

Field Evaluation of *in Situ* Aerobic Cometabolism of Trichloroethylene and Three Dichloroethylene Isomers Using Phenol and Toluene as the Primary Substrates

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The Moffett field site was used for further evaluation of *in situ* biotransformation of chlorinated aliphatic hydrocarbons with phenol and toluene as primary substrates. Within the 4 m test zone, representing a groundwater travel time of less than 2 days, removal efficiencies for 250 $\mu\text{g/L}$ TCE and 125 $\mu\text{g/L}$ *cis*-1,2-dichloroethylene were greater than 90%, and that of 125 $\mu\text{g/L}$ *trans*-1,2-dichloroethylene was $\sim 74\%$, when either 9 mg/L toluene or 12.5 mg/L phenol was used. Phenol and toluene were removed to below 1 $\mu\text{g/L}$. Vinyl chloride removals greater than 90% were also noted. However, only 50% of the 65 $\mu\text{g/L}$ 1,1-dichloroethylene was transformed with phenol addition, and significant product toxicity was evident as concomitant TCE transformation was here reduced to $\sim 50\%$. Hydrogen peroxide addition performed as well as pure oxygen addition to serve as a required electron acceptor.

Introduction

Trichloroethylene (TCE) has been widely used as a solvent for the past 50 years. Because of uninformed disposal practices for spent solvents in the past, it has become a major groundwater contaminant. Under certain anaerobic conditions in groundwater, TCE and another commonly used solvent, tetrachloroethylene (PCE), are known to be dehalogenated to produce the lesser chlorinated alkenes *cis*-1,2-, *trans*-1,2-, and 1,1-dichloroethylene (*c*-DCE, *t*-DCE, and 1,1-DCE), and vinyl chloride (VC) (1). There has been much interest in the potential of aerobic *in situ* biotransformation processes for the degradation of such chlorinated aliphatic hydrocarbons (CAHs) since Wilson and Wilson (2) first demonstrated cometabolism of TCE in soil columns where natural gas and oxygen were added to stimulate the growth of native microorganisms. In cometabolism, an enzyme (oxygenase), used by the microorganisms for initiating primary substrate oxidation, fortuitously transforms many CAHs (3). Field-scale evaluations of *in situ* biodegradation of CAHs have been undertaken since 1985 at the Moffett Federal Airfield (formerly the Moffett Naval Air Station), Mountain View, CA. Initially, methane was used as a primary substrate for aerobic cometabolism of TCE, *c*-DCE, *t*-DCE, and VC (4-8). While the methane-consuming culture developed was highly successful at transforming *t*-DCE and VC, removal efficiency was rather low with TCE and *c*-DCE. Therefore, other potential donors were sought. Phenol was then evaluated over two field seasons and was found to be quite superior to methane for *in situ* TCE and *c*-DCE degradation, providing up to 90% removal in one pass at CAH concentrations of up to 1 mg/L (9, 10).

Because of the success with phenol, additional field studies were deemed desirable to test efficiency with other CAHs (1,1-DCE, VC), to evaluate the potential of a similar alternative primary substrate (toluene), and to evaluate the effectiveness of an alternative source of oxygen (hydrogen peroxide). Results from these additional studies are presented in the following.

Materials and Methods

The *in situ* treatment evaluation was performed using the same methodology as previous studies conducted at the Moffett field site (11). In a series of stimulus-response tests, an induced gradient was created by extracting groundwater at 10 L/min and injecting chemically augmented groundwater 7 m upgradient at 1.5 L/min. The south-southeast (SSE) well leg was used for this study, the same well leg as used for the previous two evaluations of phenol-utilizing bacteria (10, 11). The stimulus was the injection of augmented groundwater, and the response was the measured concentration histories as observed in monitoring wells SSE1, SSE2, and SSE3, located 1, 2.2, and 4 m, respectively, from the injection well, in the direction of the extraction well. The spacing of these monitoring wells provided 50% breakthrough times following injection of 4, 18, and 34 h, respectively. The aquifer is a silt, sand, and gravel alluvial deposit ~ 1.4 m thick and ~ 5 m below ground surface. It is bounded on the top and bottom by silty clay layer aquitards of low permeability. The injection and extraction wells are standard 5.1 cm PVC casings with 1.52 m screens that fully penetrate the confined aquifer.

TABLE 1

Concentrations of Chemicals Added to Injected Water during Different Time Periods

time period (h)	concentration added to injected water							
	phenol ^a (mg/L)	toluene ^a (mg/L)	DO (mg/L)	H ₂ O ₂ (mg/L)	TCE (μg/L)	1,1-DCE (μg/L)	c-DCE (μg/L)	t-DCE (μg/L)
0-200	12.5	0	32	0	230	0	0	0
200-500	12.5	0	32	0	230	134	0	0
500-680	12.5	0	32	0	230	65	0	0
680-980	12.5	0	32	0	230	0	0	0
980-1316	0	9	32	0	230	0	0	0
1316-1422	0	9	32	0	230	0	119	0
1422-1465	0	9	32	0	230	0	0	0
1465-1656	0	9	32	0	230	0	0	81
1656-1680	0	9	32	0	230	0	0	0
1680-2000	0	9	0	97	230	0	0	0

^a Time-averaged concentrations.

The monitoring wells are 3.2 cm stainless steel wire-wound drive points with 0.6 m screens centered between the aquitards. A detailed description of the groundwater chemical composition and the test zone has been given previously (4). The groundwater is slightly saline with total dissolved solids of 1460 mg/L and contains some CAH contamination with measured concentrations during this study being 26 μg/L for 1,1,1-trichloroethane, 0.5 μg/L for 1,1-dichloroethane, and <5 μg/L for CFC-11. In addition, 1,1-DCE and CFC-113 are present and coelute during analysis, with a combined concentration of <5 μg/L measured as 1,1-DCE. TCE, PCE, c-DCE and t-DCE are not present.

The groundwater augmented for injection was a portion of the water extracted to induce the controlling gradient. The remainder of the extracted groundwater was air stripped before being discharged to the local storm drain system. Before chemical augmentation, the groundwater was filtered with a nominal 1 μm cotton filter and UV disinfected. It was then passed through a gas absorption column where molecular oxygen was used to purge the column, producing a dissolved oxygen (DO) concentration in the groundwater of ~32 mg/L. During the later period of the study, when H₂O₂ was used as a substitute for oxygen, helium was used to purge the gas absorption column and a 10% H₂O₂ solution was fed into the injection flow stream to produce a concentration of ~100 mg/L. For tracing groundwater flow, a 100 g/L as Br⁻ solution of NaBr was blended into the injection flow stream to produce a Br⁻ concentration of ~80 mg/L. The chlorinated alkenes were added as solute-saturated water solutions as needed: TCE was added continuously during the whole evaluation, while 1,1-DCE, c-DCE, and t-DCE were added only during the periods of their respective evaluations.

