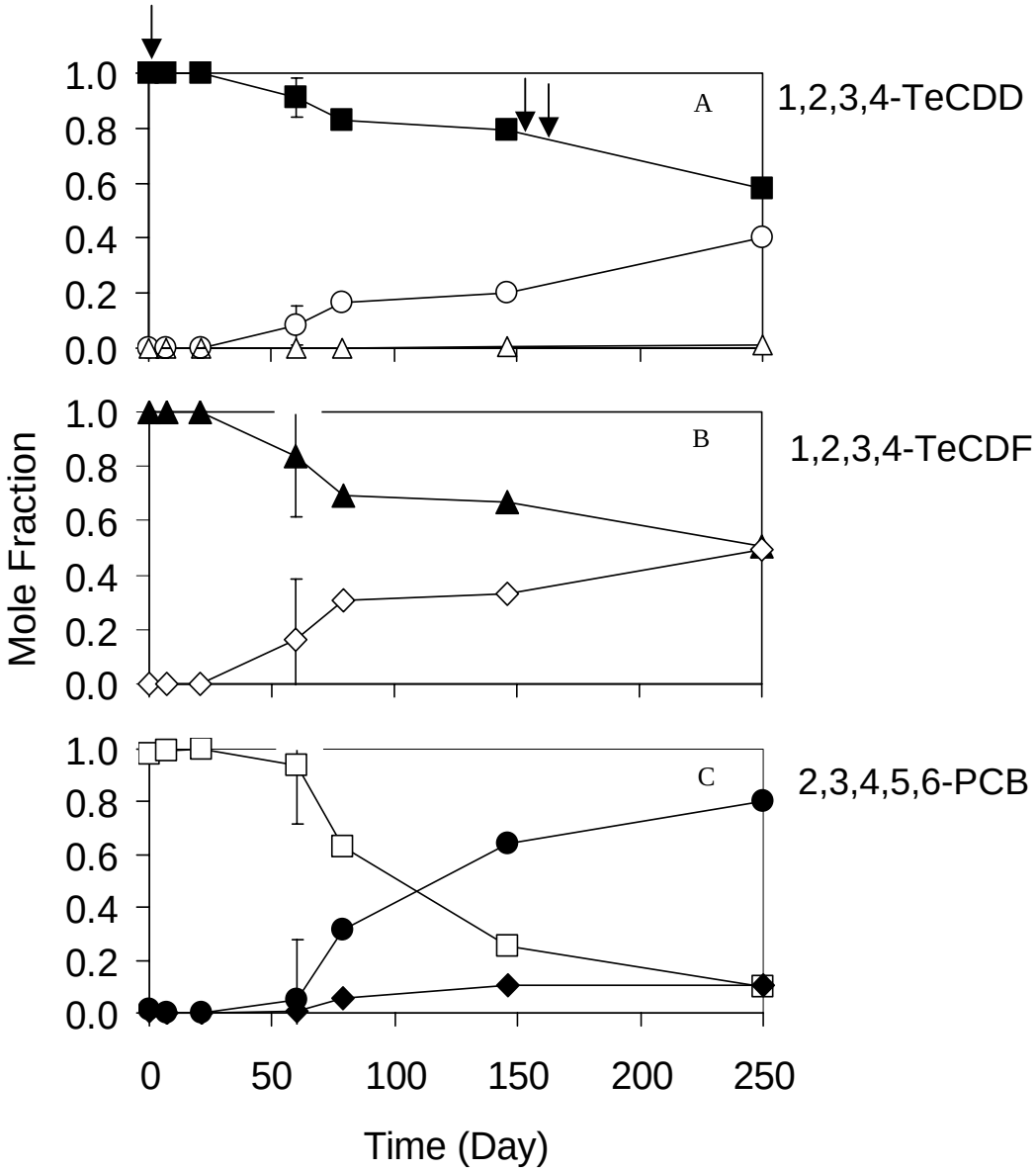


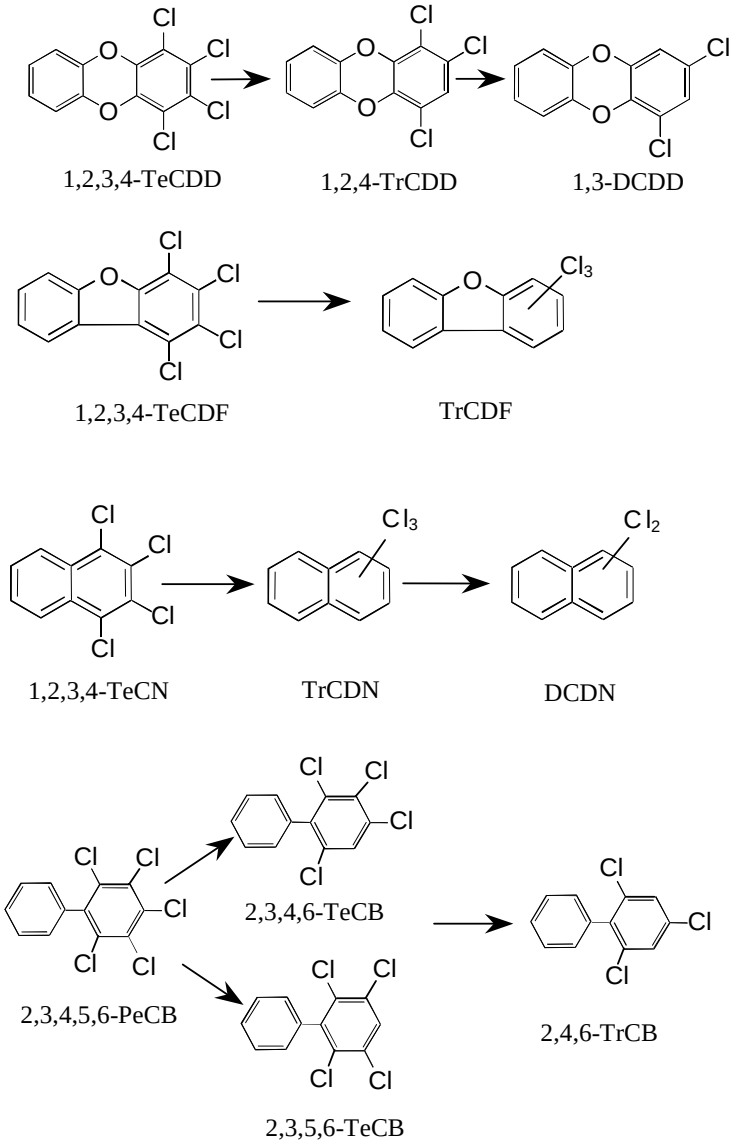
16S rRNA / DNA TRFLP Analysis of 2BP “Knock-out” Cultures



