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Effect of Contaminant Concentration on Aerobic Microbial Mineralization of DCE and VC in Stream-Bed Sediments

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Discharge of DCE and VC to an aerobic surface water system simultaneously represents a significant environmental concern and, potentially, a non-engineered opportunity for efficient contaminant bioremediation. The potential for bioremediation, however, depends on the ability of the stream-bed microbial community to efficiently and completely degrade DCE and VC over a range of contaminant concentrations. The purposes of the studies reported here were to assess the potential for aerobic DCE and VC mineralization by stream-bed microorganisms and to evaluate the effects of DCE and VC concentrations on the apparent rates of aerobic mineralization. Bed-sediment microorganisms indigenous to a creek, where DCE-contaminated groundwater continuously discharges, demonstrated rapid mineralization of DCE and VC under aerobic conditions. Over 8 days, the recovery of [1,2-^{14}C]DCE radioactivity as $^{14}CO_2$ ranged from 17% to 100%, and the recovery of [1,2-^{14}C]VC radioactivity as $^{14}CO_2$ ranged from 45% to 100%. Rates of DCE and VC mineralization increased significantly with increasing contaminant concentration, and the response of apparent mineralization rates to changes in DCE and VC concentrations was adequately described by Michaelis−Menten kinetics.

Introduction

Intrinsic bioremediation is, under suitable conditions, an environmentally effective and cost-efficient mechanism for restoration of contaminated aquifer systems. Because chlorinated ethenes are among the most prevalent groundwater contaminants in the United States, application of intrinsic bioremediation to the cleanup of sites contaminated with these compounds is especially attractive. In many chlorinated ethene-contaminated aquifer systems, regulatory acceptance of in situ remediation is hampered by the presence of the reduced daughter products, dichloroethene (DCE) and vinyl chloride (VC) that are formed during reductive dechlorination of PCE and TCE under anaerobic conditions (*1*−*7*). DCE and VC are U.S. EPA priority pollutants (*8*), and their presence in groundwater generally drives regulatory concerns for site of remediation.

The complex redox character of DCE and VC makes application of intrinsic bioremediation to DCE- and VC-contaminated aquifers problematic. Because DCE and VC are relatively reduced, their tendency to undergo further reductive dechlorination is significantly diminished (*9*), and consequently, these compounds frequently persist in anaerobic aquifers (*1*−*7*). In contrast, under aerobic conditions the potential for direct or co-metabolic oxidation of DCE and VC exists (*10*−*19*). The widely observed reductive dechlorination of polychlorinated ethenes under anaerobic conditions combined with the potential for rapid aerobic oxidation of the reduced daughter products has led several investigators to suggest that efficient bioremediation of chlorinated ethene-contaminated aquifers may occur in contaminant plumes characterized by upgradient anaerobic and downgradient aerobic zones (*1, 2, 15, 20*). Just such a fortuitous zonation of redox conditions can be found at sites where anaerobic, chlorinated ethene plumes discharge to aerobic surface water bodies. Under these circumstances, however, the potential for bioremediation depends on the ability of the stream-bed microbial community to efficiently and completely degrade DCE and VC over a range of contaminant concentrations. The purposes of the studies reported here were to assess the potential for aerobic DCE and VC mineralization by stream-bed microorganisms and evaluate the effects of DCE and VC concentrations on the rates of aerobic mineralization.

Methods

Chemicals. The ability of microorganisms to mineralize DCE and VC under aerobic conditions was evaluated in sediment microcosms using [1,2-^{14}C]DCE and [1,2-^{14}C]VC. A neat mixture of [1,2-^{14}C]DCE was obtained from Moravek Biochemicals, Inc. (Brea, CA). The [1,2-^{14}C]DCE used in this study was a mixture of 29% trans and 71% cis isomers. The radiochemical purity of the DCE mixture was determined by radiometric detection gas chromatography to be greater than 99.9%. Neat [1,2-^{14}C]VC was obtained from New England Nuclear Research Products, Du Pont (Boston, MA). The radiochemical purity of the VC was determined by radiometric/flame ionization detection gas chromatography to be greater than 97.6%. The chemical purity of both test substrates was independently confirmed by GC/FID and GC/MS.