Phenol and toluene were added in pulses during their respective evaluations, once every 8 h as controlled by a timing clock. For phenol, a solution was prepared, and an aliquot yielding 9 g of phenol was added during each 10 min or less pulse, thus producing a 12.5 mg/L time-averaged concentration. Toluene was added in a fashion similar to phenol, but as pure solute: 6.5 g was added over a 30 min period to yield a time-averaged concentration of 9 mg/L for the 8 h cycle. A pair of static mixers with 24 elements was used to mix the toluene into the injection flow stream. Although the toluene was not perfectly dissolved within the static mixers' contact time of less than 1 s, it was nevertheless very finely dispersed upon exiting the static mixers, with a pulse concentration of ~200 mg/L (toluene water solubility is ~600 mg/L).

These evaluations followed 8 months of inoperation of the field site. A summary of the injection concentrations for the chemicals of interest over this study period is given in Table 1. As in previous years, there was a prestimulation period of 3 days in which only phenol (6.5 mg/L time averaged) and oxygen (32 mg/L) were added. This was done to prevent potential biofouling of the extraction well and to provide time to correct the usual startup problems of the injection and analytical systems. Then, at time zero, the chemical augmentation of TCE and bromide began along with the increase in phenol concentration to a time-averaged 12.5 mg/L. After TCE removal approached steady state, the 1,1-DCE augmentation and evaluation began. After the termination of the 1,1-DCE addition and quasi-steady-state conditions were again achieved, the primary substrate was changed from phenol to toluene. Approximately 1 week later, augmentation with c-DCE was begun, followed by the augmentation with t-DCE. Finally, H₂O₂ was substituted for molecular oxygen as the electron acceptor.

Analytical Methods

Monitoring of the field evaluation was performed with the on-line analytical system as previously described (4, 10). The volatile organics were quantified using two channels of gas chromatography: one column, a 30 m J&W Scientific DB-5 Mega-Bore, was connected to an electron capture detector and used to quantify TCE. The other column, a 30 m J&W Scientific DB-5 Mega-Bore connected in series with a 30 m J&W Scientific 624 Mega-Bore, was connected to a PID/Hall (Tremetrics) detector to quantify toluene and the three dichloroethylenes. Bromide was quantified by HPLC anion chromatography using a Wescan detector and standard anion column (0.04 mM KHP eluant).

Phenol was quantified by reversed phase HPLC using a Spherisorb column and a UV detector (Spectra 100, Thermal Separation Products) connected in series with a fluorescence detector (FL2000, Thermal Separation Products). The UV detector provided measurements at the higher concentrations (greater than 100 μg/L) and the fluorescence detector at the lower concentrations (0.5-100 μg/L). For most of the phenol measurements, only a guard column was used with a 7.5% methanol-water eluant to eliminate production of hazardous waste. When needed, the analytical column was used with a 50% methanol-water eluant.

Dissolved oxygen and pH were measured by meters with associated probes. The meters and integrators were

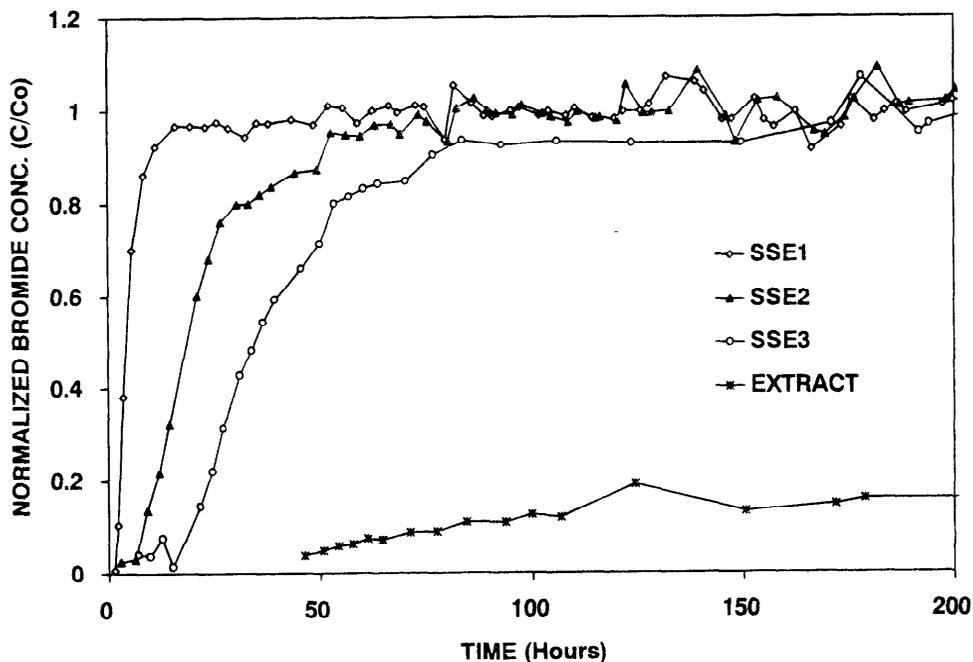


FIGURE 1. Bromide arrival at monitoring wells and extracted effluent with time following continuous injection of 80 mg/L.

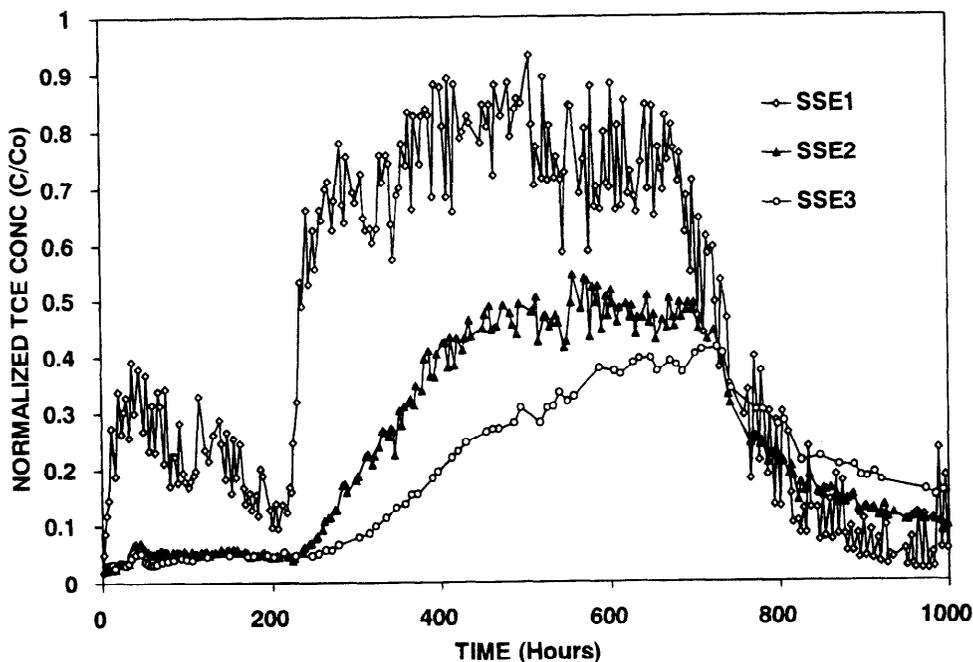


FIGURE 2. Normalized TCE concentration at the three monitoring wells following continuous injection of 230 $\mu\text{g/L}$ at 0 h with a phenol concentration of 12.5 mg/L. Concomitant injection of 1,1-DCE began at 200 h and was terminated at 680 h.

