

Trichloroethylene Concentration Effects on Pilot Field-Scale In-Situ Groundwater Bioremediation by Phenol-Oxidizing Microorganisms

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A pilot study of in-situ aerobic cometabolic degradation of trichloroethylene (TCE) through the injection of phenol and oxygen into a confined aquifer was conducted at the Moffett Field test site together with a related laboratory study. With injected phenol and dissolved oxygen concentrations of 12.5 and 35 mg/L, respectively, first-order TCE removal of 88% was obtained over a concentration range of 62–500 $\mu\text{g/L}$. With 1000 $\mu\text{g/L}$ TCE, removal was lower (77%), but increased to 90% when the phenol concentration was raised to 25 mg/L. The maximum field transformation yield of 0.062 g of TCE/g of phenol compared favorably with the highest measured resting-cell laboratory yield of 0.11 g of TCE/g of phenol. These results demonstrate high promise for in-situ aerobic cometabolic biodegradation of TCE with phenol-induced enzymes.

Introduction

Trichloroethylene (TCE) is one of the most frequently found organic contaminants in groundwater. Considerable research has been directed over the past 15 years toward finding biological methods for in-situ destruction of this compound in order to avoid pumping and treating groundwater at the surface. TCE appears not to be used as a primary energy source for bacterial metabolism, but may be destroyed through cometabolism under a variety of aerobic and anaerobic conditions. Aerobic cometabolism of TCE was first noted by Wilson and Wilson (1). Since then, TCE cometabolism has been observed by a variety of microorganisms that use different compounds to induce the enzymes (oxygenases) involved. These substrates include methane (2–7); propane (8), ethylene, and propene (9–11); toluene, phenol, cresol (12–16); ammonia (17, 18); isoprene (19); and isopropylbenzene (20). Oxygenases induced by microorganisms growing on the above substrates fortuitously oxidize TCE to TCE epoxide, which spontaneously degrades chemically to a variety of products that can be mineralized by mixed microbial communities in the environment.

The oxygenase induced by aerobic toluene or phenol utilization appeared particularly promising following a laboratory comparison, and so phenol was selected for a field study of in-situ cometabolism of TCE at the Moffett Naval Air Station, Mountain View, CA (Moffett Field) (21). The efficiency of TCE transformation was found to increase with an increase in phenol concentration. With the injection of 12 mg/L phenol, greater than 90% destruction of TCE and *cis*-1,2-dichloroethylene (*c*-DCE) was obtained. Initial concentrations of the two compounds were 40–50 $\mu\text{g/L}$. Because of the success of this evaluation,

further studies were conducted in order to determine the effect of higher concentrations of up to 1 mg/L TCE on in-situ destruction. The results are reported here together with the results from laboratory studies directed toward determining factors that might affect the rate and extent of TCE degradation by phenol-oxidizing bacteria.

Materials and Methods

Laboratory Studies. Laboratory batch studies were designed to better determine the mass ratio of TCE transformed to phenol utilized [transformation yield, T_y (2)]. Also, previous studies with methanotrophs have shown that additions of formate or methanol, which serve as sources of reducing power, increased the rate and extent of TCE utilization (2, 22, 23). The possibility of a similar effect with phenol utilizers was thus examined.

A mixed culture was used for the batch experiments. The mixed culture was grown in a 4-L reactor that was baffled, continuously stirred, and continuously fed at a total rate of 1.44 L/day, with 250 mL of liquid wasted at 4-h intervals. This provided an average detention time of about 2 days. The feed consisted of two solutions, a phenol solution and a basal salts medium (24) flowing at rates of 0.67 and 0.33 mL/min, respectively. The phenol concentration in the combined flow was 1.6 g/L, and the ammonium–nitrogen concentration was 6.7 mM. The original seed for this reactor was obtained from a 3-month-old batch-fed phenol enrichment culture developed originally from air-stripped Moffett Field groundwater. The reactor feed concentration was initially low (500 mg/L phenol) to maintain reactor phenol concentrations below toxic levels and was increased over a period of 5 months to the level designated above.

