

Project Update (April, 2004)

Title: Phytoremediation in wetlands and confined disposal facilities (CDFs)

Investigator: Dr. John H. Pardue*, Dr. William Moe*

Institutions: HSRC-South & Southwest; *Louisiana State University

EPA Project Office: Mitch Lasat

Center Director: HSRC South & Southwest, Dr. Danny Reible

Goal: The primary goal of the study is the development of a scientific basis for a plant-based remedial approach for sediments contaminated with chlorinated organic compounds.

Rationale

Hydrophobic chlorinated organics are common sediment contaminants that pose a threat to sensitive receptors. These compounds are often recalcitrant in sediments and bioaccumulate through the food chain. By contrast, rapid contaminant attenuation for certain chlorinated organics is observed in *vegetated* sediments (i.e., wetlands). In these sediments, enhanced biological processes (aerobic and anaerobic biodegradation and plant uptake) have been observed in the root zone that drives rapid natural recovery. Previous research has indicated that herbaceous wetland vegetation stimulates degradation of chlorinated organics primarily via rhizospheric biodegradation processes principally reductive dechlorination. Previously, it was widely considered that the rhizosphere was primarily an aerobic environment due to the leakage of O₂ from the aerenchymal tissues of vegetation. However, recent studies have demonstrated that the root surface is an area of intense methanogenesis and O₂ leakage only occurs at well-defined locations along the root (i.e., the root tip). Therefore, the possibility exists that a specific microbial-plant interaction exists that can be exploited to better remediate sediments.

Based on this rationale, two hypotheses are considered:

- Reductive dechlorination is enhanced in vegetated sediments because the root surface serves as a location of enhanced activities of dehalorespiring and other degrading microbial populations
- By vegetating sediment contaminated with chlorinated organic compounds, belowground root matter will serve as source of H₂, overcoming redox potential limitations in sediments.

The objectives of the proposed study are to: define the biodegradation potential of chlorobenzenes and chlorinated solvents by quantifying biogeochemical conditions in the rhizosphere. Key conditions include the specific detrital decomposition products (organic acids and hydrogen) and microbial populations that develop on and adjacent to the plant root. A second objective of the study will define other potential fate mechanisms: plant uptake and volatilization by studying the dynamics of plant uptake of chlorobenzenes in wetland sediments.

Approach:

Methodology:

Vegetated sediment core and serum bottle microcosm studies have been utilized to investigate dechlorination of chlorinated benzenes and ethenes in sediments. Methodology includes the measurements of detrital decomposition products (organic acids and ambient H₂ concentrations), parent and daughter dechlorination products and microbial populations using molecular techniques including denaturing gradient gel electrophoresis, real-time and qualitative PCR techniques.

Outputs/Accomplishments

Year One:

- Laboratory microcosm studies conducted the first year of the study established the kinetics of tetrachlorobenzene dechlorination in sediments with a range of organic matter content. In addition, the studies identified the role of H₂ as an electron donor, the expected daughter products of dechlorination and the relative role of methanogens in dechlorination. Results indicated that the ability to dechlorinate tetrachlorobenzene is widespread in sediments. 1,2- and 1,3-dichlorobenzene readily forms and subsequently these are dechlorinated to chlorobenzene and benzene.
- Microcosm studies were conducted with root material from *Phragmites communis* (common reed) and *Typha latifolia* (cat tail). In sediments, amendments of fresh root material increased dechlorination rates in direct proportion to the amount of root matter added. Ambient H₂ and methane also increased. Root turnover was, therefore, identified as a potential driver for enhancing reductive dechlorination.
- A factorial core study experiment was also performed to examine dechlorination and microbial interactions in a more realistic setting. Sediment from the PPI site was spiked with 1,2,3,4-TeCB and *Typha latifolia* and *Phragmites communis* were grown in the cores. Results demonstrated that dechlorination of 1,2,3,4-TeCB was observed throughout the core but more complete dechlorination was observed near the root.

Year Two:

- Characterization of microbial communities dechlorinating tetrachlorobenzene revealed similarities and differences in microbial populations across a range of sediment types. Denaturing gel gradient electrophoresis (DGGE) revealed differences in structure of eubacterial and archae populations. Primer based detection of 16s rDNA genes demonstrated that dehalorespiring *Dehalococcoides* populations were present in every sediment type. Certain types of these organisms have been found to link dechlorination of chlorobenzenes with production of energy.