Study Site. Microcosm experiments were initiated using creek bed sediments from a former drum disposal area at the Naval Air Station (NAS), Cecil Field located near Jacksonville, FL. Shallow groundwater at this site flows eastward from the source area and discharges 330 m downgradient into a small creek. The site is characterized by a plume of *cis*-1,2-DCE-contaminated groundwater, which extends from the source area to the creek. Dissolved concentrations of *cis*-1,2-DCE in shallow, groundwater monitoring wells range from 1900 μg/L (20 μM) at the source area to approximately 40 μg/L (0.4 μM) in a well located 7 m upgradient of the creek. Concentrations of *cis*-1,2-DCE as high as 8.9 μg/L (0.1 μM) have been detected at a depth of 20 cm in the creek bed sediments, but no contamination of the upper 10 cm has been observed. Although this site has a history of VC contamination, at the time of this writing VC concentrations throughout the plume remain at or below the detection limit of 1 μg/L (0.02 μM). At the time of sample collection, dissolved oxygen concentrations were about 2 mg/L in the shallow bed sediments and greater than 6 mg/L in the overlying surface water. For the shallow groundwater and the creek water, the pH was 7.1 ± 0.3 and the temperature was 22 °C.

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Microcosm Study. Microcosms consisted of 20-mL serum vials that were amended with 10 g of saturated bed sediment (water content = 28.6% w/w) and sealed with Teflon-lined butyl rubber stopper/base trap assemblies (*21*, *22*). Microcosms were created with a headspace of air. Killed controls were prepared as described and autoclaved twice for 1 h at 15 psi and 121 °C. Duplicate experimental microcosms and a single killed control were prepared for each substrate at six dissolved concentrations ranging from 0.2 to 57 μM for VC and from 1.4 to 80 μM for DCE. Sediment microcosms were incubated in the dark at 22 °C for a period of 8 days. Dissolved DCE and VC concentrations were determined based on independent adsorption and headspace partition experiments conducted using the same bed sediment material. At 22 °C, dimensionless Henry coefficients of 0.06 and 0.99 were determined that corresponded well with published data (*23*) for DCE and VC, respectively. No significant adsorption of DCE or VC was observed in these sediments.

To sample, the base traps of individual microcosms were rinsed with 0.5 mL of sterile distilled water and filled with 0.3 mL of 3 M KOH. After a 12-h collection period, the KOH was removed, and the amount of trapped $^{14}CO_2$ was quantified by scintillation counting. $^{14}CO_2$ production was confirmed in select vials by the addition of barium chloride as described previously (*24*). The fact that no radioactivity was detected in the base traps of sterile serum vials that contained radiolabeled substrate but no sediment indicates that trapping of radiolabeled DCE and VC was not significant (less than 1%) in experimental microcosms. The amount of radioactivity initially present in the experimental microcosms as $^{14}CO_2$ was estimated based on the percentage recovery observed in killed and sediment-free control microcosms to be less than 1% of the total radioactivity added as DCE or VC. The $^{14}CO_2$ production data were corrected for the recovery efficiency of $^{14}CO_2$ and the amount of radioactivity present initially in sediment microcosms (<1%). The efficiency of $^{14}CO_2$ recovery was determined with $H^{14}CO_3^-$ at concentrations of 1, 10, and 100 μM. The percentage recovery efficiency was quite consistent for all concentrations (52 ± 3%) but relatively low due to the circumneutral pH of the interstitial water and the nonsacrificial (no acidification) recovery method employed in this study. Because of the rapid mineralization observed in the bed-sediment microcosms and the concomitant decline in the rates of mineralization over time, the initial, apparent rates of DCE and VC mineralization at different substrate concentrations were estimated from the recovery observed over the first 24 h.

