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Degradation of halogenated aliphatic compounds utilizing sequential anaerobic/aerobic treatments

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Abstract The objective of this research was to determine if either methanogenic or sulfidogenic reductive dechlorination could survive an alternating anaerobic/aerobic sequence to biologically transform halogenated aliphatic hydrocarbons (HACs), specifically tetrachloroethylene (PCE), trichloroethylene (TCE), cis-1,2 dichloroethylene (cDCE), trans-1,2 dichloroethylene (tDCE), 1,1 dichloroethylene (1,1DCE) and vinyl chloride (VC). This ability was considered to be a necessary prerequisite for complete anaerobic/aerobic mineralization of halogenated aliphatic hydrocarbons by a single microbial consortia. Chlorinated solvents, which are among the most common groundwater contaminants, have been partially dechlorinated using single-stage anaerobic environmental treatment strategies. Various types of bacteria typically reductively dechlorinate PCE and TCE to cDCE and VC in an anaerobic environment, including methanogens, sulfidogens, and homoacetogens. The problem lies in the fact that reductive dechlorination typically leads to an accumulation of daughter compounds (cDCE, VC) which are more toxic than their parent compounds (PCE, TCE). Furthermore, PCE and (to a lesser extent) TCE, are resistant to dechlorination in aerobic environments. In contrast, VC and cDCE are readily oxidized co-metabolically in an aerobic environment by methanotrophic bacteria, and others using oxygenases (e.g. toluene oxidizers). Results from this research showed that both methanogenic and sulfidogenic reductive dechlorination could resume after transient exposures to both oxygen and hydrogen peroxide (H_2O_2). In fact, for cycles as frequent as 10 days between aerobic treatment cycles, reductive dechlorination was observed to resume at rates at least as rapid as microcosms not exposed to aerobic treatments.

Keywords Methanogenic; reductive dechlorination; sulfidogenic

Introduction

Halogenated aliphatic compounds (HACs) are among the most frequently encountered groundwater contaminants (Westrick *et al*., 1984). Tetrachloroethylene (PCE) and trichloroethylene (TCE) are HACs that are commonly used as industrial solvents, as degreasers, and in the dry cleaning industry (Fetter, 1993). The widespead occurrence of PCE and TCE in groundwater is of concern because these compounds, and their lesser chlorinated degradation products cis-1,2 dichloroethylene (cDCE), trans-1,2 dichloroethylene (tDCE), 1,1 dichloroethylene (1,1DCE), and vinyl chloride (VC), can be both toxic and carcinogenic to humans (Ensley, 1991).

Anaerobic aquifers initially contaminated with PCE, TCE, and other chlorinated solvents were found to have accumulated dechlorination products over time (Beeman *et al*., 1994). Biotransformation of PCE and TCE to less halogenated daughter products under anaerobic conditions has subsequently been observed in field studies, fixed-film reactors, microcosm studies, and pure cultures. Methanogenic bacteria are the most documented bacteria capable of reductive dechlorination of PCE, but sulfidogenic (Bagley and Gossett, 1990), and homoacetogenic (DiStefano *et al*., 1991) bacteria have also been shown capable of reductive dechlorination. These studies have shown that the predominant end product of PCE dechlorination in an anaerobic environment is cDCE and VC, even though some methanogenic bacteria are capable of complete reductive dechlorination of PCE to

ethylene (Freedman and Gossett, 1989). In general, the speed of transformation of PCE to less halogenated compounds decreases as the number of chlorines decrease. Unfortunately, the degradation products of PCE, in particular VC, are more hazardous to human health than PCE (Infante and Tsongas, 1982).

Various researchers have attempted to combine the benefits of anaerobic reductive dechlorination for highly chlorinated compounds such as PCE with aerobic co-metabolic oxidation of less chlorinated species (McCarty, 1991; Kastner, 1991; Zitomer and Speece, 1993). The problem with such a strategy lies in the fact that anaerobic bacteria, particularly methanogens, are well known for their intolerance of oxygen (Madigan *et al*., 1997). Thus most researchers propose ex-situ reactors in series or separate anaerobic and aerobic zones in-situ. Other species of anaerobic bacteria, however, are more aero-tolerant than methanogens (e.g. sulfate-reducing bacteria; Madigan *et al*., 1997). This suggests the possibility of using a single treatment zone or reactor with alternating anaerobic/aerobic conditions. Furthermore, diffusion limitations might even allow methanogens to survive aerobic phases of treatment in microanaerobic zones.