attached to a computer for automated storage of the results in the data base. The computer also controlled sample order, sample processing, and instrumentation. Detection limits were as follows: chlorinated ethylenes, 1.0 $\mu\text{g/L}$; phenol and toluene, 0.5 $\mu\text{g/L}$; bromide, 0.5 mg/L; and DO, 0.1 mg/L.

Results

Figure 1 presents the normalized bromide data (C/C_0 , monitoring well concentration/injection well concentration) for the first 200 h of the 1993 field evaluation. From these data, the 50% breakthrough time to SSE1, SSE2, and SSE3 is 4, 18, and 34 h, respectively. The concentrations at the SSE1 and SSE2 monitoring wells reached the injection concentration ($C/C_0 = 1$) within 90 h, while concentrations

at SSE3 tended to "tail" somewhat before reaching the injection concentration. Full penetration of the injected water at all monitoring wells was obtained. Thus, once sorption has reached steady-state equilibrium between aquifer solids and the groundwater, differences between organic solute concentrations measured at the monitoring wells and in the injected water should represent biotransformation. The lower normalized breakthrough concentration of 0.15 for the extraction well is the result of radial flow into the extraction well and represents the ratio of injected to extracted water flow rates (1.5:10), as anticipated.

Figure 2 presents the first 1000 h of TCE injection. Within 50 h, TCE broke through at the SSE1 well. Then, as biomass became reestablished, the normalized TCE concentration at SSE1 decreased from ~30% break through to ~12% by

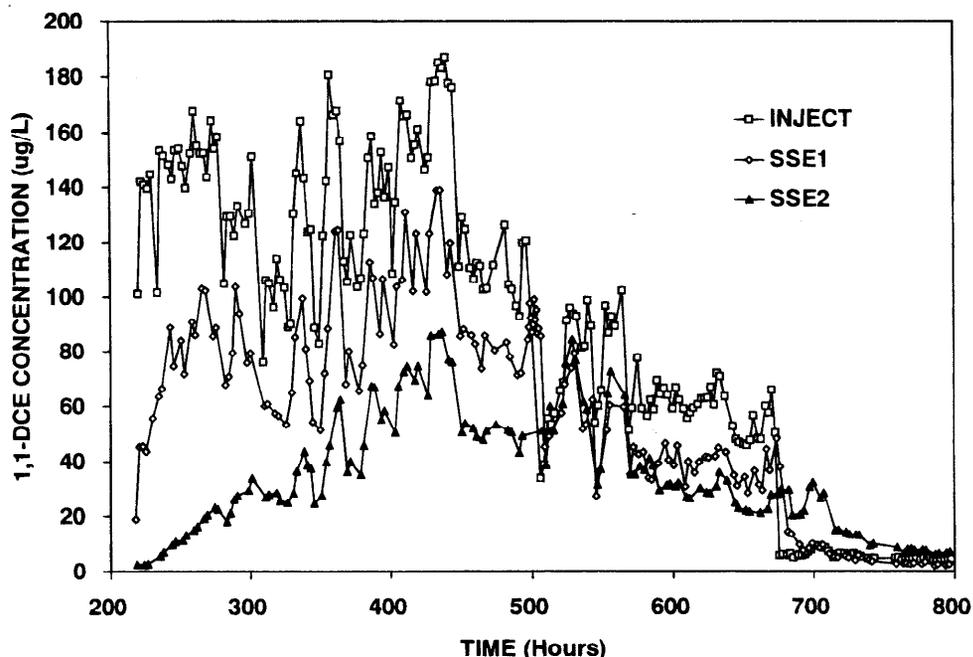


FIGURE 3. Concentration of 1,1-DCE in injection stream and at two monitoring wells following 1,1-DCE injection at 200 h and termination at 680 h.

200 h. For the SSE2 and SSE3 monitoring wells, there was an increase of only a few percent above residual concentrations from the prior year's evaluation (initially $\sim 5 \mu\text{g/L}$). The wide oscillations in normalized TCE concentrations at the SSE1 well illustrates the effect of competitive inhibition from phenol, the primary substrate, when added in pulses as found in previous studies (10, 11). As the phenol pulse passes a monitoring well, the measured chlorinated alkene concentrations there always increase. The resulting concentration variation is heavily dampened at the SSE2 and SSE3 monitoring wells. Here, monitoring data indicate the primary substrate is essentially absent at these locations.

At 200 h, the injection solution was supplemented with $134 \mu\text{g/L}$ 1,1-DCE, while TCE addition continued at $230 \mu\text{g/L}$. The effect on TCE removal at SSE1 was immediate; biotransformation of TCE decreased from nearly 88% to $\sim 15\%$. Because of this severe adverse effect on TCE removal, at 500 h the 1,1-DCE injection concentration was reduced 50% to $65 \mu\text{g/L}$. The TCE concentration at the SSE2 and SSE3 monitoring wells also increased as a result of the 1,1-DCE addition. The retardation of TCE, as affected by advection time and sorption, can be seen in the response at these downgradient wells. At SSE2, TCE biotransformation decreased from approximately 95% to 53%. TCE at SSE3 did not reach a quasi-steady-state concentration before 1,1-DCE addition was terminated, but little improvement would be expected between SSE2 and SSE3 since only trace levels of phenol passed by SSE2 to support bioactivity down gradient.

The addition of 1,1-DCE was terminated at ~ 680 h, following which the biotransformation of TCE at SSE1 rapidly increased to $\sim 94\%$, somewhat better than before the 1,1-DCE addition began. Concentrations remained higher for some time at SSE2 and SSE3 while previously sorbed TCE desorbed into the passing water. The significant adverse effect on TCE removal by a relatively small concentration of 1,1-DCE is readily apparent from these data.

