In Situ Bioremediation of Chlorinated Solvents

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Chlorinated solvents and their natural transformation products are the most frequently observed groundwater contaminants in the United States. *In situ* bioremediation using anaerobic or aerobic co-metabolic processes is a promising means of cleaning up contaminated aquifers. Studies show that under natural conditions trichloroethylene can be anaerobically degraded to dichloroethylene, vinyl chloride, and ethylene. Pilot scale field studies of *in situ* aerobic co-metabolic transformations have shown that indigenous microbes grown on phenol are more effective at degrading trichloroethylene and *cis*-1,2-dichloroethylene than microbes grown on methane. Modeling studies support field observations and indicate that the removal of trichloroethylene and *cis*-dichloroethylene results from the biostimulation of an indigenous microbial population. Field tests and modeling studies indicate that, at high TCE concentration, degradation becomes stoichiometrically limited. — Environ Health Perspect 103(Suppl 5):101–105 (1995)

Key words: trichloroethylene, dichloroethylene, co-metabolism, transformation, anaerobic, aerobic, modeling, field studies, phenol, methane

Introduction

Chlorinated solvents and their natural transformation products are the most frequently observed groundwater contaminants in the United States. These solvents, consisting primarily of chlorinated aliphatic hydrocarbons (CAHs), have been widely used as degreasing agents for industrial purposes and were improperly disposed of at waste and refuse sites or have leaked from underground storage tanks. They are relatively recalcitrant to degradation, fairly mobile, and denser than water when present as a separate phase. This has resulted in the generation of contaminant plumes that are often large in aerial extent. Methods commonly used for remediation of groundwater, such as pump-and-treat, have been somewhat effective, but these methods are costly to operate, they have resulted in the transfer of contaminants to other environmental compartments, or they require surface treatment. They often do not achieve final treatment levels. Thus, innovative treatment methods are being explored that may prove to be more effective and less costly. This paper will discuss the potential for *in situ* bioremediation of CAHs via the process of co-metabolism. Presented will be the results from field studies in which both natural (intrinsic) and enhanced transformation have been observed.

The major solvents that are frequently observed as groundwater contaminants include carbon tetrachloride (CT), tetrachloroethylene (PCE), and trichloroethylene (TCE). They can be transformed by chemical (abiotic) and biological (biotic) processes in the subsurface to a range of products, including chloroform (CF), methylene chloride (MC), *cis*-1,2-dichloroethylene (*c*-DCE), *trans*-1,2-dichloroethylene (*t*-DCE), 1,1-dichloroethylene (1,1-DCE), vinyl chloride (VC), 1,1-dichloroethane, and chloroethane. Many of the chemicals can be degraded biologically; however, microorganisms generally cannot obtain energy for growth from the transformation (*1*). For both aerobic and anaerobic transformations, the presence of a cosubstrate as a carbon and energy source is needed. Thus, transformations can be brought about by co-metabolism or through interactions of the CAHs with enzymes and cofactors produced by microorganisms for other purposes. Much of the effort of *in situ* bioremediation of CAHs is centered on promoting co-metabolism. Promoting CAH co-metabolism in the subsurface may entail adding the appropriate growth substrate and electron donor, such as oxygen, to simulate the microbial population, while effectively contacting target contaminants with the stimulated population. To date, there has not been a documented full-scale application of an *in situ* co-metabolic process to guide the design and application of this technology.

The potential for CAH biotransformation through primary substrate utilization or through co-metabolism is presented in Table 1. Most of the CAHs are degraded via co-metabolism. The potential for aerobic co-metabolism of completely substituted CAHs, such as CT and PCE, is essentially zero, while it is very high for less saturated compounds, such as MC and VC. The general trend for aerobic co-metabolism indicates a better potential the lower the degree of chloride substitution. The rates of aerobic co-metabolism are very compound specific. For example, rates for the dichloroethylene (DCE) isomers vary greatly. Aerobic transformations can result in complete degradation of CAHs to carbon dioxide, water, and chloride.

Anaerobic conditions in general show an opposite trend, with a greater transformation potential the greater the degree of substitution. A potential problem with the anaerobic processes is that CAHs are reduced to less substituted intermediates that are often transformed at slower rates and thus may accumulate. TCE can be reduced anaerobically to DCE, which can be transformed into VC (*3*). Recently, however, it has been demonstrated that PCE and TCE can be anaerobically degraded to ethylene (*4–6*), which is a nontoxic end product. This finding has sparked new interest in *in situ* CAH bioremediation via anaerobic processes.

