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Relationship between *Dehalococcoides* DNA in ground water and rates of reductive dechlorination at field scale

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ABSTRACT

Certain strains of *Dehalococcoides* bacteria can dechlorinate chlorinated ethylenes to harmless products. This study was conducted to determine if there is a valid association between the density of *Dehalococcoides* DNA in ground water and the observed rates of reductive dechlorination at field scale. *Dehalococcoides* DNA in water from monitoring wells was determined using the quantitative real time polymerase chain reaction (q-PCR) with DNA primer set specific for *Dehalococcoides* organisms. Dechlorination rate constants were extracted from field data using the BIOCHLOR software. Of the six conventional plumes surveyed, "generally useful" rates of dechlorination (greater than or equal to 0.3 per year) of *cis*-dichloroethylene (*cis*-DCE) and vinyl chloride (VC) along the flow path were observed at three sites where *Dehalococcoides* DNA was detected, and little attenuation of *cis*-DCE and VC occurred at two sites where *Dehalococcoides* DNA was not detected. At the two sites where there was no net direction of ground water flow, the relationship between the density of *Dehalococcoides* DNA in ground water and the trend in concentrations of chlorinated ethylenes over time in monitoring wells was not so consistent as that observed for the conventional plumes. A comparison of our study to a field study performed by Lendvay and his coworker indicated that monitoring wells did not efficiently sample the *Dehalococcoides* organisms in the aquifer.

1. Introduction

The extensive use of chlorinated solvents such as perchloroethylene (PCE) and trichlorothylene (TCE) in the past by industry and government have led to widespread contamination of the soil and groundwater all over the world (National Research Council, 1994; Hendrickson et al., 2002). Biological reductive dechlorination of PCE and TCE can be an important mechanism for the removal of chlorinated solvents from many anoxic aquifers; however, there appears to be a significant variation in the rates and extent of dechlorination. One explanation is that different populations of microbial species are responsible for dechlorination of chlorinated ethylenes at different sites (Flynn et al., 2000). While many bacteria can convert PCE and TCE to *cis*-1,2-dichloroethylene (*cis*-DCE) (Major et al., 2003), to date, only two bacteria, *Dehalococcoides ethenogenes* strain 195 and BAV1, have been isolated in pure culture that are able to dechlorinate *cis*-DCE and vinyl chloride (VC) to ethylene (Maymo-Gatell et al., 2001; He et al., 2003a, b). Strain BAV1 was identified as a *Dehalococcoides* species, similar to the *Dehalococcoides ethenogenes* strain 195 (He et al., 2003a, b). All mixed cultures that are capable of complete dechlorinaion of PCE or TCE contain organisms closely related to *D. ethenogenes*, and all studies

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reported to date have shown that if *Dehalococcoides* species are absent, then dechlorination past *cis*-DCE to VC or ethylene does not occur (Major et al., 2003).

The use of genetic analysis to detect *Dehalococcoides* organisms provides a rapid method for investigating the distribution of *Dehalococcoides* at a field site (Fennell et al., 2001). The study performed by Hendrickson et al. (2002) demonstrated that there was a strong association between the presence of *Dehalococcoides* DNA and complete dechlorination to ethylene. Furthermore, Lendvay et al. (2003) reported a quantitative relationship between *Dehalococcoides* DNA and ethylene production. Simply because *Dehalococcoides* DNA is present in ground water may not indicate that the bacteria are active and degrading the chlorinated solvents. On the other hand, the failure to detect *Dehalococcoides* DNA in ground water does not prove that the aquifer does not contain *Dehalococcoides* sp. or other bacteria that can degrade chlorinated solvents.

Many evaluations of chlorinated solvent contamination in ground water use mathematical models to project the future behavior of the contamination. The models are very sensitive to the rate constant for attenuation (Newell et al., 2002). The purpose of this research was to determine the relationships between *Dehalococcoides* DNA in ground water and the observed rates of dechlorination of different chlorinated ethylenes at field scale. The approach was entirely empirical. Rate constants for attenuation of chlorinated ethylenes were extracted from monitoring data at eight field sites, and ground water from the sites was examined for the density of *Dehalococcoides* DNA. The rates of degradation were compared to the density of *Dehalococcoides* DNA to see if there was a valid association between the two parameters.