The objective of this laboratory research was to determine if either methanogenic or sulfidogenic reductive dechlorination could survive an alternating anaerobic/aerobic sequence to biologically transform halogenated aliphatic hydrocarbons (HACs), specifically tetrachloroethylene (PCE), trichloroethylene (TCE), cis-1,2 dichloroethylene (cDCE), trans-1,2 dichloroethylene (tDCE), 1,1 dichloroethylene (1,1DCE) and vinyl chloride (VC). This ability was considered to be a necessary prerequisite for complete mineralization of halogenated aliphatic hydrocarbons by a single microbial consortia using sequential anaerobic/aerobic conditions.

Methods and materials

The sequential anaerobic/aerobic environment methanogenic microcosm experiments consisted of 46 biotic microcosms, four abiotic autoclaved and $HgCl_2$-dosed control microcosms, and six biotic control microcosms dosed with 50 mM of BES to suppress methanogenic activity (Pavlostathis and Zhuang, 1991). The 46 biotic microcosms consisted of 16 microcosms to be used as anaerobic environment controls and 30 microcosms to be used with a sequenced anaerobic environment and aerobic environment. The 30 microcosms consisted of three sets of ten microcosms using different aerobic environment strategies. Two of the aerobic environment strategies utilized hydrogen peroxide addition into the microcosm liquid phase at low (15 ppm) and high (75 ppm) doses in order to initiate the aerobic environment. The third aerobic environment strategy utilized the injection of atmospheric air into the microcosm headspace in order to initiate the aerobic environment.

The sequential anaerobic/aerobic environment sulfidogenic (SI) microcosm experiments consisted of a total of 50 biotic microcosms and 10 autoclaved and $HgCl_2$ dosed control microcosms. The experimental plan for the SI microcosm series called for an initial anaerobic phase followed by multiple periodic aerobic treatments of varying oxygen concentrations. The length of the initial anaerobic phase was determined experimentally through the monitoring of both biotic and abiotic microcosms for chlorinated solvent concentrations, volatile fatty acid (VFA) concentrations (both acetic and propionic acids were utilized as substrates), and biogenic gas production. The goal of the SI microcosm series was to initiate aerobic treatments once partial reductive dechlorination was shown to have occurred within biotic microcosms. Out of the 50 total biotic microcosms, 10 were sampled in order to establish partial dechlorination during the initial anaerobic phase. An additional ten microcosms were maintained anaerobically over the entire length of the study in order to serve as a basis of comparison with those biotic microcosms undergoing aerobic treatments. The remaining 30 microcosms were split into two groups of 15 and

exposed to varying types of aerobic treatments. Each of these groups was further subdivided into three groups of five (in order for aerobic treatment strategy to have five microcosms run in parallel) and exposed to three different aerobic treatment strategies. The types of aerobic treatments included a low H_2O_2 dose (10 ppm), a high H_2O_2 dose (100 ppm) and the replacement of the microcosm headspace with air. One of the groups of 15 microcosms received aerobic treatments every ten days following the establishment of partial reductive dechlorination in the initial anaerobic phase, and the other group of 15 microcosms received aerobic treatments every seven days. Upon sampling, microcosms were either discarded, or resealed with a silicone sealant and stored inverted.

Microcosms were constructed in 120 mL amber glass bottles crimp sealed with PFTE butyl-lined septum tops. Initial liquid volume for the microcosms was 100 mL with 20 mL headspace. The biomass inocula for the microcosms came from a methanogenic environment reactor that was originally seeded with biomass from a wastewater treatment plant anaerobic digester (South Regional Wastewater Treatment Plant, Orlando, FL). Initial substrate concentrations for all of the sequential environment microcosms and controls were 500 mg/L acetic acid and 400 mg/L propionic acid. Each microcosm included 1.0 mL of Wolfe's vitamin solution and 1.0 mL of Wolfe's mineral solution (Atlas, 1993). 50 mg/L of $Na_2S{\cdot}9H_2O$ (reducing agent) was also added. In addition to the components listed above, the sulfidogenic microcosms contained 300 mg/L as SO_4 and 50 μM of BES, a methanogen suppressor. Initial pH and ORP values measured after the addition of the reducing agent were 7.4 and –6 mV, respectively. All microcosms were maintained at 30°C for the duration of the experiment. Abiotic control microcosms were dosed with 20 mg/L $HgCl_2$ and autoclaved.