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Table 1. Potential for chlorinated aliphatic hydrocarbon biotransformation as a primary substrate or through co-metabolism.[a]

Compound	Primary substrate: Aerobic potential	Primary substrate: Anaerobic potential	Primary substrate: Aerobic potential[b]	Co-metabolism: Anaerobic: Potential[b]	Co-metabolism: Anaerobic: CAH product
CCl_4			0	XXXX	$CHCl_3$
$CHCl_3$			X	XX	CH_2Cl_2
CH_2Cl_2	Yes	Yes	XXX		
CH_3CCl_3			X	XXXX	CH_3CHCl_2
CH_3CHCl_2			X	XX	CH_3CH_2Cl
CH_2ClCH_2Cl	Yes		X	X	CH_3CH_2Cl
CH_3CH_2Cl			XX	[c]	
$CCl_2=CCl_2$			0	XXX	$CHCl=CCl_2$
$CHCl=CCl_2$			XX	XXX	$CHCl=CHCl$
$CHCl=CHCl$			XXX	XX	$CH_2=CHCl$
$CH_2=CCl_2$			X	XX	$CH_2=CHCl$
$CH_2=CHCl$	Yes		XXXX	X	

[a]Data from from McCarty and Semprini (*2*). [b]0, very small if any potential; X, some potential; XX, fair potential; XXX, good potential; XXXX, excellent potential. [c]Readily hydrolyzed abiotically, with half-life on order of 1 month.

Results

Anaerobic Transformations of TCE in Saint Joseph, Michigan

A detailed study of the distribution of TCE and its anaerobic transformation products was performed in a sand aquifer near the town of St. Joseph, Michigan (*7,8*). The study was undertaken to improve the understanding of the distribution of the CAHs years after the contamination occurred and to obtain information on natural mixing and transformation processes under anaerobic conditions. The industrial site, which is on the National Priority List (NPL), is approximately 750 m east of Lake Michigan. The sampling consisted of obtaining 155 groundwater samples at 5-ft intervals between the groundwater table, located within 35 to 40 ft from the ground surface, and a clay layer, located 65 to 90 ft below the ground surface.

The concentrations of the CAHs varied significantly with depth. Relatively high concentrations (several milligrams per liter of TCE, *c*-DCE, and VC) existed at all locations within 20 m of the center of the plume. The dominant DCE isomer present was *c*-DCE, with maximum concentrations of *c*-DCE, *t*-DCE, and 1,1-DCE of 133, 3.9, and 5.3 mg/l, respectively, occurring at the same locations. Methane and ethylene were also observed, with maximum concentrations of 12.3 and 6.6 mg/l, respectively. The high methane indicates methanogenic conditions existed, and the presence of ethylene indicates that some of the TCE had been completely dechlorinated.

Detailed contour analyses of these data are presented by Semprini et al. (*8*). The contaminant distributions showed consistent spatial relationships. Methane was not observed in areas of high TCE concentration. *c*-DCE was observed in a transition zone between high and decreasing TCE concentrations. TCE concentrations decreased with depth, and methane concentrations increased with depth. Vinyl chloride and ethylene were found to be associated with high methane concentrations. Sulfate was absent from areas of high methane concentration and present in zones where TCE concentration was high and methane concentration was low. The contamination also showed definite trends with depth. A typical distribution of CAHs and methane in a wellbore is shown in Figure 1. Sulfate concentrations decreased with depth. The results showed sequential dechlorination with depth, consistent with the development of more highly anaerobic conditions of methanogenesis. It is not known whether the presence of high TCE concentrations inhibited the development of methanogenic conditions at shallower depths, whether the presence of sulfate inhibited CAH transformations, or whether there were higher concentrations of a substrate contaminant at the depth to drive the aquifer to become more anaerobic.

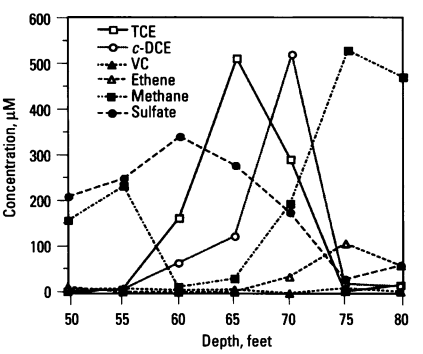

Figure 1. CAH, methane, and sulfate profile versus depth in wellbore T1-6 from the St. Joseph National Priority List site.