2. Materials and methods

2.1. Site description

Eight chloroethylene-contaminated sites from six locations within the US were surveyed in this study. These sites had good records of long-term monitoring, which made it possible to extract rate constants for attenuation of the contaminants.

The Western Processing site is located at Kent, Washington (WA). The source was an industrial landfill. The lithology is primarily sand with silt lenses. The LF-3 site and the FTA-2 site are located at Tinker Air Force Base (AFB), Oklahoma (OK). The LF-3 site originated from an industrial sludge pit located on the landfill, and the FTA-2 site originated from a fire protection training area. The contaminants are in a semi-consolidated to consolidated sandstone, siltstone, and mudstone aquifer. The North Beach site is located at the US Coast Guard Support Center at Elizabeth City, North Carolina (NC). The source was a landfill. The contaminants are in silty to fine sand. The SS-17 site is located at Altus AFB, OK. The source was a TCE spill in a clay and shale aquifer. The Target Area 1 site is at Dover AFB, Delaware (DE). The source was surface impoundments that released contaminants to an aquifer in fine and course sand. The Area 800 site and the Area 2500 site are at the former England AFB, Louisiana (LA). The sources were solvent spills into clayey sand and course sand.

The plume at the Western Processing site was extracted with a pump and treat system from September 1988 through April 2000. The plume at site LF-3 has been extracted by a pump and treat system since early 1999. The plume at site SS-17 has been extracted with a two-phase vacuum extraction system since September 1996. At the North Beach Landfill Site, the source was removed in 1999. There are no engineered remediation actions at the other sites.

2.2. Analyses of Dehalococcoides DNA

Dehalococcoides DNA in groundwater samples was analyzed by SiREM (Guelph, Ontario). The test employs polymerase chain reaction (PCR) analysis using a primer set specific to DNA sequences in the 16S rRNA gene of the *Dehalococcoides* group. The primer set was designated SiREM Primer set 1. It targeted variable regions S4/S7 in the 16S rRNA gene. A second primer set amplified sequences from most members of the True *Bacteria* was used as a control to confirm that PCR amplifiable-DNA was successfully extracted from the sample. To capture Bacteria, 1 L of ground water sample was filtered using a 0.45 μm sterile Nylon filter. The biomass was removed collected from the filter, and DNA was extracted using the FastDNA® Spin Kit for Soil (Bio 101, Carlsbad, CA, USA).

The density of *Dehalococcoides* in the water samples was determined using quantitative real time PCR (q-PCR). The assays were performed for SiREM in the laboratory of Elizabeth Edwards at the University of Toronto, Ontario, Canada. The primer used was identical to those used in the commercial test (Quantitative Gene-Trac, SiREM, Guelph, Ontario). Q-PCR reactions (50 μL) were performed in duplicate using 25 μL of 2 × DyNAmo SYBR Green qPCR Master Mix (MJ Research Inc., MA, USA), 1.0 μL of 25 pmol of each primer and 19 μL of DNase and RNase-free water (Sigma) and 4 μL of template DNA which were gently mixed at room temperature and transferred into a 96-well plate (Opticon™ Systems) and sealed with eight-strip Ultraclear caps (MJ research Inc., Waltham, MA, USA). Q-PCR was performed with a DNA Engine Opticon 2 System (MJ Research Inc., MA, USA) with initial denaturation at 94 °C for 10 min; 45 cycles of 94 °C for 45 s, annealing at 60 °C for 45 s and extension of 72 °C for 50 s. Standard curves of C_t versus $\log_{10}$ 16S rRNA gene copy number, produced using known quantities of cloned *Dehalococcoides* 16S rRNA genes, were used to estimate the 16S rRNA gene copy number in unknown samples. Verification of the specificity and identity of the PCR products was determined by melting curve analysis performed between 72 and 95 °C using the Opticon Monitor Software. The effective detection limit of the q-PCR reaction was 500–5000 gene copies of *Dehalococcoides* DNA per liter of ground water extracted.