For batch experiments, TCE transformation was measured in 250-mL amber glass bottles sealed with Mininert Teflon-lined caps. To each bottle, 20 mL of deionized water containing an organic compound was added together with 3 mL of suspended culture from the reactor, followed by 2.0 mL of TCE-saturated water (resulting in an aqueous TCE concentration of about 27 mg/L). The initial TCE mass in the bottles was calculated from measurements of TCE concentrations in head-space samples taken after 1 min of vigorous hand shaking, using estimates of the equilibrium distribution of TCE between the gas and liquid phases (2). The bottles were then placed in a 21 °C environmental chamber on a Lab-Line circular action shaker table rotating at approximately 200 rpm. Fifty-microliter gas samples were periodically withdrawn for TCE analysis using a Hamilton CR700-200 syringe. Samples were taken for 148 h, at which time TCE degradation appeared to have ceased in all bottles. From the total amount of TCE degraded, the transformation capacity (g of TCE transformed/g of cell dry weight) and transformation yield (g of TCE transformed/g of phenol used to grow cells) were determined (2). The TCE-

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saturated aqueous solution used was prepared as previously described (2). The organic chemicals added to different bottles included the following: formate (Fischer Scientific), acetate and benzoate (J. T. Baker), lactate (Sigma), salicylate and tryptophan (MCB), phenol (EM Science), and catechol (ChemService). No organic chemicals were added to the controls.

In-Situ Studies. The in-situ evaluation was performed using the same methodologies as described previously (21). Here, a series of stimulus-response tests were conducted under induced gradient conditions of injection and extraction of groundwater, using the south southeast (SSE) leg of the test site as in the previous study with phenol (21). This consisted of an injection well (SSEI) and an extraction well (P2) located 7 m apart, with three monitoring wells between. The monitoring wells (SSE1, SSE2, and SSE3) were spaced 1, 2.2, and 4 m, respectively, from the injection well. All well screens were placed in a silt, sand, and gravel aquifer that was about 1.4 m thick and located about 5 m below ground surface. The aquifer was confined above and below by a silty clay layer of low permeability. A detailed chemical description of the groundwater and the test zone was given previously (21, 25).

In order to create a dominant groundwater gradient between the injection and extraction well, 10 L/min of groundwater was removed from the extraction well and was air-stripped to remove volatile compounds prior to discharge to receiving waters. A portion of extraction water was pumped before air stripping into the injection well at 1.5 L/min, after being amended with phenol (12.5–25 mg/L), dissolved oxygen (35 mg/L), bromide (76 mg/L), and TCE (60–1000 µg/L). On the basis of studies with the bromide tracer, groundwater travel times from the injection well were approximately 4, 12, and 30 h to the SSE1, SSE2, and SSE3 monitoring wells, respectively. Further details on the system were provided previously.

This study of the effect of TCE concentration on its degradation was initiated during the second field season of phenol injection following 8 months of inoperation. The aquifer was prestimulated with the addition of 5 mg/L phenol (time averaged) and 35 mg/L oxygen to the injection water for 5 days before initiating the TCE addition. Then, phenol, oxygen, bromide, and 60 µg/L TCE were added together to the injection water at time zero. All chemicals except phenol were added continuously. Phenol was pulse-injected by adding over a 15–40-min period out of each 8 h a concentrated phenol solution containing 9 g of phenol, giving a time-averaged phenol injection concentration of 12.5 mg/L. This concentration was used for the first 1000 h of operation. After that, phenol concentration was increased 50% to a time-averaged concentration of 19 mg/L for 1 week and then increased again to 25 mg/L, or twice the initial concentration.

After 1 week of operation, the injected TCE concentration was increased to 125 µg/L, then to 250 µg/L after another week, 500 µg/L during the subsequent week, and then 1000 µg/L following that.