Figure 3 presents the concentration history for 1,1-DCE. Initially, after injection began at 200 h, the 1,1-DCE concentration rapidly increased and stabilized at $\sim 70\%$ of the injection concentration at the SSE1 monitoring well; thus $\sim 30\%$ of the 1,1-DCE was transformed within the first meter of travel. At SSE2, biotransformation reached $\sim 54\%$. No further biotransformation took place between SSE2 and SSE3 (data not shown).

At 500 h, after the concentration of 1,1-DCE was decreased to $65 \mu\text{g/L}$, there may have been a slight improvement in biotransformation at SSE1, but none of note at SSE2. The measured 1,1-DCE injection concentration of $4.5 \mu\text{g/L}$ after addition was terminated at 680 h is largely due to a mixture of CFC-113 (a coeluting peak and groundwater contaminant) and recycled 1,1-DCE from the extraction well. After 680 h, the higher concentration of 1,1-DCE at SSE2 and SSE3 is expected due to slow desorption from the aquifer solids.

Figure 4 presents the measured phenol concentrations at SSE1 and SSE2 for the period when phenol was used as the primary substrate. The phenol was added in short pulses of $\sim 200 \text{ mg/L}$ once every 8 h to produce the time-averaged concentration of 12.5 mg/L . Even initially, phenol concentration never reached the time-averaged injection concentration, indicating a significant phenol-utilizing biomass remained from the previous year. At SSE2, phenol concentration never exceeded $200 \mu\text{g/L}$ after the first day. However, once 1,1-DCE addition was begun, phenol concentrations at SSE1 quickly increased and reached a peak at 500 h that was near the time-averaged injection concentration of 12.5 mg/L . Then, when 1,1-DCE concentration was decreased to $65 \mu\text{g/L}$, phenol concentration also decreased. Termination of 1,1-DCE addition at 680 h resulted in a slow decrease in SSE1 phenol concentration and 200 h later reached levels noted prior to 1,1-DCE addition.

At SSE2 during the 1,1-DCE addition, phenol was frequently detected at its highest concentration there of $\sim 2 \mu\text{g/L}$. Here, the oscillations in TCE concentration were

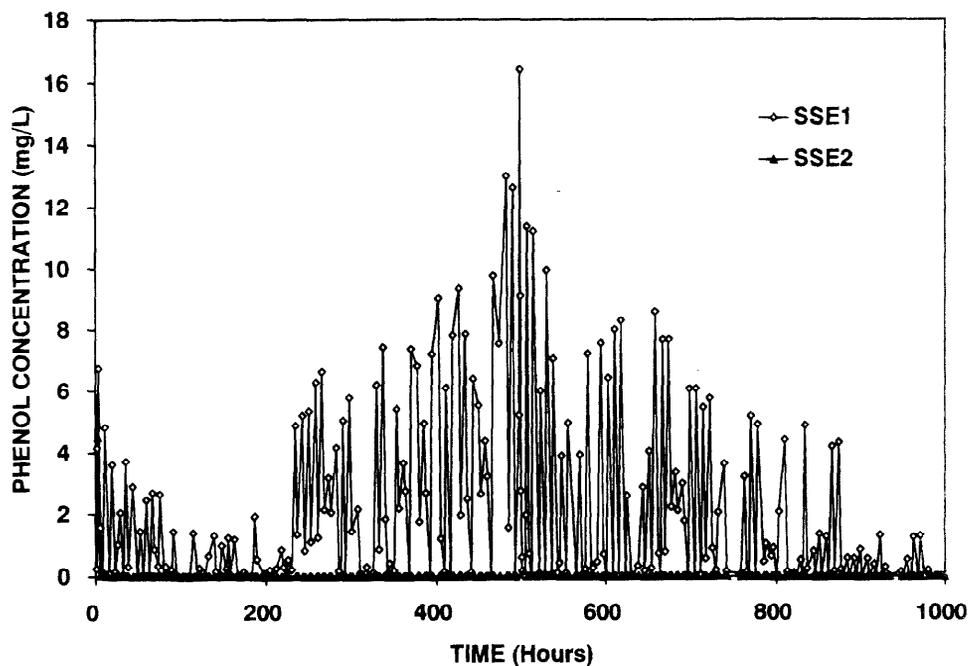


FIGURE 4. Phenol concentration at the first two monitoring wells during the first 1000 h of injection.

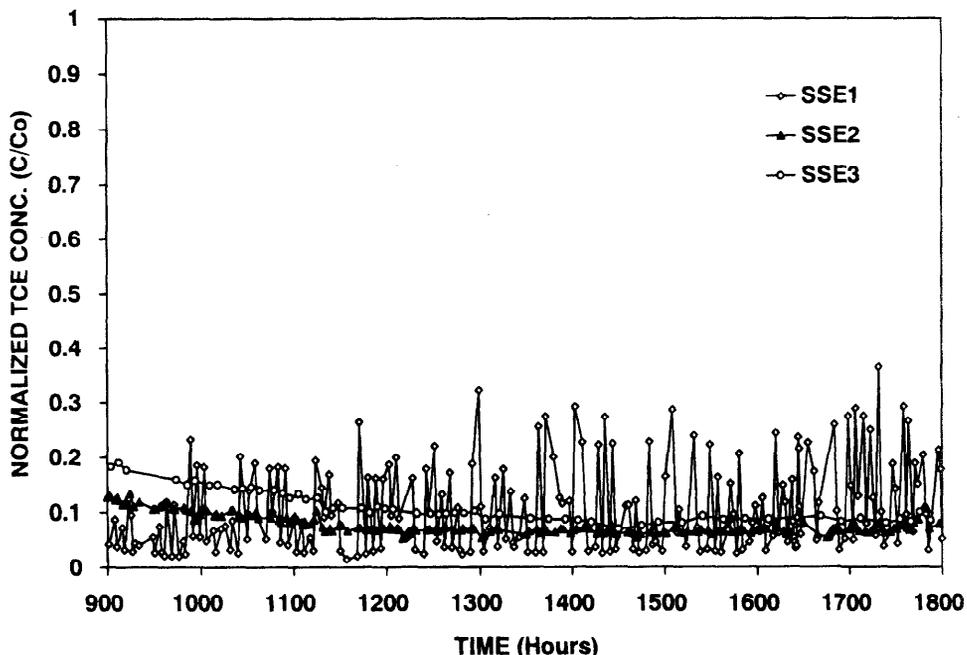


FIGURE 5. Normalized TCE concentration at various monitoring wells following transition of primary substrate from phenol to toluene at 980 h.

much greater than in other portions of the study, perhaps as a result of greater competitive inhibition caused by a greater penetration of the primary substrate. After 1,1-DCE addition was terminated, phenol was detected at SSE2 in only a few samples, and then at less than $1 \mu\text{g/L}$. By 800 h, phenol was always below the $0.5 \mu\text{g/L}$ detection limit at SSE2, indicating that phenol utilization in the aquifer was then very efficient (more than 99.995% removal).