2.3. Chemical analysis

Groundwater samples for the analysis of chlorinated solvents were prepared in an automatic static headspace sampler. The samples were analyzed by gas chromatography with a mass spectrometer detector. The reporting limits were 1.0 μg/L for all the analytes. The concentrations of ethylene and methane in ground water samples were determined using a headspace equilibration technique. The gaseous components in the

headspace were separated by gas chromatography and then measured with a thermal conductivity detector. The reporting limits were 0.001 mg/L in the original aqueous phase for both gases. The concentration of dissolved H_2 was measured on a RGA3 Reduction Gas Analyzer equipped with a 60/80 molecular sieve 5A column and a reduction gas detector. The reporting limit was 1.0 nM in the water originally sampled. Nitrate plus nitrite were analyzed using Lachat Flow Injection Analyses. The reporting limit was 0.1 mg/L as nitrogen. Sulfate and chloride were analyzed using Waters Capillary Electrophoresis. The reporting limits were 0.5 mg/L for sulfate and 1.0 mg/L for chloride. Total organic carbon was analyzed using a Dohrman DC-80 Carbon Analyzer. The reporting limit was 0.5 mg/L. Ferrous iron was determined in the field using a Chemetrics Kit Model K-6010D. The reporting limit was 0.1 mg/L. The dissolved oxygen, pH, oxidation–reduction potential and conductivity were measured by corresponding electrodes and meters.

2.4. Calculation of dechlorination rates

The BIOCHLOR decision support system was used to calculate the rates of reductive dechlorination of chlorinated ethylenes (available at: http://www.epa.gov/ada/csmos.html) (Aziz et al., 2000). The BIOCHLOR software simulates remediation by natural attenuation of dissolved solvents at chlorinated solvent release sites. It is based on the Domenico analytical solutions to the solute transport equation and has the ability to simulate one-dimensional (1D) advection, 3D dispersion, linear adsorption, and sequential, first-order biotransformation via reductive dechlorination. It assumes biotransformation follows a pseudo-first-order rate law. Since BIOCHLOR incorporates a number of simplifying assumptions, it is not a substitute for the detailed mathematical models that are necessary for making final regulatory decisions at complex sites. It is offered as a screening tool to determine whether it is appropriate to invest in a full-scale evaluation of natural attenuation at a particular site. It is very easy to use as compared to other transport and fate model.

At six sites in five locations (see Table 2), the contamination in ground water formed a conventional plume. Each plume had a region of high contamination associated with the source and a region with lower concentrations extending away from the source in the direction of ground water flow. BIOCHLOR was calibrated to field data on contaminant concentrations from single sampling events. Site-specific information was collected from reports or papers on the study sites (Acree and Ross, 2003; Altus Air Force Base, 2002; Dover Air Force Base, 2003; Landau Associates, Inc., 1995, 2002; Tinker Air Force Base, 1999, 2002; Wilson et al., 1997). Parameters used to calibrate BIOCHLOR to the plumes are listed in Table 1 in the supplemental material. To avoid errors in fitting rate constants for natural attenuation, the rate constants were fit to data that were collected prior to initiation of any engineered remedies. A detailed example of one of the calibrations is provided in the supplemental materials.

At the Areas 800 and 2500 sites at England AFB, Louisiana, there was no discernable overall direction of ground water flow in the plumes. A bayou that communicated with the contaminated aquifer overlies the sites. Groundwater in the aquifer flowed toward the bayou or away from the bayou depending on the seasons and recent precipitation events. As a result, concentration isopleths of TCE, *cis*-DCE and VC were arranged in concentric circles that were centered about the source area. Therefore, time series data on contaminant concentrations and dummy variables for hydrologic properties (seepage velocity 100 ft/yr, longitudinal dispersivity 10 ft, and retardation factor 1) were inserted into BIOCHLOR to extract the rates of dechlorination over time in particular wells. A detailed example of one of the calibrations is provided in the supplemental materials.

2.5. Definition of "generally useful" rates of biological reductive dechlorination

McNab et al. (2000) evaluated the distribution of contaminants in more than 200 chlorinated solvent plumes. Ninety percent of the plumes had maximum concentrations of chlorinated hydrocarbons less than 70,000 μg/L. There is no legally mandated time frame for monitored natural attenuation of contaminants in ground water (US Environmental Protection Agency, 1999). For purposes of discussion, a time frame for remediation of 30 years will be assumed. The maximum contaminant levels (MCLs) for PCE and TCE in drinking water are 5 μg/L. The rate of natural biodegradation necessary to reduce concentrations from 70,000 to 5 μg/L in 30 years would be 0.32 per year (calculated by a first-order kinetic model). Similarly, the rates of natural biodegradation necessary to reduce 80%, 50%, and 10% of the plumes from their maximum concentrations to 5 μg/L in 30 years would be 0.27, 0.23, and 0.13 per year, respectively.