The amount of DCE and VC biodegraded in the bed-sediment microcosms during the incubation period was estimated at the completion of the study from the difference in headspace concentrations between experimental and killed control microcosms (*21*). The quantities of DCE isomers and VC remaining in bed-sediment microcosms after the final base collection were determined by headspace analysis using flame ionization detection gas chromatography. Throughout the study period, ethene and ethane production were monitored by headspace analysis using thermal conductivity detection gas chromatography (detection limits were 50 nmol L^{-1} headspace).

Results and Discussion

The microorganisms indigenous to the creek bed sediments at NAS Cecil Field demonstrated rapid mineralization of VC under aerobic conditions (Figure 1). Over 8 days, the recovery of [1,2-^{14}C]VC radioactivity as $^{14}CO_2$ ranged from 45% to 98% in the experimental microcosms (Table 1). For all treatments, VC mineralization was primarily attributable to biological activity, because the recovery of $^{14}CO_2$ in killed control microcosms was 6% or less (Table 1). The high, observed $^{14}CO_2$ recoveries are consistent with headspace VC analyses,

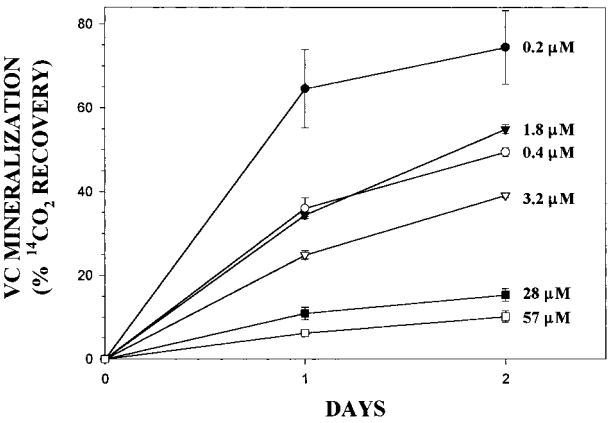

FIGURE 1. Percentage recovery of [1,2-^{14}C]VC radioactivity as $^{14}CO_2$ in bed sediment microcosms over a 48-h period. Data are means ± standard deviations for duplicate experimental microcosms.

TABLE 1. Final Percentage Recovery of Initial Radioactivity as $^{14}CO_2$ after an 8-Day Incubation Period

substrate	concn (μM)	experimental[a] (%)	control[b] (%)
[1,2-^{14}C]DCE	1.4	98 ± 12	3
	2.8	57 ± 2	7
	7.6	53 ± 4	5
	15	29 ± 5	6
	40	21 ± 1	4
	80	17 ± 4	3
[1,2-^{14}C]VC	0.2	98 ± 13	0
	0.4	97 ± 1	3
	1.8	92 ± 9	3
	3.2	68 ± 1	6
	28	52 ± 1	1
	57	45 ± 2	1

[a] Experimental data are means ± standard deviations (SD) for duplicate microcosms. [b] For each substrate concentration, control data are from single killed control microcosms.

TABLE 2. Concentration of DCE and VC Remaining in the Headspace of Experimental Microcosms after 12 Days[a]

substrate	concn (μM)	experimental[b] (%)
[1,2-^{14}C]DCE	1.4	16 ± 13
	2.8	12 ± 12
	7.6	32 ± 14
	15	77 ± 1
	40	70 ± 3
	80	88 ± 18
[1,2-^{14}C]VC	0.2	0 ± 0
	0.4	5 ± 4
	1.8	6 ± 9
	3.2	8 ± 3
	28	6 ± 6
	57	2 ± 1

[a] Final experimental concentrations are expressed as a percentage of the final concentrations observed in sterile control microcosms. [b] Experimental data are means ± standard deviations for duplicate microcosms. VC, ethene, and ethane were not detected in DCE microcosms. Ethene and ethane were not detected in VC microcosms.

