Title: Phytoremediation in wetlands and confined disposal facilities (CDFs)
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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 O_2 from the aerenchymal tissues of vegetation. However, recent studies have demonstrated that the root surface is an area of intense methanogenesis and O_2 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 H_2, 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 H_2 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 H_2 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 H_2 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 H_2 concentrations coupled with methane concentrations could effectively identify dehalorespiring microbial activity against a background of other H_2-utlizing 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.

- 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 to date:

- 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

Proposed Efforts over the next year:

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 H_2 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 H_2 for dehalorespiring organisms in the soil via root exudates or root turnover or whether the roots provided important intensely 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 will allow 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 H_2 thresholds for different anaerobic, H_2-utilizing processes. The proposed effort in the next year would combine techniques developed in Year Two with the basic experimental set-ups in Year One. Two sets of studies are proposed.

- A factorial core study will be performed with several treatments: sediments vegetated with *Typha*, sediments vegetated with *Phragmites* and unvegetated sediments. The sediment is mineral-dominated prepared with known quantities of labile and desorption-resistant tetrachlorobenzene. Cores will be sacrificed and roots and bulk soil will be separated by careful sectioning and sieving. Bulk soil and root tissue will be analyzed for parent tetrachlorobenzene and daughter dechlorination products. DNA extracts of bulk soil and root matter will be performed followed by application of several molecular techniques including DGGE and real-time PCR of *Dehalococcoides* and methanogens. Extent of dechlorination will be compared with the relative size of labile and desorption-resistant pools.
- A supporting set of microcosm studies will also be performed. Replicate cores will be used to prepare microcosms, prepared separately with bulk soil and root matter. Repeated additions of chlorobenzenes will be made and daughter products, H_2 and methane monitored over time. Relative magnitude of chlorobenzene dechlorination will be compared between the bulk soil and root material and various measures of the population size and composition (DGGE and real-time PCR).

The expected results of the studies proposed above would be 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 higher reductive dechlorination, vegetating sediments in CDFs with species with dense root mats may serve as a passive but effective approach to remediating portion of the bed and minimizing flux.

Supplemental Keywords: marshes, natural attenuation, wetlands

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