Wiedemeier et al. (1999) reported that typical values for the decay coefficients in the field were 0.07–1.20 per year for PCE, 0.05–0.9 per year for TCE, 0.18–3.3 per year for *cis*-DCE, and 0.12–2.6 per year for VC. The assumed rates in this study were in the range reported by Wiedemeier et al. For the purpose of discussion, a rate of 0.3 per year can be considered a "generally useful" rate constant for monitored natural attenuation of most chlorinated hydrocarbon plumes. If the initial concentrations of chlorinated ethylenes are low, or if the time allowed to reach MCLs is long, a slower rate might be acceptable at a particular site.

2.6. Statistical analysis

The Spearman correlation (a nonparametric analysis) was used to analyze the relationship between the density of *Dehalococcoides* DNA in ground water and the dechlorination rates of *cis*-DCE and VC at field scale. At sites where the dechlorination rates were extracted from multiple sampling events, the average dechlorination rates were used. The analysis were performed using the statistical software SPSS for windows (SPSS Inc., USA).

3. Results and discussion

3.1. Site geochemistry

Table 1 compares the geochemistry of the ground water at the various sites. Based on the concentration of soluble electron

Table 1 – Overall geochemical environment of the sites in the survey

Site	Well	Geochemisty[a]	O_2 (mg/L)	NO_3+NO_2-N (mg/L)	Fe(II) (mg/L)	SO_4 (mg/L)	CH_4 (mg/L)	H_2 (nM)
Western Processing	T4	Iron reducing and methanogenic	0.2	0.04	7.5	<0.1	20.7	2.38
LF-3	2-259B	Iron reducing	0.2	0.03	9	<0.1	3.1	—
North Beach	GM3-30	Sulfate reducing	0.2	<0.1	0.5	59.7	0.060	<0.4
	MW-6	Sulfate reducing	0.3	<0.1	0.2	5.72	0.055	7.97
FTA-2	2-62B	Nitrate reducing, sulfate reducing, methanogenic	0.1	5.32	—	138	0.938	7.69
Target Area 1	MW212D	Nitrate reducing, methanogenic	0.4	2.17	<0.1	0.71	3.26	1.19
	DM353D	Oxic	0.7	0.53	<0.1	0.36	0.247	10.47
Area 2500	Well #23	Iron reducing, methanogenic	0.1	0.08	12	<0.1	3.11	2.27
Area 800	A39L009PZ	Iron reducing, sulfate reducing	0.1	0.08	7	18.6	0.19	6.54
	SS45L001MW	Oxic, iron reducing	3.8	0.22	2.5	35.7	0.505	—
SS-17	WL080	Oxic or nitrate reducing	0.7	1.58	<0.1	1670	0.003	<0.4

[a] Oxic: dissolved oxygen was greater than 0.5 mg/L; iron-reducing: iron II was greater than 0.5 mg/L; methanogenic: methane was near or greater than 1 mg/L; nitrate-reducing: nitrate plus nitrite –N was greater than 0.5 mg/L and oxygen was not available; sulfate reducing: oxygen was not available and the concentration of sulfate was greater than 20 mg/L.

acceptors and reduced metabolic products, the ground water at each site was classified and assigned to arbitrary categories (see Table 1). Most of the water samples fell into more than one category. This may reflect spatial heterogeneity in the aquifer, with water from different geochemical environments contributing to the water sampled from the well. It may also reflect concurrent geochemical processes occurring in the aquifer. In either case, the rate of attenuation of chlorinated ethylenes in the ground water plumes in this survey could not be associated with a unique electron accepting process.

The SS-17 site and the Area 800 site were included in the survey as controls where reductive dechlorination and the presence of *Dehalococcoides* DNA were not expected. With the exception of well A39L009PZ at the Area 800 site, the water at these two sites is oxic, and the concentration of *cis*-DCE and VC are a small fraction of the concentration of TCE. Water in the lower plume at Target Area 1 is reducing in wells near the source (MW212D in Table 1), but oxic in a down gradient well (DM353D in Table 1). Water in the other plumes was iron-reducing, sulfate-reducing, or methanogenic.