Analytical Methods. For the laboratory studies, culture dry-mass concentration was determined by filtering 50 mL of culture through a Whatman GF/F glass fiber filter, drying overnight at 103 °C, cooling and weighing, followed by combustion for 20 min in a 550 °C oven, and again cooling and weighing. Dry solids concentration was determined from the weight loss during combustion. TCE

Table I. TCE Transformation Capacities and Yields by Phenol-Grown Cells in Presence of Various Organic Compounds^a

compound added	amount added (mM)	T_c (g of TCE/ g of cells)	T_y (g of TCE/ g of phenol)
resting cells (no added compound)		0.24	0.11
formate	10	0.38	0.17
formate	20	0.31	0.14
formate	40	0.25	0.11
lactate	3.3	0.38	0.17
acetate	5	0.27	0.12
benzoate	1.3	0.20	0.09
tryptophan	0.9	0.19	0.08
catechol	1.5	0.14	0.06
salicylate	1.4	0.04	0.02
phenol	1.4	0.03	0.01

^a Concentration of compounds = 40 mM electron equivalents, equivalent to 20 mM formate, unless otherwise indicated.

concentration was determined by electron-capture gas chromatography as previously described (2), except that 50 µL of headspace gas was withdrawn with a Hamilton CR700-200 constant-rate syringe and immediately analyzed, and standards were prepared by adding TCE to isooctane in vials sealed with Mininert Teflon-lined caps.

Monitoring of the field experiment was performed using an on-line automated data acquisition system as described in detail previously (25). The system included automated gas chromatography (GC) with both electron-capture and Hall conductivity detectors for TCE analysis, a high-performance liquid chromatograph (HPLC) for phenol analysis, ion chromatography for bromide analysis, and probe and meter for dissolved oxygen (DO) analysis. The lower limits for the analysis were as follows: DO, 0.1 mg/L; bromide, 0.5 mg/L; TCE 0.5 µg/L; and phenol, 0.025 mg/L.

Results

Laboratory Studies. At steady state, the 1.6 g/L phenol-fed laboratory reactor produced a dried-cell density of approximately 700 mg/L, giving a calculated net growth yield of 0.44 g of cells/g of phenol consumed. The mixed culture consisted primarily of about 0.5 µm diameter and 2 µm long Gram-negative rods. Analysis of these cells as described previously (26) indicated the storage polymer poly(β -hydroxybutyrate) (PHB) was present at a level of about 12% of cell dry weight. PHB appears to be useful as a source of reducing power for TCE transformation (26).

Results of studies to determine cell TCE transformation yield and capacity are summarized in Table I. No TCE was utilized in the controls, and TCE transformation in the resting cell culture (no compound added) began immediately but leveled off with time as typically observed in such systems with methane or phenol-oxidizing cultures. The transformation capacity (T_c) measured here was 0.24 g of TCE transformed/g of dry cell weight.

The extents of TCE transformation were lowered when aromatic compounds, including phenol, were added. However, TCE degradation tended to be enhanced by the presence of the aliphatic compounds, the greatest en-

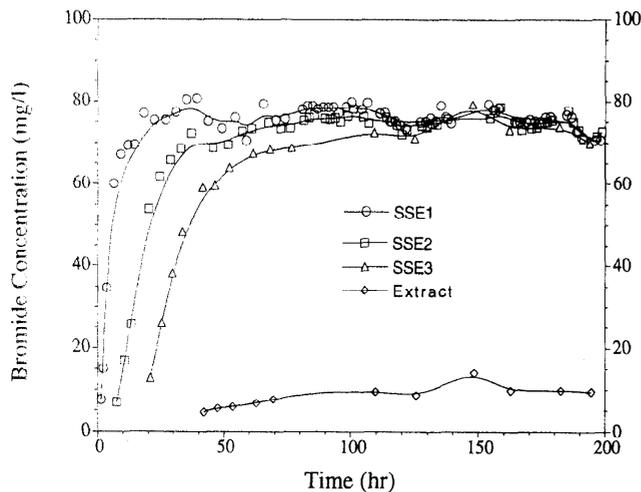


Figure 1. Bromide tracer concentrations with time at monitoring locations following initial injection of 76 mg/L.