which indicated that final experimental microcosm VC concentrations were less than 10% of the final control concentrations (Table 2). The fact that the changes in headspace VC concentrations were significantly greater than the recovery of $^{14}CO_2$ may reflect incorporation of the radiolabel into the biomass. Utilization of VC for growth has been reported previously (*10*, *11*). The present results are consistent with previous reports of microbial degradation

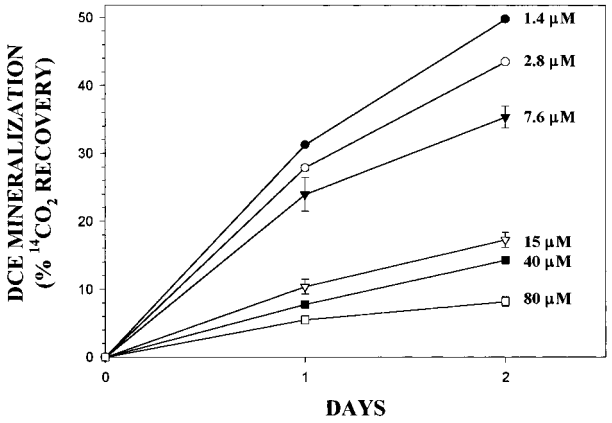

FIGURE 2. Percentage recovery of [1,2-^{14}C]DCE radioactivity as $^{14}CO_2$ in bed sediment microcosms over a 48-h period. Data are means ± standard deviations for duplicate experimental microcosms.

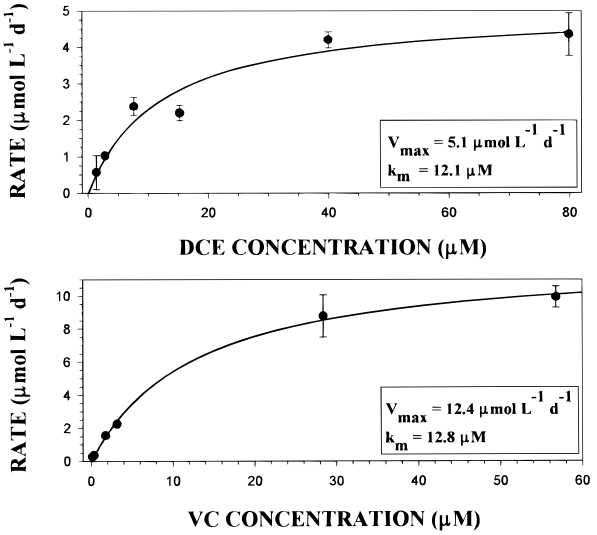

FIGURE 3. Change in the rate of microbial mineralization of DCE and VC (μmol L^{-1} d^{-1}) as a function of contaminant concentration (μM). For each dissolved concentration, the data are means ± standard deviations for duplicate experimental microcosms. Data have been corrected by subtracting the rate of mineralization observed in killed control microcosms at each concentration. Michaelis−Menten parameter estimates were obtained by nonlinear regression analysis.

of VC under aerobic conditions (*10*−*13*, *21*, *25*). The rapid aerobic vinyl chloride mineralization observed in the bed sediments in this study (6.2−58% in 24 h) is similar to that reported for chlorinated ethene-contaminated aquifer sediments (6.3−36% d^{-1}) (*21*) and for vinyl chloride acclimated cultures of Rhodococcus (9−12% d^{-1}) (*13*, *25*). These results demonstrate that the bed-sediment microbial community is capable of rapid, aerobic VC mineralization and represents a significant bioremediation potential under aerobic conditions.