3.2. *Calculation of dechlorination rates in conventional plumes*

The first-order rate constants were estimated by calibrating BIOCHLOR to field data following a forward, trial-and-error process until all the predicted concentrations of all the chlorinated ethylenes best matched their field data. At a real field site, concentrations in wells can be affected by the relationship between the screened intervals of the wells and the vertical distribution of contaminant concentrations. The actual rates of biotransformation probably vary from one location to the next in an aquifer. As would be expected, there was scatter in the field data. A sensitivity analysis revealed that if the rates of transformation varied by a factor of three, it was possible to clearly identify a rate that was the best fit to the data in the calibrations. Calibrations were examined at rate constants of 0.01, 0.03, 0.1, 0.3, 1, 3 and 10 per year. The rate constant producing the best match to the field data for each chlorinated ethylene was selected as the best estimate of the rate. Fig. 1 is an example of the sensitivity analysis. It shows the correspondence between the measured values for *cis*-DCE and VC at the North Beach site in 1997 and the concentrations that were predicted by calibrating BIOCHLOR using three different values for the first-order rate constant for biotransformation. The dotted line was considered as the best calibration to the field data.

At the Western Processing site, field data before implementation of a pump and treat system were used for calibrating BIOCHLOR. The rate constants compared very favorably with the rate constants derived from the half-lives for *cis*-DCE (1.1 years) and VC (0.55 year) obtained by Lehmicke et al. (2000) at the other sites, when possible, field data from independent sampling events were used to calibrate BIOCHLOR. This was done to evaluate the variability over time of the rate constants that were extracted from the field data. There was agreement in the rate constants extracted for the LF-3 plume from data collected in 1997 before the operation of extraction system and in 2002 after the operation of extraction system. The rate we extracted from data on the FTA-2 plume was faster than a

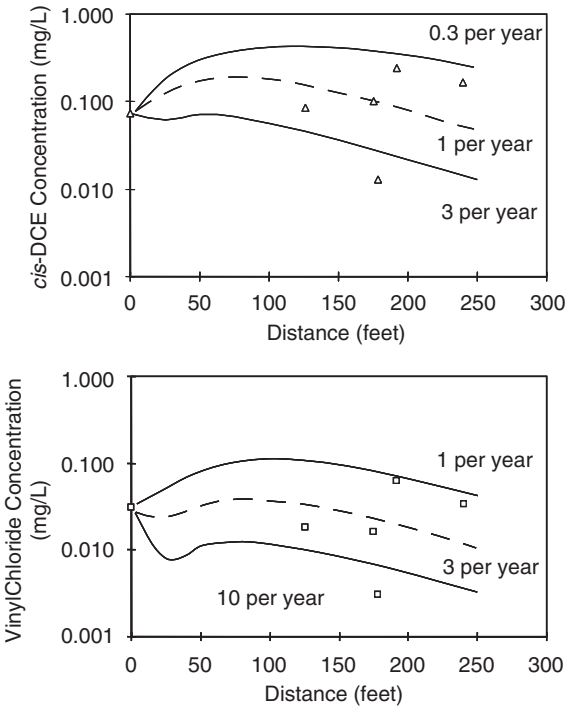

Fig. 1 – Correspondence between the measured values for *cis*-DCE and vinyl chloride at the North Beach site in 1997 and the concentrations that were predicted by calibrating BIOCHLOR using three different values for the first-order rate constant for biotransformation. The dotted line was considered as the best calibration to the field data.

rate constant extracted at the same site in 1999 by a contractor for the US Air Force (Parsons, Inc., cited in Tinker Air Force Base, 1999). The rate constants extracted from the North Beach site using data collected in 2002 after source removal was lower than in 1997 before source removal. The lower plume in the Target Area 1 is currently attenuating more rapidly near the source areas than in the far field. As a result, the current profile of concentrations with distance from the source is inverted. Therefore, we chose to calibrate BIOCHLOR to historical data collected in 1997. There was good agreement between the rate constants extracted using BIOCHLOR and rates previously extracted by Ei et al. (2002) using a transect approach or well-to-well comparisons.

