

## Stimulation of Trichloroethene Degradation with Natural Organochloride Amendment

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**Background/Objectives.** Several strategies for enhancing the bioremediation of chlorinated compounds in groundwater and sediments have been developed and implemented, particularly biostimulation with organic carbon and bioaugmentation. The electron-accepting stimulation of dechlorinating bacterial communities, however may provide a more strategic and targeted mechanism of biostimulation for enhanced degradation of chlorinated contaminants. In this research, a synthesized mixture of "natural organochlorides" were produced from enzymes and tested for their ability to stimulate the degradation of TCE under anaerobic conditions in microcosm studies.

**Approach/Activities.** A synthesized organochloride mixture was produced using a chloroperoxidase enzyme reacting on a soil organic matter extract. Synthesized organochlorides were then amended to triplicate slurry microcosms (100 mL total volume) with an anaerobic headspace. A non-chloroperoxidase reacted organic matter extract was used in control microcosms, and these amendments were compared to controls not amended with any exogenous soil organic matter. Inoculum was an uncontaminated forest soil. Vitamins, 100  $\mu$ L of neat methanol, and 0.1 mM of trichloroethene were amended to all microcosms. TCE and degradation products were analyzed using gas chromatography with a micro-electron capture detector.

**Results/Lessons Learned.** After a lag time of 1.5 months, the entirety of the trichloroethene degraded within 7 days in both the sets of microcosms amended with synthesized organochlorides and with the organic matter extract. TCE was then re-amended in these microcosms, and after another lag time of 1.5 months, it was again degraded within 7 days. No accumulation of dechlorination products were measured above detection limits. After four months, the TCE in the non-amended microcosms failed to degrade, indicating that organic matter extract amendment was stimulative of TCE degradation. The reaction of organic matter with chloroperoxidase enzyme made no significant difference in TCE degradation rates or lag times, however, indicating that the enrichment of organic matter with synthesized organochlorides did not improve the stimulation of TCE degradation. This may at least partly be a result from the processing of organic matter extracts and synthesized organochlorides, which in many steps were biased towards the more hydrophobic fractions. Thus, the chloroperoxidase reaction may not have significantly increase the *bioavailable* pool of organochlorides for stimulation. Follow up experiments underway will elucidate if a water-soluble fraction of chloroperoxidase produced organochloride-enriched organic matter stimulates over non chloroperoxidase-treated controls. The mechanism of stimulation from the extracted organic matter is not fully understood. Amendments of readily bio-available carbon sources used by dechlorinating bacteria, such as methanol or acetate, has failed to stimulate TCE degradation with this soil amendment; thus, the stimulation from natural organic matter extract is not purely from the availability of electron donors or carbon sources. It is possible that specific congeners of organochlorides that were present in the organic extract but not produced/enriched from chloroperoxidases provided biostimulation. Further experiments investigating organochlorides produced from other chlorination enzymes may elucidate the possibility that only a narrow and specific set of organochlorides provide stimulatory activity. Other stimulatory mechanisms of the organic matter extract will be elucidated from meta-transcriptomic analysis of the microcosms.