Another primary goal of this study was an evaluation of the effectiveness of toluene as an alternative primary substrate. At 980 h, pulse feeding of phenol was terminated and that of toluene was begun. Here, in order to obtain equivalent DO consumption, the time-averaged concentration for toluene was set at 9 mg/L . From Figure 5 results, it is clear that the microorganisms previously fed phenol

immediately switched to toluene as they continued to transform TCE. The most significant change in response was a greater amplitude in the TCE concentration variation at SSE1. From these data, it is not clear whether the TCE variation noted was the result of stronger competitive inhibition kinetics with toluene or because the toluene pulses took longer to pass the sample location due to retardation or possibly the slow or incomplete dissolution of toluene; consequently, toluene might be present for a longer period. The lower TCE concentrations found after the toluene pulse had passed were approximately the same as when phenol was injected.

Coinciding with the transition to toluene, the presence of a new compound at SSE1 was indicated by the HPLC UV detector. This compound disappeared by the time the

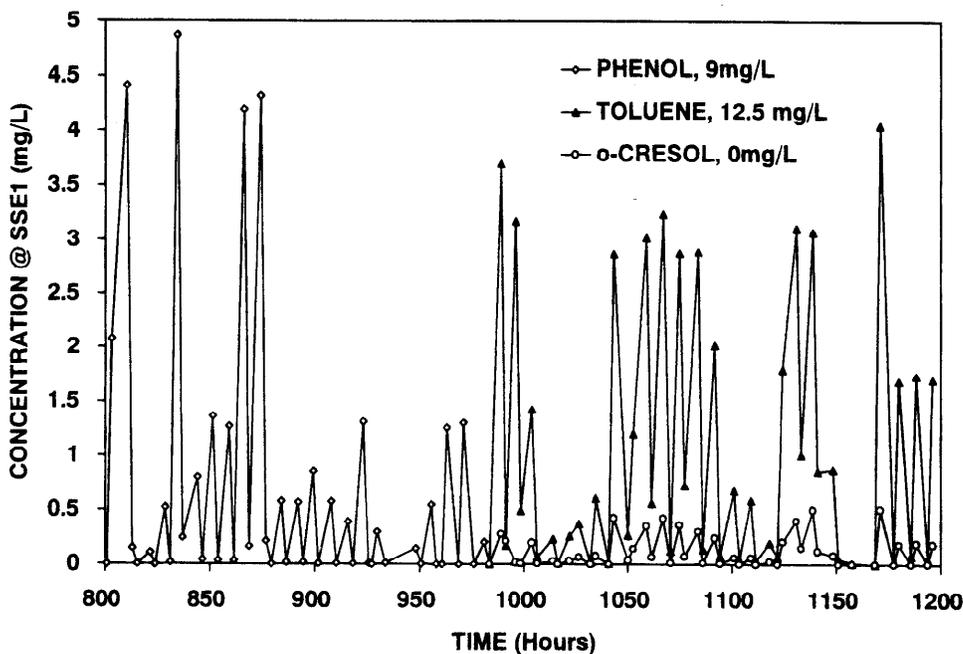


FIGURE 6. Concentrations of phenol, toluene, and *o*-cresol at the first monitoring location before and after the transition of primary substrates from phenol to toluene at 980 h.

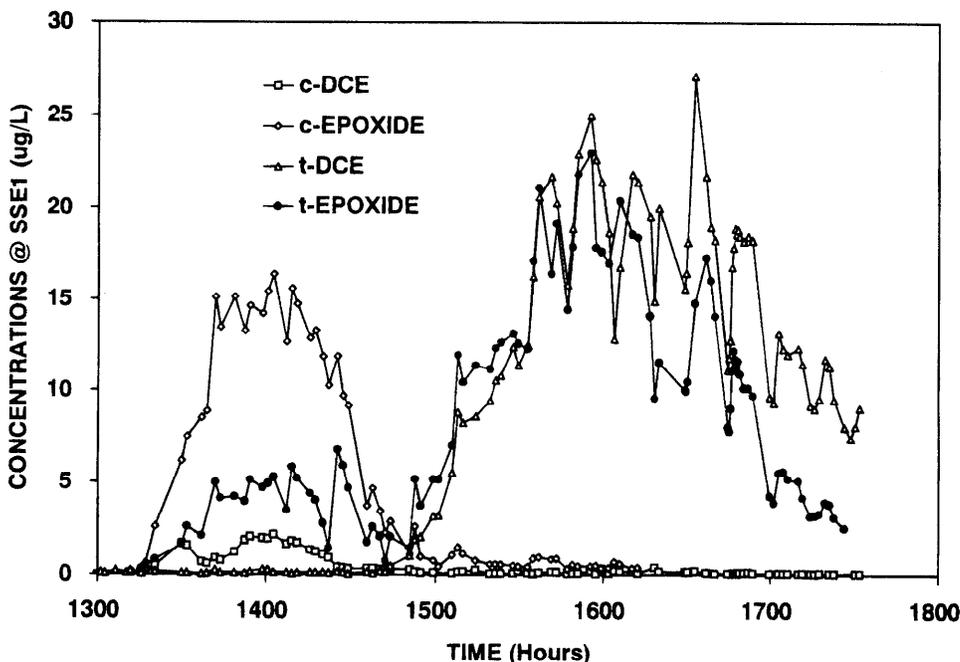


FIGURE 7. Presence at the first monitoring well of *cis*-dichloroethylene epoxide and *trans*-dichloroethylene epoxide during addition of 119 $\mu\text{g/L}$ *c*-DCE between 1316 and 1422 h and addition of 81 $\mu\text{g/L}$ *t*-DCE between 1465 and 1656 h.

injected water reached SSE2. The elution pattern for this compound was found to coincide with that for *o*-cresol but not for *m*- or *p*-cresol. The appearance of *o*-cresol is good evidence of toluene and hence TCE conversion by toluene orthomonooxygenase (TOM), which is one of the five oxygenases known to initiate aerobic toluene metabolism (12). Figure 6 illustrates the measured concentrations at SSE1 of phenol, toluene, and *o*-cresol during the transition period between the primary substrates. At the SSE3 monitoring well, toluene remained below the detection limit of 1 $\mu\text{g/L}$ throughout the study.