3.3. *Relationship between Dehalococcoides DNA and dechlorination rates at conventional plumes*

Dehalococcoides DNA was measured and determined to be present in 24 contaminated wells at six sites where the contamination in ground water formed conventional plumes. In nine wells at three of the sites (Western Processing, LF-3 and North Beach), *Dehalococcoides* DNA was unequivocally detected. The locations of the monitoring wells and the density of *Dehalococcoides* DNA at the three sites were shown in Table 2 in the supplemental materials.

Table 2 shows the relationship between the concentrations of chlorinated ethylenes in ground water, their apparent rates of attenuation along a flow path in the aquifer, and the detection of bacterial DNA and the density of *Dehaloccoides* DNA in water samples from a monitoring well in the plume. There did not appear to be any consistent relationship between the concentrations of chlorinated ethylenes and their rates of biotransformation. A wide variety of concentrations were represented in the survey; however, a relatively narrow range of rate constants was deduced.

A consistent association between the presence of *Dehaloccoides* DNA and "generally useful" rates of dechlorination of lower chlorinated ethylenes was observed. At the three sites where *Dehalococcoides* DNA was detected in at least one monitoring well, the dechlorination rates of *cis*-DCE and VC were greater than or equal to 0.3 per year. The FTA-2 site did not have significant degradation of *cis*-DCE, and a lack of *Dehalococcoides* DNA was consistent with this observation.

The SS-17 site was oxic, and reductive dechlorination was not expected. This site was included in the survey as a control. The only extracted rate constant was very low, 0.01 per year. As would be expected, *Dehalococcoides* DNA was not detected.

"Generally useful" rate was also observed at a site where *Dehalococcoides* DNA was not detected. At the Target Area 1 site, in the lower plume, there was little *cis*-DCE degradation near the source, as evidenced by no VC, and no *Dehalococcoides* DNA. However, a rate of 0.3 per year was observed for the attenuation of VC along the flow path of the plume. Since the geochemistry data indicated that the water in the down gradient of the plume was oxic (see DM353D in Table 1), it is likely that the obtained rate of attenuation of VC was caused by aerobic degradation.

For a number of reasons, there was no quantitative correlation between the density of *Dehalococcoides* DNA in ground water and the rates of dechlorination of *cis*-DCE and VC. Nevertheless, by Spearman correlation analysis (a non-parametric analysis), the density of *Dehalococcoides* DNA in ground water had good positive correlations with the dechlorination rates of *cis*-DCE ($r = 0.89$, $p = 0.02$, $n = 6$) and VC ($r = 0.94$, $p = 0.01$, $n = 6$).

3.4. *Relationship between Dehalococcoides DNA and dechlorination rates in particular wells*

At the Areas 800 and 2500 sites at England AFB, there was no net direction of ground water flow. The dechlorination rates were extracted based on the time series data in the monitoring wells. Table 3 shows the relationship between the concentrations of chlorinated ethylenes in ground water, their apparent rates of dechlorination over time, and the detection of bacterial DNA and the density of *Dehalococcoides* DNA in water sampled from monitoring wells at England AFB, Louisiana. Two values are entered under "Date Collected" in Table 3. The first date is the earliest date in the data set used to extract rate constants. The second date is the last date used to extract rate constants and the date that the samples were collected to assay for *Dehalococcoides* DNA. In many wells it was not possible to calibrate BIOCHOR and extract rate constants because the chlorinated ethylenes were absent, or because concentrations increased over time. When it was possible to extract rate constants, there was not a consistent relationship between the concentrations of chlorinated

Table 2 – Relationship between the concentrations of chlorinated ethylenes in ground water, their apparent rates of dechlorination along a flow path in the aquifer, and the detection of bacterial DNA and the density of *Dehalococcoides* DNA in water samples from a monitoring well in the plume