hancement being with formate or lactate addition. Indeed, formate and lactate increased the transformation capacity by as much as 60% over the base capacity. Also of significance, the phenol-grown mixed culture had a much higher capacity to degrade TCE than the methanotrophic bacterial culture reported by Alvarez-Cohen and McCarty (2), which had a T_c of 0.036 and 0.073 g of TCE/g of cell dry weight, and a T_y of 0.013 and 0.026 g of TCE/g of methane without and with formate, respectively.

Moffett Field Studies. Figure 1 illustrates normalized bromide results at the monitoring wells following the first injection of bromide at a concentration of 76 mg/L. In this figure, as well as in all others except Figure 3, the lines shown represent running averages for the data as obtained by a locally-weighted least-squares error method. At all wells the bromide reached the injection concentration ($C/C_0 = 1$) within a few days or less, indicating the full penetration of injected water past each monitoring well, ensuring that removal efficiency for TCE could be estimated at each monitoring well by a comparison with the injected TCE concentration, once sorption had reached steady-state equilibrium between groundwater and aquifer solids. No dilution by noninjected water occurred at any of the monitoring locations; the dilution noted at the extraction well is indicative of radial flow into that well as anticipated.

Figure 2 illustrates the normalized DO concentration at the monitoring wells. DO was present in the water at a concentration greater than 30% of the injected concentration throughout most of the study at all monitoring wells, thus ensuring that aerobic conditions were present. The exception was when a high phenol concentration (25 mg/L) was injected; here, DO approached zero at times. The great variation in DO concentration at the first monitoring well (SSE1) resulted from the pulsed addition of phenol, which was accompanied by the periods of greatest DO depletion. As demonstrated in all previous studies, this pulsing effect was dampened by dispersion at all subsequent monitoring wells. Initially, DO reduction on average at the first monitoring well was less than at SSE2, but no further reduction occurred downgradient of SSE2. This suggests, and phenol measurements confirmed, that phenol was consumed to levels below 50 $\mu\text{g/L}$ by the time the injected water reached SSE2. After about 300 h of operation, the average DO depletion was similar at all monitoring locations. Due to the increased biomass

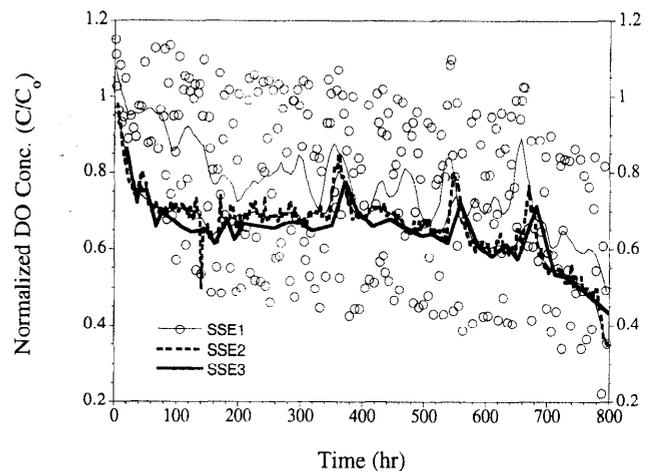


Figure 2. Normalized measured dissolved oxygen concentrations at monitoring locations following the pulsed injection of phenol at a time-averaged concentration of 12.5 mg/L. Data are shown only for SSE1 to illustrate large concentration fluctuations resulting there from pulsed phenol addition. In addition, running averages are provided for all locations as this better illustrate trends.