Two common daughter products of anaerobic transformation of TCE in groundwater are *c*-DCE and *t*-DCE. The potential for aerobic cometabolism of these compounds

was evaluated previously at the Moffett field site with methane and phenol as primary substrates (5, 11), and further evaluation with toluene was believed desirable. *c*-DCE was added to the injection solution at an average concentration of 119 $\mu\text{g/L}$ from 1316 to 1422 h along with 230 $\mu\text{g/L}$ TCE. Over the 2.2 m distance to the SSE2 monitoring well, greater than 98% of the *c*-DCE was transformed (Figure 7). At SSE2, an equivalent of ~13% of the *c*-DCE transformed was detected as the *cis*-1,2-dichloroethylene epoxide and ~4% as the *trans*-1,2-dichloroethylene epoxide, assuming the response factors of the Hall detector for the epoxides were the same as that for the parent compounds. Verification that one of the peaks does indeed represent the *trans*-dichloroethylene epoxide was

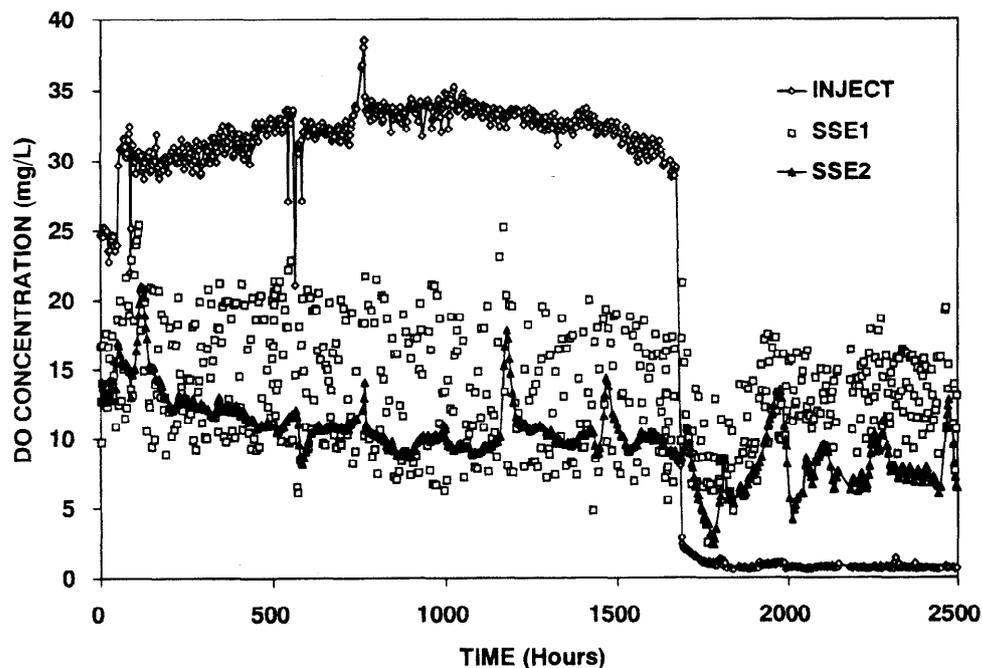


FIGURE 8. DO concentration in the injection flow and at the first two monitoring wells before and after transition from addition of 32 mg/L pure oxygen to 97 mg/L hydrogen peroxide at 1680 h.

presented previously (5). However, the other peak that appeared was assumed to represent *cis*-dichloroethylene epoxide since this epoxide is the logical byproduct from *c*-DCE transformation and this particular peak was found only during this portion of the evaluation. Next, *t*-DCE was added at an average concentration of 81 $\mu\text{g/L}$ from 1465 to 1656 h and *c*-DCE was terminated. Here, only 75% of the *t*-DCE was transformed, and the equivalent of 20% of the transformed *t*-DCE was detected as the *trans*-dichloroethylene epoxide. Interestingly, no *cis*-dichloroethylene epoxide was detected during the addition of *t*-DCE. During the period when the two 1,2-DCE isomers were added, TCE was also added continuously, but unlike when 1,1-DCE was added, there was no discernible impact on TCE biotransformation efficiency.

The final goal of this study was to evaluate whether hydrogen peroxide would be a suitable substitute for molecular oxygen as an electron acceptor. This study was initiated at 1680 h by substituting helium gas for oxygen gas in the gas absorption column, and a 10% H_2O_2 solution was added at a flow rate of 1.5 mL/min to the 1500 mL/min injection flow stream, yielding 97 mg/L H_2O_2 in the injection solution. When hydrolyzed in the aquifer, this should yield 0.5 mol of O_2 /mol of H_2O_2 , or ~ 46 mg/L DO. The response in DO values in the injection stream and the monitoring wells is presented in Figure 8. While, as expected, no DO was then detectable in the injection stream, hydrolysis of H_2O_2 in the aquifer to produce DO is evident from the DO concentrations measured at SSE1 and SSE2. Over the first 300 h following the transition to H_2O_2 , the injection system stopped automatically several times as a result of a 3.4 kPa (0.5 lb/in.²) drop in injection wellhead pressure. This safety control had been installed previously to prevent chemical addition in case of injection system failure. It is believed this significant pressure drop resulted from disinfection by H_2O_2 near the injection well, which is actually a beneficial effect. Overall, this stoppage only created a temporary imbalance in the toluene to oxygen ratio. No adverse effect from the change in oxygen source occurred to TCE removal,

as evidenced in Figure 9 by the consistent TCE concentration at monitoring wells before and after H_2O_2 addition at 1680 h. Thus, H_2O_2 was found to be a suitable alternative electron acceptor to molecular oxygen when added at a concentration that will not permit oxygen to exceed its solubility in water.

Discussion

The Moffett field site has now been used to evaluate at field scale the removal efficiencies through cometabolism for several chlorinated ethenes by microorganisms that use methane, phenol, or toluene as primary substrates (11, 13). Moffett field site results from the current and previous studies are summarized in Table 2. There are several cautions that must be exercised when these data are evaluated. First, the concentration of each CAH treated was in general less than 250 $\mu\text{g/L}$. In the case of methane, the concentrations tested were generally less than 100 $\mu\text{g/L}$. In a previous study with phenol, TCE concentrations up to 1000 $\mu\text{g/L}$ were evaluated (10). Here, the removal efficiency with 500 $\mu\text{g/L}$ TCE was similar to that shown in Table 2, but at 1000 $\mu\text{g/L}$ TCE, the removal efficiency decreased. This is consistent with the maximum laboratory-measured transformation yield of 0.11 g of TCE/g of phenol consumed. Thus, with 12.5 mg/L phenol added, a maximum transformation of 1400 $\mu\text{g/L}$ TCE might be obtained. The field TCE removal was about half of this maximum or ~ 0.062 g of TCE/g of phenol. By doubling the amount of phenol added to 25 mg/L, the efficiency of TCE removal was increased to 90%. While higher phenol concentrations thus resulted in a larger supported biomass in the aquifer and higher TCE removal efficiency, the amount of phenol that could be added was limited by oxygen availability.