Fennell *et al.* 2004 *AEM*

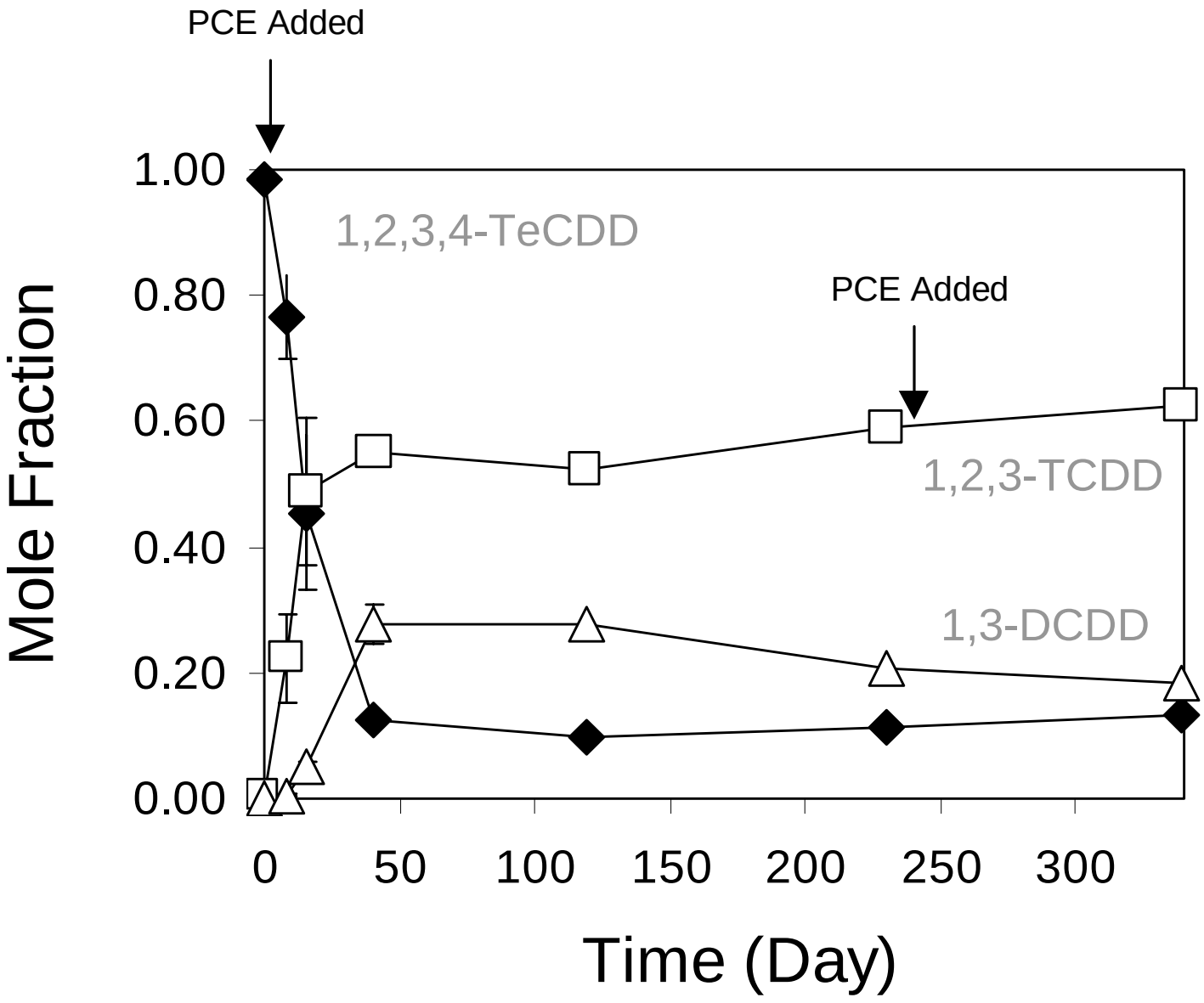
Dechlorination by *Dehalococcoides ethenogenes* 195





Fennell et al. 2004, *ES&T*

Dechlorination of 1,2,3,4-TeCDD by mixed culture containing *Dehalococcoides ethenogenes* strain 195



CONCLUSIONS

- The capacity for anaerobic PCDD/F dechlorination appears to be widely distributed in estuarine/marine environments
- The activities and interactions of diverse microbial populations will affect the fate of organohalides
- *Dehalococcoides ethenogenes* strain 195 is able to dechlorinate PCDD/Fs and other halogenated aromatic compounds
- Activity of dehalogenating bacteria can be stimulated with select “haloprimers” to enhance bioremediation of contaminated sediments