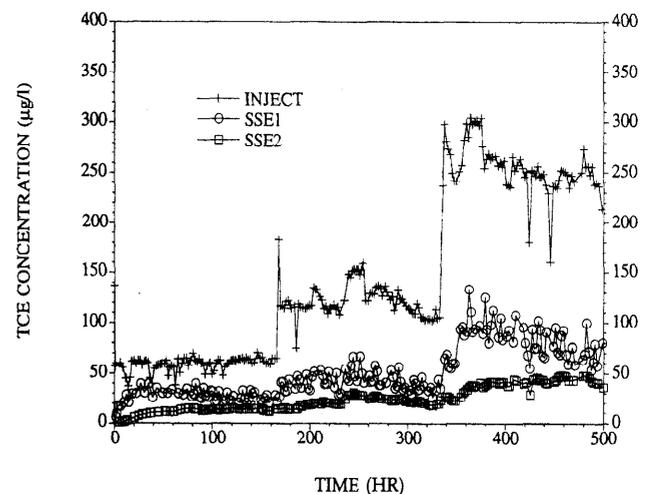


Figure 3. TCE concentrations with time in injection water and at sampling locations following the injection of 12.5 mg/L time-averaged phenol concentration.

concentration with time near the injection well, phenol utilization was then essentially complete by the time the groundwater had reached SSE1. Thus, most biological activity became confined within this 1-m zone, in spite of efforts to spread phenol further into the aquifer by use of the pulsing strategy.

Figure 3 illustrates TCE concentration at the injection well and at the monitoring wells SSE1 and SSE2 during the first 500 H of operation. A sufficiently large phenol-oxidizing population remained from the previous year's operation so that phenol and TCE utilization began immediately. With the initial TCE injection concentration of 62 $\mu\text{g/L}$, at least 40% removal was observed at the SSE1 well and 65% at the SSE2 well. After 40 h, the SSE1 TCE concentration continued to decrease until the injection concentration was increased to about 125 $\mu\text{g/L}$ after 168 h of operation. A near-steady-state concentration at SSE2 was also achieved by the end of the first week. Following each subsequent concentration increase, TCE concentration at SSE1 again increased somewhat for 3-4 days and then decreased. A similar effect occurred at SSE2. These results suggest that 7 days was sufficient time to reach a

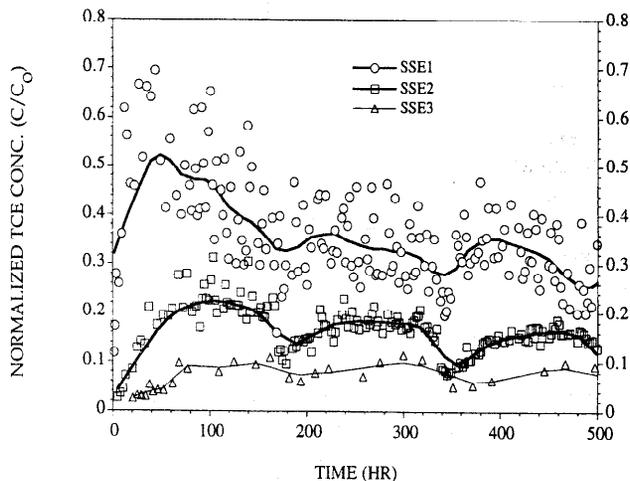


Figure 4. Normalized TCE concentrations with time during injection of 12.5 mg/L time-averaged phenol concentration, while TCE injection concentration (C_0) was step increased from 62.5 to 250 $\mu\text{g/L}$. Lines represent running averages of data to better indicate trends.

pseudo-steady-state condition at which conservative estimates of removal efficiency could be made.

Some of the different transport and removal processes occurring can be observed in Figures 3 and 4. Figure 3 illustrates measured concentrations, while Figure 4 illustrates concentrations at the monitoring wells normalized with respect to the injection concentration. The lines shown with Figure 4 represent running averages of the data. Following each increase in the injection concentration of TCE, the response at SSE1 was rapid, with a resulting increase there taking place for a few days. This can best be seen following the increase to 250 $\mu\text{g/L}$ after 336 h. The additional 30 h required to reach a maximum TCE concentration at SSE1 compared with the 4 h advective transport time appears to have been related partly to the sorptive response of TCE to aquifer material. After about 40 h, TCE concentration at SSE1 decreased somewhat. This response might be attributed to the slowly increasing mass of phenol-oxidizing microorganisms in the test zone. A similar response was observed at SSE2, but the time periods are longer, the maximum concentrations reached are lower, and the oscillations in TCE concentration that resulted largely from the pulse injection of phenol and resulting competitive inhibition response are greatly dampened. Concentration variations at SSE3 (Figure 4) are even smaller. This dampening effect presumably results from the combination of transport, dispersion, sorption, and lowered phenol competition as described previously for methane injection (27).