A question to be asked is whether the CAH removals noted might be partially the result of other processes, such as dilution, sorption, or volatilization. Although not generally shown, bromide tracer was added continuously to confirm that near 100% of the injected water passed each of the monitoring wells, thus confirming that dilution of

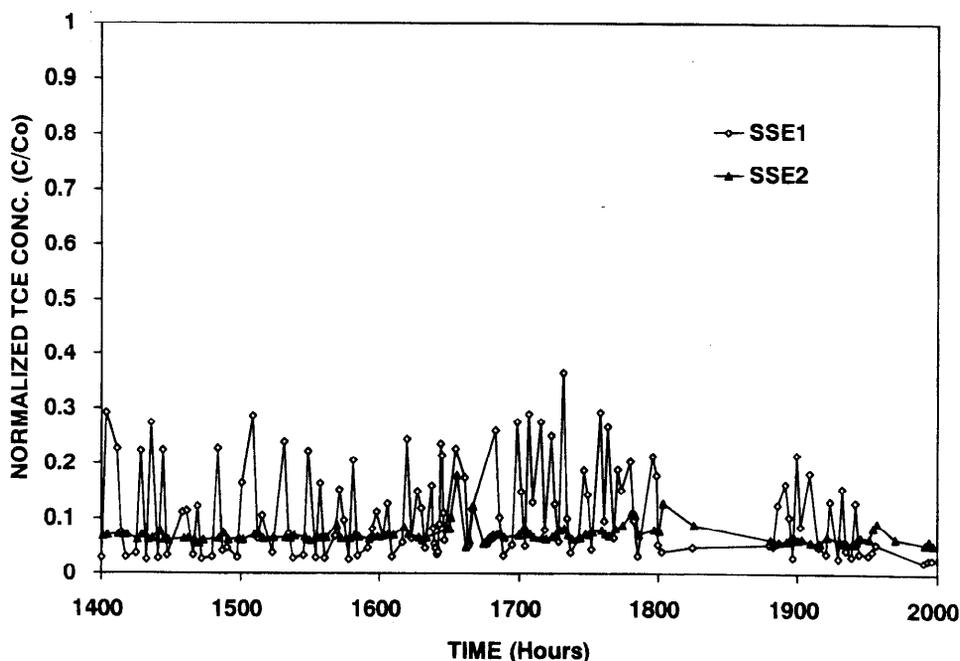


FIGURE 9. Normalized TCE concentration at the first two monitoring wells before and after changeover from pure oxygen to hydrogen peroxide addition at 1680 h.

TABLE 2
Efficiency of Chlorinated Aliphatic Hydrocarbon Removal Obtained at the Moffett Field Site with Different Primary Substrates

primary substrate	concn (mg/L)	DO consumed (mg/L)	% removal ^a				vinyl chloride
			TCE	1,1-DCE	c-DCE	t-DCE	
methane ^d	6.6	20	19	ne ^b	43	90	95
phenol ^c	12.5	30	94	54	92	73	>98
toluene	9	28	93	ne	>98	75	ne

^a Results for the CAH concentration range from 60 to 250 $\mu\text{g/L}$.
^b ne, not evaluated. ^c Results for TCE and 1,1-DCE from this current study, that for c-DCE from a previous study (11), and that for VC and t-DCE from the same previous study, but not reported until now.
^d Ref 5.

CAHs was not a factor. Extensive previous studies on the significant extent of sorption and its impact on retardation of the rate of movement of CAHs in the Moffett aquifer have helped understand the effect sorption may play in CAH removal. These previous studies give confidence that the steady-state removals at SSE2 noted in Figure 5 over a 900 h period are the result of biological degradation and not sorption. Indeed, the TCE response to 1,1-DCE injection (Figure 2) illustrates that biodegradation or lack thereof explains most of the response noted, although sorptive effects play a large part in the slow TCE decrease following discontinuation of 1,1-DCE injection at 680 h. The sorption impact can also be noted in Figure 5, which illustrates the long time it takes for the TCE concentration at SEE3 to reach the lower concentration measured at SEE2. Volatilization losses are insignificant, as illustrated by the fact that in previous studies the CAHs do reach injection concentration, given sufficient time to reach sorption equilibrium. Following injection of primary substrate, the highly soluble carbon dioxide is produced, but even if this gas were insoluble in water, the estimated 6 mL produced per liter of injected water is much too small to result in measurable stripping of the CAHs. The same is true for the

amount of oxygen injected or that resulting from hydrogen peroxide hydrolysis; the resulting DO solution concentration in either case was well below saturation. Also, the quantities of oxygen lost per unit mass of primary substrate consumed as noted in Table 2 are what one would expect from the biological oxidation of the primary substrate. Thus, the evidence suggests oxygen ebullition from the injected water did not and would not occur. Finally, the Moffett aquifer is confined above and below with a clay layer so that diffusion of CAHs out of the groundwater into a gaseous vadose zone cannot occur. Indeed, the residence time of groundwater between injection and extraction is too short for such loss to be significant in any case. Thus, from a variety of perspectives one can conclude that the major factor affecting CAH decreases with biostimulation was biodegradation.

As indicated in Table 2, ~2.4 g dissolved oxygen is required for each gram of phenol. Since the solubility of pure oxygen at 1 atm of pressure is ~45 mg/L, the concentrations of primary substrate added in the studies indicated in Table 1 may be near the upper practical limit for addition in a field system that is similar to the one used at Moffett field. Because of the limits set by both transformation yield and oxygen-dependent biomass concentrations, removal efficiencies for TCE in concentrations higher than 1000 $\mu\text{g/L}$ are likely to be significantly lower than indicated in Table 2.

The removal efficiency for VC with phenol was not reported previously, but stems from results following the previous year's study of TCE concentration effects (10). The procedure followed for evaluation was essentially the same as that followed during the first year with phenol (11) but with a TCE concentration of 250 $\mu\text{g/L}$ and a VC concentration of 60 $\mu\text{g/L}$.

The removal efficiencies for TCE listed in Table 2 apply when TCE was alone or when present in combination with a similar concentration of either c-DCE, t-DCE, or VC. All other CAHs evaluated were added together with TCE. No significant competitive inhibition between TCE and these

CAHs was noted in the field. However, this was definitely not the case with 1,1-DCE, which exhibited a high degree of transformation product toxicity. With 65 $\mu\text{g/L}$ 1,1-DCE, ~50% removal of 1,1-DCE resulted. The adverse impact this had on TCE degradation, which was present at ~250 $\mu\text{g/L}$, is readily apparent from Figure 2. TCE removal efficiency decreased from over 90% to only ~50%. The significant transformation product toxicity with 1,1-DCE was found previously in laboratory studies with methane as a primary substrate (14), and thus, it was felt important to determine the impact in this field study with phenol. Although 1,1-DCE was not evaluated in the field with either methane or toluene, there is no reason to expect it would behave any differently from that found here with phenol.