Liquid phase chlorinated solvents were measured using a Tekmar Co. purge-and-trap fitted to a Hewlett Packard gas chromatograph (GC) Model 5890 equipped with a flame ionization detector (FID) and a Vocol capillary column. Chlorinated solvents in the gas phase were calculated using Henry's Law. Volatile fatty acid (VFA) concentrations were measured using a Shimadzu 14-A GC fitted with a FID and a 3 mm internal diameter glass column with 60/80 Carbopack C/0.3% Carbowax 20M/0.1%H_3PO_4 packing (Supelco Inc., Bellefonte, PA). Biogenic gases were measured using a Shimadzu 14-A GC fitted with a thermal conductivity detector and a 15 ft × 1/8″ internal diameter stainless steel support packed with 60/80 Carboxen 1000 (Supelco Inc., Bellefonte, PA).

Results and discussion

The laboratory research investigated the possibility of sustaining reductive dechlorination of HACs despite intermittent exposure to aerobic/oxic environmental conditions. This is a necessary prerequisite for establishing a single microbial consortia capable of biologically transforming HACs in both anaerobic and aerobic conditions. Both methanogenic and sulfidogenic experiments were conducted during this research. Initial anaerobic PCE to cDCE transformation rates averaged 9.3×10^{-2} μM/day for methanogenic microcosms and 3.75×10^{-1} μM/day for sulfidogenic microcosms relative to abiotic controls. After the establishment of anaerobic reductive dechlorination of PCE, microcosms were exposed to various aerobic treatments, including injection of air into the microcosm headspace and injection of hydrogen peroxide (H_2O_2) in varying concentrations. Results indicated that in methanogenic experiments reductive dechlorination of PCE and TCE resumed or continued after the initiation of aerobic treatments. Figure 1 shows an example of a methanogenic anaerobic/aerobic treatment experiment. After the injection of 15 ppm H_2O_2 on day 35, reductive dechlorination successfully resumed, particularly indicated by the decrease in PCE and the increase in TCE between day 118 and 130 in Bottle #2. Note that Figure 1 indicates that additional PCE was injected into Bottle #2 and Bottle #3 and was rapidly dechlorinated.

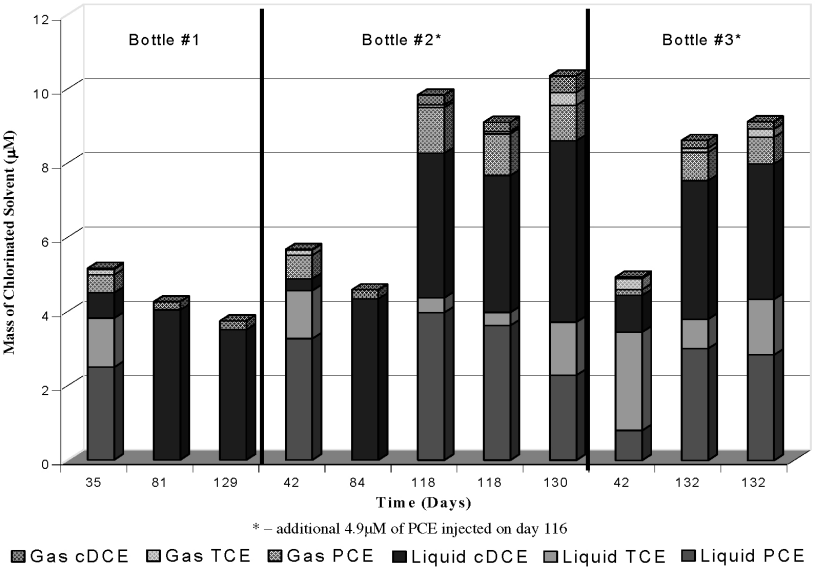

Figure 1 Hydrogen peroxide experiment (15 ppm), selected methanogenic microcosms

An example of a sulfidogenic microcosm series undergoing a cycle of aerobic treatments is shown on the next page (Figure 2). Specifically, the effect of adding ambient air in ten-day or longer intervals within individual anaerobic microcosms (labeled SI-21 to SI-25) capable of dechlorinating PCE in a sulfate-reducing environment is shown. Figure 2 shows that daughter products of PCE were detected within the sulfidogenic microcosms SI-21 to SI-25 after the initiation of aerobic treatment. Trichloroethylene and cDCE, not PCE, were the dominant halogenated species present after day 83. Additionally, cDCE appears in the liquid phase of SI-21 to SI-25 much earlier than cDCE does for the biotic anaerobic controls (not shown). Not until day 123 is cDCE detected within the biotic