Figure 5 presents a summary of normalized TCE concentration for the period of 0–1000 h over which the TCE concentration was increased from about 62 to 1000 $\mu\text{g/L}$. With the increase to 1000 $\mu\text{g/L}$, the removal efficiency at SSE2 decreased from about 80% to about 60% and that at SSE3 from better than 90% to 78%. As long as the TCE concentration was 500 $\mu\text{g/L}$ or less, the overall removal in the system exceeded 85%.

With TCE at 1000 $\mu\text{g/L}$, phenol concentration was then increased to 10 mg/L at 1008 h, and then to 25 mg/L at 1176 h. The results are illustrated in Figure 6. TCE removal increased following the increases in phenol injection. Since no controls were available, it is not possible to conclude the degree to which improved removals with

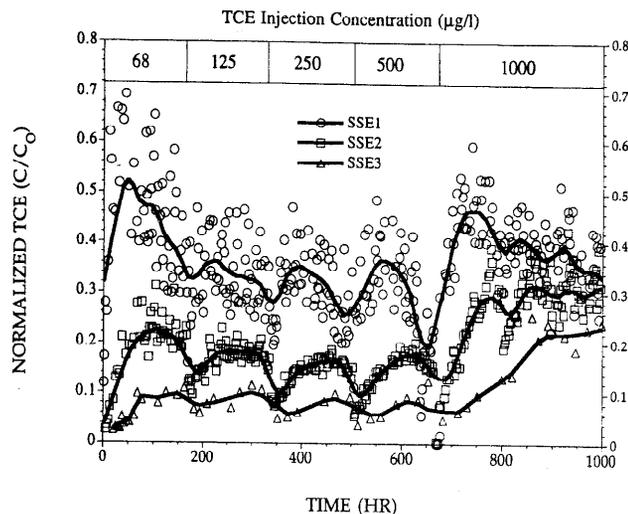


Figure 5. Normalized TCE concentrations with time at monitoring locations during phenol injection at a time-averaged concentration of 12.5 mg/L, during which TCE injection concentration (C_0) was raised in steps from 62.5 to 1000 $\mu\text{g/L}$.

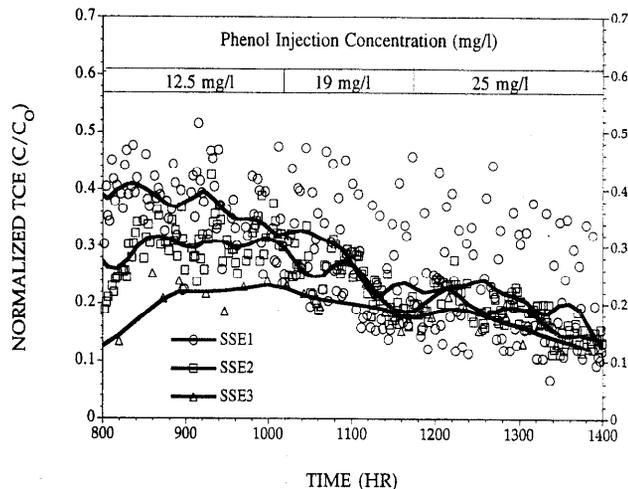


Figure 6. Normalized TCE concentrations with time at monitoring locations with injected TCE concentration (C_0) of 1000 $\mu\text{g/L}$, during which the time-averaged phenol concentration was increased in steps from 12.5 to 25 mg/L.

time were a direct result of phenol concentration increases or to other factors such as better adaptation to the TCE. In any event, at the end of this study, TCE removals of about 90% were being obtained with an injection TCE concentration of 1000 $\mu\text{g/L}$ and phenol concentration of 25 mg/L.