CAH removal efficiencies with phenol or toluene for the compounds studied were similar. Indeed, the same microorganisms were no doubt operating as no lag or change in TCE removal efficiency was experienced during the sudden change in primary substrate from phenol to toluene. However, with methane as a primary substrate, the removal effectiveness with various CAHs was significantly different. Here, TCE and *c*-DCE removals were relatively poor, but *t*-DCE and VC removals were good. With phenol and toluene, TCE, *c*-DCE, and VC (studied with phenol only) were very good, but *t*-DCE removal was only moderate. The interesting difference between the methane and aromatic hydrocarbon systems is in their relative efficiencies for *c*-DCE and *t*-DCE removal. In anaerobic groundwaters, transformation of the chlorinated solvents, TCE, to *c*-DCE and VC frequently occurs, so that these intermediates are often found at contaminated sites. It is thus of significance that these compounds were also quite effectively removed with either phenol or toluene as primary substrates.

Frequently, 1,1-DCE is found at contaminated sites. Its primary origin appears to be from the abiotic elimination reaction with 1,1,1-trichloroethane (TCA), which is another common solvent contaminant of groundwaters. Whenever TCA is present as a major contaminant, 1,1-DCE is likely to be found as well (15). As illustrated in this study, its presence at even fairly low concentrations is likely to hinder aerobic cometabolic TCE removal. Indeed, the native 1,1-DCE concentration in groundwater at the Moffett field site of ~2 $\mu\text{g/L}$ may have had an adverse impact on the results obtained there. Methods to help circumvent this potential problem are needed.

As noted above, the microbial consortium developed *in situ* at Moffett field on phenol could also utilize toluene as a primary substrate. In a previous microcosm study conducted at the Moffett field site it was found that the microorganisms grew faster on phenol than on toluene; 7 vs 20 days, respectively, were required for establishment of an effective primary-substrate-removing culture (11). When phenol was first added *in situ*, ~8 days was required for the development of a population sufficient for significant phenol removal, about the same as in the microcosm study. After an additional 8 days, phenol removal was about complete. Based upon the laboratory studies, a longer time for development of a suitable culture on toluene can be expected. The previous microcosm study, however, indicated that TCE removal with toluene was better than with phenol. This was not indicated in the *in situ* results, although TCE removal was so high with either substrate that it may be difficult here to distinguish clearly between their relative effectiveness.

There are at least five different pathways by which toluene can be metabolized aerobically, each requiring a different oxygenase for initiating toluene oxidation (12). With the switch in primary substrate from phenol to toluene, measurable concentrations of *o*-cresol appeared at the SSE1 monitoring location. This is strong evidence that TOM was the dominant oxygenase present. This is the same oxygenase used by *Pseudomonas cepacia* G4 for phenol and toluene oxidation (16). This was the first aromatic hydrocarbon-utilizing microorganism identified to be effective at TCE cometabolism. Phenol- and toluene-degrading populations were obtained from a screen fabric bag containing glass beads and hung in a fully penetrating well between SSE1 and SSE2 during the phenol addition phase of this study before toluene addition (17). A high percentage of isolates were found to utilize both phenol and toluene, and the TOM pathway was dominant among them. These studies not only provide further evidence for the dominance of the TOM pathway by the microorganisms established through phenol injection at the Moffett field site but are also consistent with the finding of no lag period in toluene utilization when first injected into the aquifer.

Phenol and toluene are both toxic and regulated chemicals. A question thus often arises about the desirability of their addition to groundwater for cometabolism of CAHs. However, removal efficiencies for both at the Moffett field site were exceptional. Once the phenol-using culture was well established, the measured phenol concentration at the intermediate monitoring well, SSE2, was always well below the instrument detection level of 0.5 $\mu\text{g/L}$ as long as 1,1-DCE was not being added. But even when 1,1-DCE was added, the phenol concentration at SSE2 never exceeded 2 $\mu\text{g/L}$. Likewise, when toluene was added as the primary substrate, the concentration at SSE2 never exceeded the detection level of 1 $\mu\text{g/L}$. This is well below the U.S. EPA drinking water maximum contaminant level (MCL) and maximum contaminant level goal (MCLG) for toluene, which are the same or 1000 $\mu\text{g/L}$ (18). It is also well below the toluene taste and odor threshold of 40 $\mu\text{g/L}$ (18). There are no MCL or MCLG values established for phenol, but the 1962 U.S. PHS drinking water standard for phenol was 1 $\mu\text{g/L}$ based of taste and odor problems from chlorinated phenols that often formed during water disinfection. Taste and odor should be the major concern from phenol and toluene injection.

The Moffett field site experience (10) and biological treatment theory (19) indicate that higher removal rates and greater CAH removal efficiencies would be obtained by maintaining a higher biomass in the aquifer system. This could be obtained by adding higher concentrations of primary substrate. However, a limit exists because higher concentrations of dissolved oxygen would be needed as well. Higher oxygen concentrations might be obtained if an alternative to molecular oxygen, such as hydrogen peroxide, were used. Hydrogen peroxide has the added advantage in that it is easier to add *in situ* to groundwater than molecular oxygen. In this study, the substitution of hydrogen peroxide on an equivalent basis for molecular oxygen was demonstrated to be as effective as molecular oxygen. There was no significant change in biotransformation efficiency for TCE when the transition to hydrogen peroxide was made. Another possible advantage was the noted sudden drop in injection water pressure following addition of 97 mg/L hydrogen peroxide, suggesting a resulting reduction in clogging potential. This requires

additional experimentation for confirmation since this pressure drop may simply have been coincidental. While hydrogen peroxide may have advantages, it is more costly than pure oxygen. In addition, if too much is added, there is the added danger that, upon hydrolysis, the saturation level for dissolved gases may be exceeded and gas formation may occur in the aquifer, thereby causing a potential clogging problem. The tradeoffs between different forms of oxygen needs to be examined in any application.

While highly efficient TCE removal was obtained in this study with both phenol and toluene additions, a final caution needs to be stated. There are many different species of microorganisms that can use phenol or toluene as primary substrates, but there are also many different pathways exhibited and different potentials for TCE transformation. Even for a given pathway, the oxygenase involved may have a different susceptibility for CAH degradation. Thus, with the current state of knowledge, the ability of phenol- or toluene-degrading microbial cultures at a given site to efficiently degrade TCE and other CAHs of interest should be thoroughly evaluated in the laboratory before a full-scale effort is undertaken. The direct applicability of the Moffett field results to another site should not be assumed without a confirmatory study.

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