Detailed mass flux estimates based on the contour analysis indicated that ethylene represented 10 to 20% of the CAH flux. Analysis of the substrate(s) driving the anaerobic conditions was not performed. The CAH results are consistent with the COD distribution reported by McCarty and Wilson (*7*), which shows high upgradient COD levels and a reduction in COD in the region where methanogenic conditions exist. The results indicate that significant intrinsic anaerobic transformation of TCE has been and probably still is occurring at the site.

Studies of Enhanced Aerobic Transformations of CAHs at the Moffett Naval Air Station

In situ aerobic co-metabolism of CAHs has been studied in a shallow aquifer at a pilot test facility at the Moffett Naval Air Station, California. *In situ* biotransformation of TCE, *c*-DCE, *t*-DCE, and VC was observed by a microbial population grown on methane and oxygen (*9,10*). At the same site, but along a different experimental leg, the biotransformation of TCE, *c*-DCE, and *t*-DCE was recently observed by a microbial population grown on phenol and oxygen (*11,12*).

These pilot scale experiments were performed under induced gradient conditions of injection and extraction. Groundwater was extracted at a rate of 10 l/min and amended at the surface with the chemicals of interest, including the growth substrates methane and dissolved oxygen (DO) or phenol and DO and the target CAH contaminants, and then injected into the test zone at a rate of 1.5 l/min. The injection and extraction wells were 6 m apart. TCE and *c*-DCE, the two CAHs common to both studies, were not present in the groundwater and were continuously added in a controlled manner. The concentration response of methane, phenol, DO, TCE, and *c*-DCE was monitored at the downgradient monitoring wells located 1, 2.2, and 3.8 m from the injection well. The growth substrates and DO were added in alternating pulses to limit biofouling near the injection well and to distribute the microbial growth. Methane was added at a time-aver-

aged methane concentration of 6 mg/l. In the initial phenol study (*11*), phenol was added at a time-averaged concentration of 6.25 mg/l, which after 500 hr of addition was increased to 12.5 mg/l. The CAHs were added in a concentration range of 40 to 120 mg/l. In the subsequent study (*12*), phenol was injected at concentrations ranging from 12.5 to 25 mg/l, and TCE concentrations were varied from 62 to 1000 µg/l.

Comparisons of test results and model simulations for the methane studies are presented by Semprini and McCarty (*13,14*). Good agreement was obtained between model simulations and field results using a porous media transport model that included nonsteady-state processes of microbial growth, electron donor and acceptor utilization, and the co-metabolic transformation of contaminants using competitive inhibition kinetics. Transport processes of advection, dispersion, and rate-limited sorption and desorption were also included. The results showed that VC and *t*-DCE were transformed at rates similar to the methane growth substrate, while *c*-DCE and TCE were transformed at rates approximately a factor of 10 and 100 times lower than methane, respectively. In the 2-m biostimulated zone, the following degrees of transformation were achieved: TCE, 20%; *c*-DCE, 50%; *t*-DCE, 90%; and VC, 95% (*9*).

Simulations of the initial phenol experiment have also been performed using the same model (*15*). Figure 2 presents the field observations and model simulations for *c*-DCE and TCE response at monitoring well SSE1 (1 m from the injection well) due to biostimulation through phenol addition. The model simulations agree with the field observations and indicate that decreases in CAH concentrations with time resulted from the biostimulation of the phenol-utilizing population. The further decrease in concentration after 24 days resulted from the increase in the phenol-utilizing population due to the increase in the phenol injection concentration. The oscillation in the concentration of TCE and *c*-DCE results from the pulsed injection of phenol, where phenol competitively inhibits the transformation of the TCE and *c*-DCE. With prolonged phenol injection, over 95% of the *c*-DCE and 90% of the TCE were degraded in the 2-m biostimulated zone.

Comparison of the methane and phenol experiments indicates that phenol was a much better co-metabolic growth substrate for the degradation of TCE and *c*-DCE at the Moffett test site. Methane was a better growth substrate for degrading *t*-DCE. Model simulations of both experiments indicate that phenol was a better substrate for several reasons. Phenol addition resulted in a greater stimulated biomass. More phenol could be added since it required less DO consumption. It also had a higher yield coefficient than methane. The rate coefficients for the co-metabolic transformation for TCE and *c*-DCE were also a factor of two to three greater for the phenol-users compared to the methane-users.