The phenol concentration was measured at all monitoring locations throughout this study, except for the injection stream, which was calculated based upon the mass added. Phenol was frequently detected at SSE1, but concentrations found there were generally less than 0.5 mg/L when 12.5 mg/L was injected. However, detections at SSE2 and SSE3 were infrequent. The HPLC with fluorimeter detection that was used to measure phenol had a visible response to a phenol concentration as low as 10 $\mu\text{g/L}$, but the quantifiable detection limit was about 25 $\mu\text{g/L}$. The phenol concentrations at the SSE2 and SSE3 monitoring locations were generally too low to give a visible response on the detector, and thus they are 25 $\mu\text{g/L}$ or below. Therefore, phenol removal was excellent and at least 99.8% in this system.

Table II. Average Removal Efficiencies for TCE at Various Monitoring Well Locations and Transformation Yields

phenol added (mg/L)	TCE added ($\mu\text{g/L}$)	removal (%)			transformation yield (g of TCE/g of phenol)
		SSE1	SSE2	SSE3	
12.5	62	60	78	89	0.0044
12.5	125	68	82	87	0.0087
12.5	250	70	82	88	0.018
12.5	500	68	84	88	0.035
12.5	1000	68	70	77	0.062
19	1000	75	82	85	0.045
25	1000	78	85	90	0.036

Discussion

The laboratory studies indicated that the addition of aromatic compounds together with TCE during batch experiments lowered the TCE transformation capacity. This reduction in TCE degradation by aromatic compound addition may have resulted from competitive inhibition or toxicity. The high final carbon dioxide content in the gas of all bottles containing aromatic compounds except salicylate (data not shown) suggests that the compounds, with the exception of salicylate, were degraded by the mixed cultures, and so compound toxicity may not have been the important factor. However, the extent of TCE degradation was enhanced by the presence of formate, lactate and, to a small extent, acetate. They probably function as noncompetitive external sources of reducing power as found with methanol and formate for methanotrophic bacteria. Hence, their addition in the field may be an effective means to increase TCE degradation efficiency (27). The higher T_c found with 10 mM formate addition compared with that at the higher formate concentrations studied (Table I) suggests that further studies are needed of factors affecting the optimal concentrations of external sources of reducing power considered for addition in the field.

The results of the Moffett Field study are summarized in Table II. The percentage removals listed are based upon average values at the end of a period following a change in concentration. Considering results at the SEE3 well, removals of better than 87% were obtained with TCE injection concentrations of up to 500 $\mu\text{g/L}$ and an injected phenol concentration of 12.5 mg/L. The percentage removals were essentially the same at all the lower injected TCE concentrations, indicating the removal tended to be first order with respect to concentration. At 1000 $\mu\text{g/L}$, removal efficiency was a lower 77% with 12.5 mg/L phenol injection. The lower percentage removal may have resulted because TCE concentration was nearer to the K_s value, and thus deviation from first-order kinetics occurred here, TCE transformation product toxicity was beginning to have a measurable effect, or there was insufficient reducing power available to carry out the transformation when the lower phenol concentration was used.

When the phenol concentration was increased above 12.5 mg/L, TCE removal improved as had been found previously (21). Because of the lack of suitable controls, this improvement cannot be attributed with confidence solely to the increase in phenol concentration. At the highest concentration of phenol used, about 90% TCE removal was obtained with an injection concentration of 1000 $\mu\text{g/L}$. Thus, this study has demonstrated that high efficiency removals can be obtained in-situ by phenol and

oxygen injection, even with relatively high TCE concentrations.