Facility/location	Date	PCE	TCE	*cis*-DCE	VC	PCE	TCE	*cis*-DCE	VC	Bacterial DNA primer	*Dehaloccoides* DNA Cell density (Cells/L)
		Concentration near source (μg/L)				Rate of dechlorination (per year)					
Western Processing Kent, WA	1988[a]			10,000	460			0.6	3		
	1999			26	44			0.6[b]	1[b]		
	4/2003			0.34	1.59						2.3×10^6
LF-3 (landfill) Tinker AFB, OK	9/1997[c]	5.3	97	38,000	23,000			1	3		
	11/2002	8	28	28,400	20,400			1	3		2.7×10^6
North Beach, USCG Support Center Elizabeth City, NC	1997[d]	2000	105	74	30.8	0.1	1	1	3		
	10/2002	561	52	25	<1	0.1	0.6	0.3	1		1.8×10^5
FTA-2 (fire training) Tinker AFB, OK	8/1997	6.1	9440	1200	1.7		0.1[e]				
	11/2002	7.5	9330	977	2.9		0.3			Detected	
SS-17 Site Altus AFB, OK	3/2003	38.5	8160	264	4.28		0.01			Trace	
Target Area 1 (lower plume) Dover AFB, DE	7/1997	680	2400	560	<1	0.1	0.1	0.1	0.3	Detected	
	1997[f]	Transect TA to TB				0.26	0.23	0.31	0.3		
		Transect TB to TC				0.18	0.07	0.27			
		Wells TA to TB				0.14	0.06	0.08	0.2		
		Wells TB to TC				0.48	0.22	0.28			

[a] Data collected before the operation of pump and treat system.
[b] Data calculated from the half-lives for *cis*-DCE (1.1 years) and VC (0.55 year) obtained by Lehmicke et al. (2000).
[c] Data collected before the operation of extraction system, except that data near source was from the earliest date available (August 2001).
[d] Data collected in May or December of 1997 before source removal.
[e] Rate estimate obtained by Parsons (Tinker Air Force Base, 1999).
[f] Data obtained by Ei et al. (2002).

Table 3 – Relationship between the concentrations of chlorinated ethylenes in ground water, their apparent rates of dechlorination over time, and the detection of bacterial DNA and the density of *Dehalococcoides* DNA in water samples from monitoring wells at England AFB, Louisiana

Facility/location	Site/well	Date collected	TCE	*cis*-DCE	VC	TCE	*cis*-DCE	VC	Bacterial DNA primer	*Dehaloccoides* DNA Cell density (Cells/L)
			Concentration (μg/L)			Rate of dechlorination (per year)				
Area 2500	A39L011PZ	9/1997	<1	189	59.2		1			4.0×10^6
		4/2003	<1	<1	102					
Area 2500	A39L010PZ	6/1997	<1	9.5	549		0.3	0.3		1.2×10^6
		4/2003	<1	<1	95.5					
Area 800	A39L009PZ	6/1997	35.8	80.3	<1	0.1	0.1	1		6.7×10^5
		4/2003	20.8	52.6	5.2					
Area 2500	Well #5	3/1999	<12	39	120		3		Not Detected	
		4/2003	<1	<1	166					
Area 2500	Well #3	3/1999	<1	<1	<1				Not Detected	
		4/2003	<1	25.6	39.1					
Area 2500	Well #23	5/2000	<1	<1	<1					4.8×10^5
		4/2003	<1	92	60.6					
Area 2500	A39L015PZ	2/2002	<1	<1	<1				Not Detected	
		4/2003	<1	<1	0.4					
Area 800	SS45L001MW	6/1997	451	4.3	0.5	0.1	1	3	Detected	
		4/2003	249	126	4.5					
Area 800	Well #17	3/1999	9.8	<1	<1				Detected	
		4/2003	20.5	30.1	3.9					
Area 800	Well #19	3/1999	<1	<1	<1				Detected	
		4/2003	<1	<1	3.8					

ethylenes in ground water and the rates of dechlorination over time.

The relationship between the presence of *Dehalococcoides* DNA and the trend in concentrations of chlorinated ethylenes over time was not so consistent as that observed for the conventional plumes. Of the four wells where *Dehalococcoides* DNA was detected, only one well (well A39L010PZ at Area 2500) exhibited "generally useful" rates of dechlorination for both *cis*-DCE and VC. In well A39L011PZ at Area 2500, the concentration of VC increased over time; the dechlorination rate of *cis*-DCE was greater than 0.3 per year, correlating to the presence of *Dehalococcoides* DNA. In well A39L009PZ at Area 800, the dechlorination rates of TCE and *cis*-DCE were very slow, however, the dechlorination rate of VC was 1 per year, correlating to the presence of *Dehalococcoides* DNA. Well #23 at Area 2500 contained *Dehalococcoides* DNA, but the concentrations of both *cis*-DCE and VC were increasing over time.