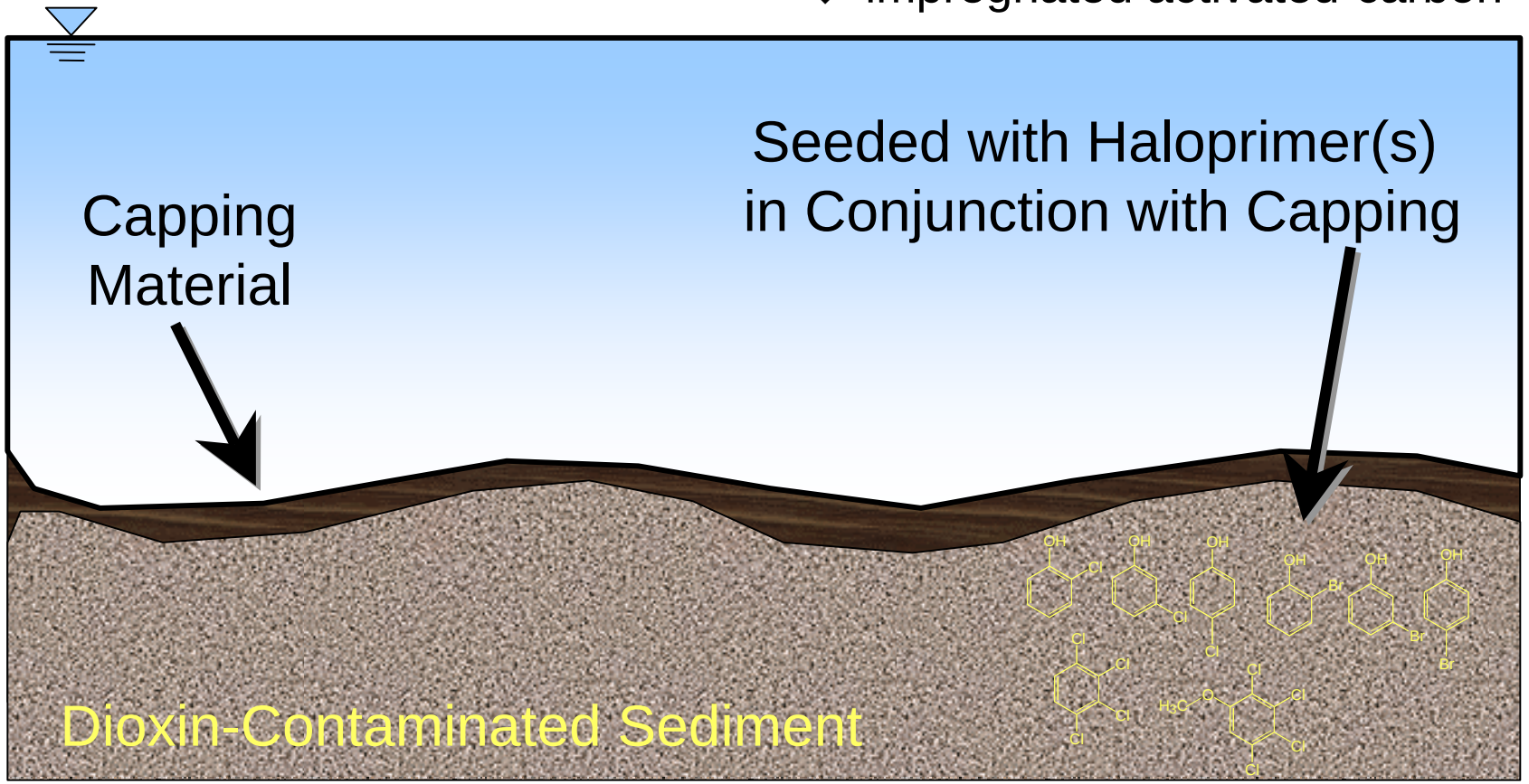
In Situ Haloprimer Application

In situ Amendment to Sediment

- with or without capping
- detoxification in place
- detoxification prior to dredging