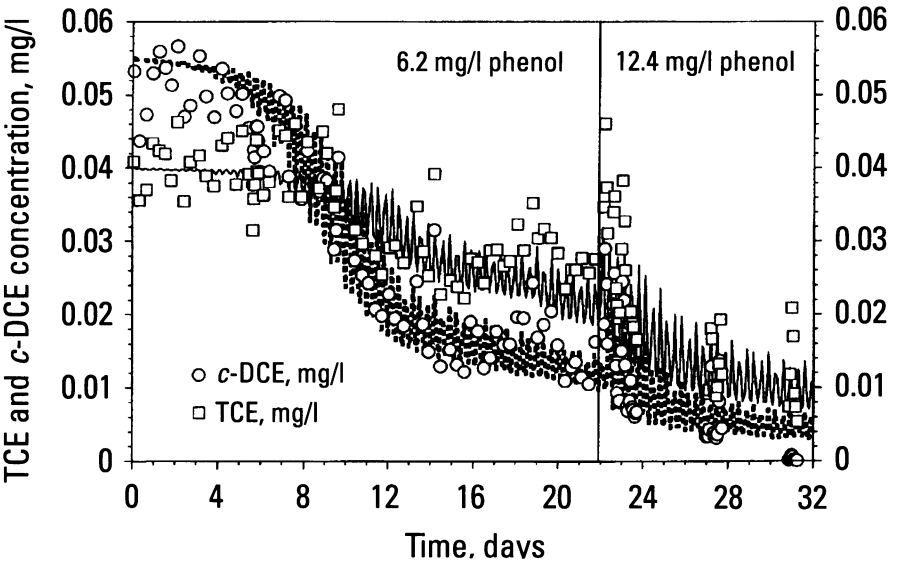

Figure 2. TCE and *c*-DCE response at monitoring well SSE1 due to biostimulation with phenol and oxygen at Moffett Field and comparison model simulations.

The results of the subsequent phenol experiments in which TCE concentration was gradually increased from 62.5 to 1000 µg/l are shown in Figure 3. TCE removals

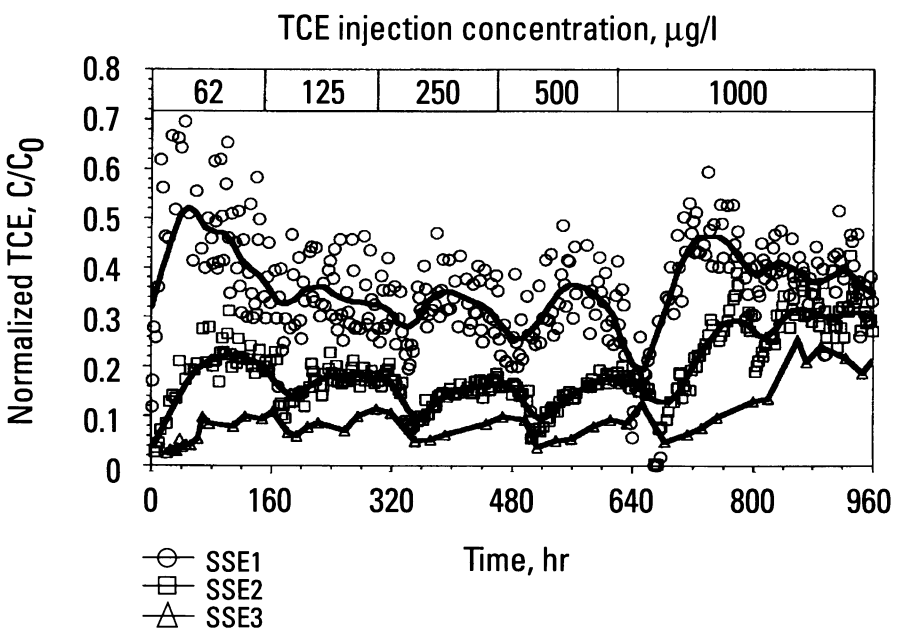

Figure 3. Normalized TCE concentrations with time at monitoring locations during phenol injection at a time-averaged concentration of 12.5 mg/l, during which TCE injection concentration was raised in steps from 62.5 to 1000 µg/l. Adapted from Hopkins et al. (*12*).