Bed-sediment microorganisms were also capable of rapid mineralization of cis- and *trans*-DCE (Figure 2). For all substrate concentrations, DCE mineralization was primarily attributable to biological activity, because the recovery of $^{14}CO_2$ in killed control microcosms was 7% or less (Table 1). Final microcosm headspace analyses indicated that both cis and trans isomers of DCE were degraded by the bed-sediment microorganisms in approximately equimolar amounts (data not shown). The percent loss of DCE by the end of the incubation was in good agreement with the $^{14}CO_2$ recovery results (Tables 1 and 2). The results of the present study are consistent with a recent report of aerobic mineralization of DCE in surface soils and aquifer sediments (*14*). Klier et al. (*14*) reported greater than 95% mineralization of *cis*-DCE within 70 days and about 38% mineralization of *trans*-DCE within 60 days for soil microorganisms. DCE mineralization by aquifer microorganisms was found to be much slower with only 3−10% of [1,2-^{14}C]*cis*/*trans*-DCE recovered as $^{14}CO_2$ in aquifer microcosms after 180 days (*14*). In the present study, the recovery of [1,2-^{14}C]DCE radioactivity as $^{14}CO_2$ in bed-sediment microcosms ranged from 15% to 100% after an 8-day incubation period (Table 1). These results demonstrate that the bed-sediment microbial community is capable of rapid, aerobic DCE mineralization and represents a significant bioremediation potential under aerobic conditions.

No accumulation of reduced daughter products was observed in bed-sediment microcosms exposed to VC or DCE. Specifically, ethene and ethane were not detected in VC microcosms, and VC, ethene, and ethane were not detected in DCE microcosms. The facts that greater than 97% of the radioactivity added as [1,2-^{14}C]DCE and [1,2-^{14}C]VC was recovered as $^{14}CO_2$ and that $^{14}CO_2$ was the only product of biodegradation observed in this study indicate that degradation of VC and DCE involved oxidation to CO_2. The mechanism of oxidation may have been direct (*10*−*13*) or co-metabolic oxidation (*13*−*19*). Utilization of VC as a carbon source for growth and energy production has been reported previously (*10*−*13*). Although direct oxidation of DCE has not been verified, mineralization of DCE without the addition of a carbon cosubstrate has been documented for soil and aquifer microorganisms (*14*). Alternatively, co-metabolic oxidation of DCE and VC under aerobic conditions has been demonstrated for a variety of cosubstrates including methane (*15*, *17*, *18*, *26*), phenol (*16*, *19*, *27*, *28*), toluene (*19*), and propane (*13*). In the present study, potential cosubstrates for aerobic co-metabolism of DCE and VC include methane and numerous natural aromatic and aliphatic compounds associated with bed-sediment detritus. GC analyses of bubbles that continuously outgas from the bed sediments at the site indicated that the shallow bed-sediment microbial community is exposed to aerobic conditions and high concentrations of methane simultaneously as the result of oxygen supply from the water column and methane production in underlying anaerobic sediments. The presence of trace concentrations of methane in the bed-sediment microcosms suggests the possibility of methanotrophic co-metabolism under these conditions. In addition, the bed sediments contained 2−5% organic matter, consisting in large part of partially degraded plant material.

The apparent rates of DCE and VC mineralization changed significantly with increasing substrate concentration (Figure 3). A first-order rate expression did not accurately describe DCE and VC mineralization in this study, because the rates of mineralization did not increase linearly with increasing substrate concentration (*29*). Similarly, a zero-order rate expression did not adequately describe DCE and VC mineralization in this study, because the rate was not constant over the range of substrate concentrations investigated (*29*). Consequently, a nonlinear regression package (Sigmaplot 3.0, Jandel Scientific, San Rafael, CA) was used to estimate the Michaelis−Menten kinetic parameters, V_{max} and k_m, for aerobic mineralization of VC and DCE. For DCE, V_{max} was 5.1 ± 0.6 μmol L^{-1} d^{-1} (±standard error of the estimate) and k_m was 12.1 ± 4.1 μM. For VC, V_{max} was 12.4 ± 0.4 μmol L^{-1} d^{-1} and k_m was 12.8 ± 1.3 μM. The results indicate that, under these study conditions, the maximum rate of VC mineralization by the bed-sediment microbial community was approximately 2.4 times that for DCE. However, the bed-sediment microbial community exhibited a similar