- Microcosm experiments revealed that measurement of H₂ concentrations coupled with methane concentrations could effectively identify dehalorespiring microbial activity against a background of other H₂-utilizing bacteria such as methanogens in sediments. The method effectively identified complete dechlorination of *cis*-1,2-dichloroethene in wetland sediments via dehalorespiration while complete dechlorination of 1,2-dichloroethane was identified as cometabolic, either by dehalorespirers or methanogens <see attached manuscript>.
- A real-time PCR method was developed for measurement of *Dehalococcoides* sp. and archae bacteria on the root surface and in bulk sediment. Methods were tested on vegetated cores that have been exposed to chloroethenes for over 2 years. Method development for several type II methanotrophs is progressing.

Summary of results from Year One and Year Two:

- Demonstrated the stimulatory effects of root matter on reductive dechlorination of chlorobenzenes
- Showed that hydrogen measurements coupled with methane measurements could identify dehalorespiration in a complex matrix of anaerobic microbial processes
- Demonstrated rapid and complete dechlorination of chlorobenzenes and chloroethenes in planted sediments

Year 3 update:

The initial year of the project was utilized to perform some basic experiments on the effect of vegetation on degradation of chlorobenzenes and chloroethenes. These experiments identified some interesting trends. Freshly-added root matter stimulated reductive dechlorination of chlorobenzenes, enhancing H₂ production and methanogenesis. In sediments spiked with chlorobenzenes that were then planted, more rapid and complete dechlorination of chlorobenzenes was observed in sediments near the root versus the bulk soil. In upflow core experiments and microcosm studies, rapid dechlorination of chloroethenes and ethanes were observed in the rhizosphere and appeared to be linked with large populations of dehalorespiring bacteria. While these studies suggested a role for vegetation, our existing techniques lacked the resolution to determine the nature of the interaction. It was unclear whether vegetation simply provided a source of H₂ for dehalorespiring organisms in the soil via root exudates or root turnover or whether the roots provided important intensely reducing surfaces for dehalorespiring bacteria to grow.

Based on suggestions from the scientific advisory committee, method development of higher resolution, quantitative techniques were performed including real-time PCR techniques that could more directly quantify population size of dehalorespiring and

methanogenic populations. This has allowed us to better separate indirect vegetation effects from direct changes in microbial populations in the rhizosphere and on the root surface, itself. Other indirect methods were also developed to identify activities of dehalorespirers based on known H₂ thresholds for different anaerobic, H₂-utilizing processes.

Three sets of studies were performed during Year Three.

- A factorial core study was performed with several treatments: sediments vegetated with *Typha*, sediments vegetated with *Phragmites* and unvegetated sediments. The sediment was mineral-dominated and prepared with known quantities of labile and desorption-resistant hexachlorobenzene. Cores have been sacrificed and roots and bulk soil were separated by careful sectioning and sieving. Bulk soil and root tissue were analyzed for parent hexachlorobenzene and daughter dechlorination products. DNA extracts of bulk soil and root matter were performed followed by application of several molecular techniques including real-time PCR of *Dehalococcoides* and methanogens.
- A supporting set of microcosm studies was also performed. The same sediment used in the core study was used to prepare microcosms spiked with 1,2,3,4-tetrachlorobenzene. Sediments were subjected to an artificial “aging” procedure following spiking, to remove labile, sorbed 1,2,3,4-tetrachlorobenzene. Subsequently, root matter from *Typha* and *Phragmites* were added to replicate microcosms and daughter products, H₂ and methane monitored over time. This experiment was conducted to test the hypothesis that “aged”, desorption-resistant chlorobenzenes would also be bioavailable to dechlorinating organisms and subject to stimulation with root matter.
- A second microcosm study has examined the quality of the root matter and its effect on dechlorination. In this study chloroethene-degrading populations were tested for stimulation by 10 different wetland plants, including reeds, sedges and grasses. TCE dechlorination was followed by measuring the daughter products or dechlorination and gas production (H₂ and CH₄). This experiment was performed to test the hypothesis that the quality of root matter, by species of plant, alters the stimulatory effect on the dehalorespirers.