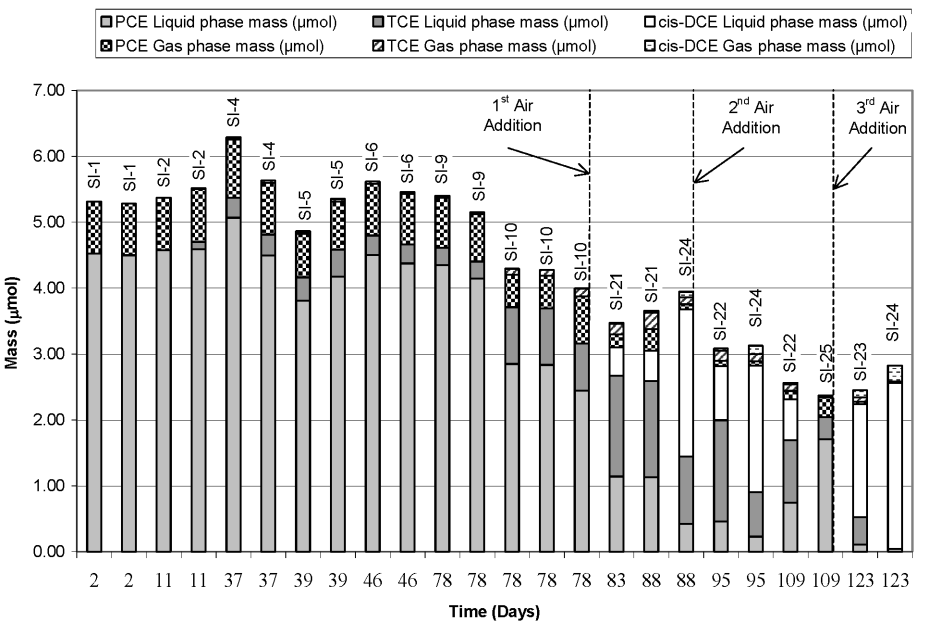

Figure 2 Chlorinated solvent partitioning in sulfidogenic microcosms after initiation of aerobic treatments with air

anaerobic controls (not shown). These results would seem to indicate that the addition of ambient air to an anaerobic culture capable of reductive dechlorination in a sulfate-reducing environment did not preclude further dechlorination. In fact, the addition of ambient air seemed to enhance the dechlorination process, as evidenced by the earlier appearance of cDCE in SI-21 to SI-25 than in the SI biotic anaerobic controls. Kastner (1991) also observed an enhancement of reductive dechlorination rates following an aerobic treatment cycle during a similar microcosm study. The presence of PCE daughter products in levels in excess of those found in the biotic anaerobic controls strongly implies, but does not prove conclusively, that reductive dechlorination was able to continue after the initiation of aerobic treatments in SI-21 through SI-25. Therefore, it is probable that anaerobic bacteria were able to resume reductive dechlorination following periodic aerobic treatments in SI-21 to SI-25.

Conclusion

- Reductive dechlorination of PCE was observed under both methanogenic and sulfidogenic conditions in all unsuppressed anaerobic environment microcosms. PCE was observed to be sequentially reduced to TCE and to cDCE.
- Results indicated that reductive dechlorination of PCE and TCE resumed and/or continued after injection at the low H_2O_2 concentrations (15 ppm) in methanogenic microcosms.
- Less conclusive results indicated reductive dechlorination resumed after a long reacclimation period for the injection of the higher H_2O_2 concentration (75 ppm) in methanogenic microcosms.
- Limited reductive dechlorination was observed in methanogenic microcosms following exposure to air.
- Under sulfidogenic conditions, reductive dechlorination was resumed after both dosing with 100 ppm of H_2O_2 and with air. In fact, with 10 days elapsing between aerobic treatment cycles, reductive dechlorination was observed to resume at rates more rapid than sulfidogenic microcosms not exposed to aerobic treatments.
- No conclusive evidence was observed to indicate that aerobic degradation of cDCE took place during any of the aerobic phase techniques. This was probably due to the microcosm inocula not containing methanotrophs, or other aerobes involved in co-metabolism of chlorinated ethanes.

Acknowledgements

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