Oxygen usage tended to increase with time, even though the injected phenol concentration was maintained constant for the first 1000 h of operation. While part of this increase might be attributed to oxygen usage for TCE oxidation, the amount for this would be negligible, less than 0.4 mg/L for oxidation of 1000 $\mu\text{g/L}$ TCE. The major portion of the increased oxygen usage is for cell respiration. With time after phenol addition, the cellular mass in the aquifer would increase and so would its demand for oxygen for respiration. Following the initial phenol injection, the demand was about 1.5 g of oxygen/g of phenol injected, and after 1000 h it was about 2.0 g of oxygen/g of phenol injected. The oxygen:phenol ratio decreased again following the increase in phenol concentration to 25 mg/L, at which time DO became limiting. The theoretical amount of oxygen required for complete phenol oxidation to carbon dioxide and water is about 2.4 g oxygen/g of phenol. The lower oxygen requirement than this theoretical amount can be attributed to partial phenol synthesis into cellular material.

Another measure of importance is the transformation yield. Based upon values listed in Table II, the highest transformation yield found in the field was 0.062 g of TCE/g of phenol, which is over 50% of the value found with the resting (non-fed) laboratory culture of 0.11 g of TCE/g of phenol. With lower TCE concentrations, or with the use of higher phenol concentrations to increase the percentage removal, the transformation yield was lower. In the laboratory a much higher TCE concentration was used so that the value obtained there is perhaps near the maximum that could be obtained without the addition of an external source of noncompetitive reducing power such as formate. Also, in the field, phenol was continuously added, resulting in competitive inhibition. This could lower the transformation yield, as was found in the laboratory study when phenol was present. As another factor, the laboratory culture appeared to be nitrogen-limited as PHB formation was high, providing an internal source of energy for TCE transformation. In the field, the 0.40 mM nitrate present in the groundwater provided excess nitrogen so that PHB formation would probably be less. The exact effect of this on transformation yield is not known, but it could reduce it as well.

The transformation yield provides useful information to help estimate the amounts of phenol and oxygen that would need to be injected into groundwater in order to obtain a given removal of TCE, and thus it is an important cost component of the treatment system. The much higher transformation capacity and yield with phenol-oxidizing bacteria compared with methane-oxidizing bacteria, both with and without exogenous sources of reducing power such as formate, perhaps best explain the better Moffett Field results for TCE found with phenol than with methane (21).

The close agreement found between transformation yields in the field and laboratory is encouraging. Since primary substrate (electron-donor) and electron-acceptor addition are likely to be among the major cost components of an in-situ treatment system, methods to increase transformation yield are important. The laboratory study suggest that the addition of a noncompetitive external source of reducing power, such as formate or lactate, may help increase the transformation yield by providing necessary energy without inhibiting TCE degradation.

The Moffett Field facility effectively simulates an in-situ treatment system where contaminated groundwater is pumped to the surface, phenol and oxygen are added to the water, and the mixture is injected back into the ground for biodegradation. The Moffett Field case represents essentially a one-dimensional view of an in-situ bioremediation system where the aquifer acts as a bioreactor. Another approach effectively simulated by the Moffett Field system is a doubled-screened recirculation well with one screen located in the aquifer at a higher elevation and another screen located at a lower elevation (28-30). A pump operates within the well to draw contaminated water into the well through one screen and injects it back into the aquifer through the other screen. Phenol and oxygen (perhaps in the form of hydrogen peroxide) are then mixed with the recirculating groundwater in the well for injection into the aquifer. Such a system avoids bringing the groundwater to the surface, an advantage both in reducing human contact with contaminants and in reducing costs for pumping. In either case, the aquifer itself serves as the bioreactor, so an above-ground system is not needed.

On the basis of the results of this study, high percentage removals of TCE in groundwater appear possible by multiple recirculations through an extraction/injection system as described above. However, caution needs to be taken in the extrapolation of the results obtained at Moffett Field to other sites as phenol-oxidizing bacteria with less ability at TCE degradation could be present. Studies on the characteristics of microorganisms present at other sites should be conducted prior to full-scale implementation.

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