Summary of results from these studies (some are still ongoing)

- Results from the factorial study have indicated that *Dehalococcoides* are present at higher densities on the root than in the bulk sediment (an example result is presented in Table 1). An enhancement of 2-orders of magnitude was observed for both *Typha* and *Phragmites*. Although enhancement of Eubacteria and Archae were observed as well, ratios of *Dehalococcoides* to these bacterial groups

indicate that enhancement was selective for these dehalorespiring organisms on the root surface.

Table 1. 16S rDNA gene copies per gram of sediment (or root) in *Typha* and *Phragmites*-planted sediment columns spiked with HCB

Bacterial groups	<i>Typha</i>		<i>Phragmites</i>	
	Roots	Sediment	Roots	Sediment
Eubacteria	3.25×10^7	7.74×10^7	1.17×10^5	1.37×10^5
<i>Dehalococcoides</i>	4.24×10^5	1.53×10^5	4.90×10^3	3.54×10^3
Methanogens	1.15×10^7	1.57×10^7	4.71×10^5	3.92×10^5
Ratio: Dhc/Eubacteria x 100%	1.30	0.20	4.2	0.26
Ratio: Dhc/Archae x 100%	3.70	0.97	1.0	0.9

- Results from the first microcosm study have demonstrated that “aged” 1,2,3,4-tetrachlorobenzene can dechlorinate even when the more labile form is extracted. In sediments where roots were present, dechlorination of 1,2,3,4-TeCB was faster ($k=0.11/d$ for *Typha*, $k=0.34/d$ for *Phragmites* and $k=0.006/d$ for the control with no root). H_2 and CH_4 production was higher in systems with roots than in those with no roots present. Dechlorination of 1,2,3,4-TeCB to benzene as a final dechlorination product occurred.
- Results from the second microcosm study demonstrated again the enhancement of root matter on the dechlorination process for TCE. Rate constants of TCE dechlorination were statistically more rapid for some plant species, although the nature of the stimulation (increased H_2 production, effect on the dehalorespiring population) will require additional investigation to elucidate. Complete dechlorination to ethene was observed consistent with the previous results with this microbial population.

Proposed Work for Coming Year:

The results of the studies performed in Years 1-3 have led to a better understanding of the scientific basis behind the use of vegetation to remediate sediments contaminated with chlorinated benzenes, PCBs and dioxins. If the live root surface serves as a locus for

enhanced reductive dechlorination, vegetating sediments in CDFs with species with dense root mats may serve as a passive but effective approach to remediating portions of the bed and minimizing flux. There are still some uncertainties with regard to the application of the technology to real sediments. Questions include: what are the characteristics of the starting microbial population that will lead to a successful level of treatment? What characterization steps need to be performed to understand whether the approach has a chance for success? What needs to be monitored to determine whether the approach is working? Therefore, work during the (likely) final year of funding will be to answer the questions for a historically contaminated sediment in a greenhouse study to bridge the gap between these smaller scale studies and a field pilot.

Sediment will be obtained from a site historically contaminated with chlorobenzenes. We have access to sediments from the Petro Processors Superfund site near Baton Rouge, but other sites will be considered, if materials are available. Sediments will be removed and transported to the greenhouse and placed in replicated large aquaria (50 gallons), prior to planting. Then, characterization, treatment and monitoring steps will be performed following a protocol for applying the plant-assisted bioremediation technology developed based on the results of these lab studies.

- Sediment will be characterized with a range of microbial (i.e., real-time PCR for *Dehalococcoides*, Archae and Eubacteria, DGGE) and chemical (i.e., size and status of redox-sensitive electron acceptor pools such as microbially reducible iron, soot concentrations, nutrient concentrations, contaminant distribution and type, organic matter concentrations) techniques. The objective of this extensive characterization will be to assess after the greenhouse “pilot” which data were useful in predicting success and the rate of success.
- Sediment will be planted with a wetland species selected on the basis of Year Two and Three studies. Sediment will be, depending on the results of the characterization work, fertilized and inoculated with a chlorobenzene dehalorespiring culture, if needed and maintained in the greenhouse.
- The system will be monitored over the course of the project period and sampled by coring and high-resolution section to assess the progress to clean-up. As before, microbial populations on the plant root matter and bulk sediment will be assessed and related to the chlorobenzene degradation products observed near the roots, themselves.

Supplemental Keywords: marshes, natural attenuation, wetlands

Students supported:

Eun-Ju Lee, Ph.D.

Elaiza Alvarez, M.S.