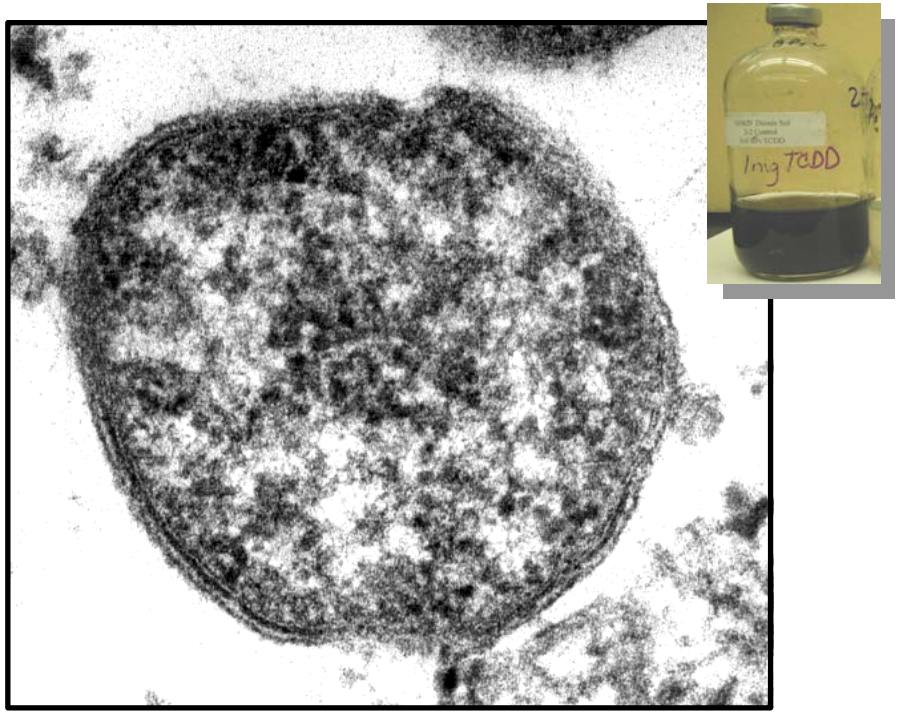
Haloprimer Addition Via

- gel encapsulation
- liquid or polymer injection
- impregnated activated-carbon



Bioaugmentation with *Dehalococcoides ethenogenes*?

Initial laboratory and pilot-scale experiments underway



The Consortium

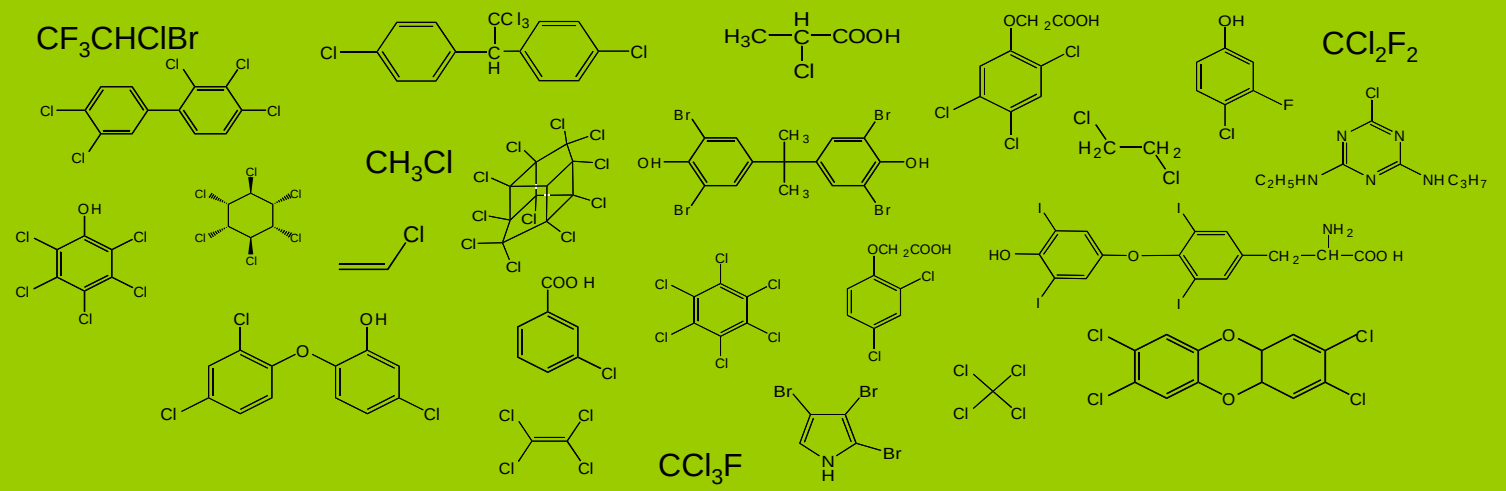
Young-Beom Ahn
James Voordeckers
Fang Liu
Sung-Keun Rhee
Victoria Knight