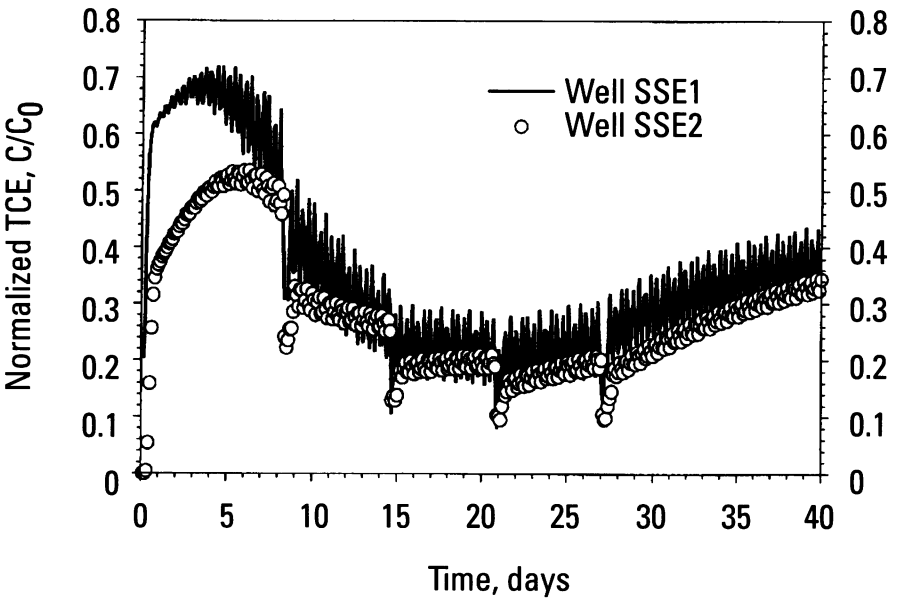

Figure 4. Model simulations of the TCE response shown in Figure 3, using a transformation capacity model.

at the furthest monitoring well SSE3 were essentially the same at about 87% for TCE concentrations up to 500 µg/l. At 1000 µg/l the removal decreased to 75%. Hopkins et al. (*12*) indicated that the lower removal at the higher concentration may have resulted from the TCE concentration being closer to the half-saturation coefficient (K_s) value, resulting in a deviation from first order kinetics; TCE transformation product toxicity began to have a measurable effect; or there was insufficient reducing power to carry out the transformation. The tests indicate that TCE concentration is an important factor to consider when evaluating *in situ* co-metabolic treatment.

Figure 4 presents model simulations of the experiments presented in Figure 3. In these simulations, the model presented by Semprini and McCarty (*13,14*) was adapted to include a transformation capacity model for TCE degradation of the form proposed by Alvarez-Cohen and McCarty (*16*) and Anderson and McCarty (*17*). The simulations agree reasonably well with the field observations. The simulations indicate that the inclusion of more complicated kinetic expressions is required to model TCE transformation over a broad range of concentrations.

Summary

At the St. Joseph site, complete transformation of TCE to ethylene occurred, with ethylene representing up to 20% of the contaminant mass flux. VC and ethylene were associated with high methane and low sulfate concentrations, which indicates that their production was associated with methanogenic conditions in the aquifer. The results are encouraging and indicate that it may be possible to enhance transformations at such sites through the controlled addition of an anaerobic growth substrate and possibly minor nutrients. Sites that should be considered are those that are actively degrading the CAHs anaerobically and that pose no risk of exposure to vinyl chloride if it is produced. Another consideration is that it may be possible to promote aerobic co-metabolism as a final polishing step to degrade DCE and VC that is formed anaerobically.

Aerobic studies indicate that for the geochemical and microbial conditions present at the Moffett site, phenol was a much better growth substrate than methane for degrading TCE and *c*-DCE. This is encouraging since TCE is present at so many sites, and *c*-DCE is often a major intermediate of the anaerobic transformations of PCE and TCE. The studies also indicate that TCE concentration is an important factor to consider. Studies at other sites are needed to determine if consistent results are obtained.

Model simulations provide a useful tool in analyzing the results from field studies in which complicated biological, physical, and transport processes are occurring. The simulations indicate that complex kinetic models are needed to describe the co-metabolic transformation of TCE over a broad range of concentrations. These models will also be of value in the design of *in situ* remediation systems.

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