affinity (approximately equal k_m) for DCE and VC. The Michaelis−Menten parameters, V_{max} and k_m, are sensitive to a number of environmental factors and vary according to in situ conditions. V_{max} is influenced by microbial biomass, metabolic acivity, and the availabilty of nutrients and substrates that support growth and metabolism (*29*). In groundwater and surface water environments, estimates of k_m are particularly sensitive to variations in substrate supply rates resulting from sediment matrix effects and differences in water flow rates (*29*). Given these limitations, accurate estimation of in situ kinetic parameters must be conducted under conditions that closely approximate the in situ environment. Because these estimates are sensitive to a number of environmental factors and because in situ conditions vary significantly over time and from site to site, the parameter values reported here are not intended as estimates of the in situ kinetic parameters. Instead, this investigation was intended to provide some indication of the potential for aerobic mineralization of DCE and VC and the general response of mineralization to changes in concentration.

Although biodegradation of groundwater contaminants is most often modeled with first-order kinetics, selection of an appropriate model for describing DCE and VC biodegradation is a matter of determining the k_m for degradation relative to the environmentally significant ranges of dissolved DCE and VC concentrations. For groundwater bioremediation, the VC concentrations of environmental significance range from the MCL of 0.02 μM (*15*) up to the solubility limit of 17.6 mM (*1*). In situ VC concentrations as high as 16, 25, and 71 μM have been reported for Dover AFB, DE (*30*), Plattsburg AFB, NY (*31*), and St Joseph, MI (*32*), respectively. For DCE, the concentration range of environmental significance is from the MCLs of 0.7 and 1 μM for *cis*- and *trans*-DCE, respectively, up to the solubility limit of about 4.1 mM (*12*). In situ DCE concentrations as high as 20, 100, 530, and 1300 μM have been reported for NAS Cecil Field (this study), Dover AFB (*30*), Plattsburg AFB (*31*), and St Joseph, MI (*32*), respectively. The present study was conducted under near static conditions intended to maximize the apparent k_m and should therefore overestimate the in situ concentration range for which DCE and VC biodegradation is pseudo-first-order with respect to concentration. Using the k_m determined in the present study for DCE as a liberal estimate of the concentration range for pseudo-first-order degradation, the results clearly demonstrate that the first-order model is not appropriate for describing changes in DCE mineralization rates as a function of contaminant concentration at NAS Cecil Field. Moreover, the fact that DCE and VC concentrations at many sites (*30*−*32*) exceed those found at NAS Cecil Field suggests the possibility that first-order assumptions are not widely applicable to chlorinated ethene-contaminated sites and emphasizes the need to examine the biodegradation kinetics of chlorinated ethenes in detail.

The possibility that DCE and VC biodegradation may exhibit Michaelis−Menten kinetics has important implications for assessments of intrinsic rates of biodegradation. Intrinsic bioremediation of groundwater contamination has been applied most often to petroleum-contaminated sites where relatively low contaminant solubilities combined with comparatively high, apparent half-saturation constants for biodegradation make the first-order degradation model appropriate for simulation of petroleum−hydrocarbon biodegradation over time (*33*, *34*). As a consequence, a laboratory investigation conducted at a single, environmentally meaningful concentration could be expected to provide a reasonable estimate of the rate of hydrocarbon biodegradation over a range of concentrations. In contrast, the high aqueous solubility of DCE and VC and their comparatively low apparent half-saturation constants indicate that in situ rates of DCE and VC mineralization may exhibit Michaelis−Menten kinetics over the range of dissolved concentrations encountered in situ. Thus, until DCE and VC biodegradation investigations have been conducted in sufficient numbers to allow some generalization about the underlying kinetics of the process, it is important that biodegradation rate assessments for DCE and VC be performed over a contaminant concentration range relevant to the specific study site and in sufficient detail to allow identification of the appropriate kinetic model.

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