Donna Fennell, Lee Kerkhof

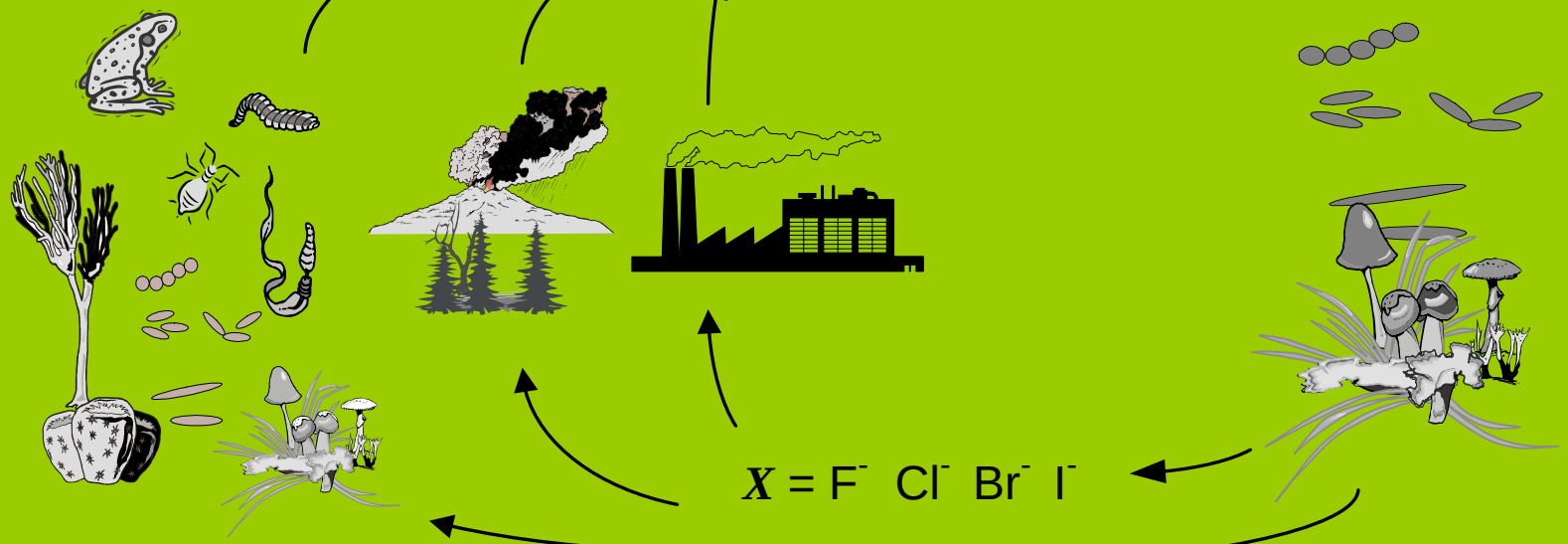
Support: DoD / SERDP
Office of Naval Research

DoD EPA DOE SERDP
Strategic Environmental Research and Development Program
Improving Mission Readiness Through Environmental Research

The Halogen Cycle



R-*X*



X = F^- Cl^- Br^- I^-

R