According to Newell et al. (2002), the rate of attenuation of chlorinated ethylenes over time in monitoring wells is strongly influenced by the rate of physical and chemical weathering of the residual contamination in the source area of the plume. It seems that the concentrations of chlorinated ethylenes in the wells at England AFB was not controlled by the biotransformation of the chlorinated ethylenes dissolved in ground water, but by the rate of dissolution or desorption of the chlorinated ethylenes from the source material.

In well SS45L001MW at Area 800, the dechlorination of *cis*-DCE and VC were rapid (1 and 3 per year, respectively), but no *Dehalococcoides* DNA was detected even though bacterial DNA was detected. The geochemistry data indicated oxic or iron-reducing condition (see well SS45L001MW in Table 1), and *Dehalococcoides* was not expected in this environment. The obtained rate constants may be caused by oxidation reaction. Even though anaerobic microzones may exist and harbor *Dehalococcoides*, it may also be possible that the PCR assay has failed to detect *Dehalococcoides* bacteria that were present in the aquifer, or the transformations have been carried out by a strain of *Dehalococcoides* bacteria that was not recognized by the PCR primer, or the transformations of *cis*-DCE and VC have been carried out by other bacteria all together.

As would be expected, *Dehalococcoides* DNA was absent in oxygenated ground water where chlorinated ethylene concentrations were for the most part below detection limits (Well #19 at Area 800).

In three wells (well #5, well #3, and well A39L015PZ at Area 2500), bacterial DNA was not detected, indicating lack of PCR amplifiable DNA in the water samples. The absence of *Dehalococcoides* DNA in these samples did not approve the absence of *Dehalococcoides* bacteria in the ground water. This revealed that there are problems associated with sampling *Dehalococcoides* via monitoring wells.

3.5. *Evaluation of sampling bias caused by sampling monitoring wells*

To evaluate the effects of any sampling bias caused by sampling monitoring wells as opposed to sampling the aquifer sediment, the density of *Dehalococcoides* DNA in monitoring wells and rates of attenuation at field scale were compared to a field study performed by Lendvay et al. (2003). In Lendvay and his coworkers' study, the density of *Dehalococcoides* DNA in sediment as measured by the quantitative real time polymerase chain reaction (q-PCR) was compared to rates of removal of chlorinated ethylenes during in situ bioremediation of a PCE plume. The comparison was performed in two plots. One was augmented with *Dehalococcoides* organisms (augment plot), and one was not (control plot). The mean first-order rates of degradation of *cis*-DCE in the augment plot and in the control plot were 19 and 12 per year, respectively. The densities of *Dehalococcoides* DNA in the sediment in the augment plot and in the control plot were 8×10^5 and 3.6×10^4 *Dehalococcoides* cells per gram wet sediment, respectively. By assuming the sediment contained 0.11 ml of pore water per gram wet weight (water filled porosity 25%), the densities of *Dehalococcoides* DNA in or exposed to the pore water in the augment plot and in the control plot were 7×10^9 and 3.2×10^8 cells/L of water, respectively.

Fig. 2 compares the densities of *Dehalococcoides* DNA in monitoring wells versus the rates of attenuation of *cis*-DCE at field scale in our study to that in the study of Lendvay and his coworkers. The gray shape in Fig. 2 bounds the area where the rate of biotransformation is proportional to the cell density in the plot that was bioaugmented with an active culture of *Dehalococcoides*, and the plot that relied on the native strains of *Dehalococcoides*. The densities of *Dehalococcoides* cells in two of the monitoring wells at the natural attenuation sites fell within the gray shape, indicating that the performance of the native *Dehalococcoides* stains in the plumes undergoing natural attenuation was roughly equivalent to the performance of the *Dehalococcoides* stains at the active in situ bioremediation plots. Based on the concentration of cells in the water from the monitoring wells, the performance in the other wells was substantially better than in the bioremediation demonstration plots. The rates were up to one order of magnitude faster than would be expected from the density of *Dehalococcoides* cells.

The solid square in Fig. 2 is the first-order rate of transformation of *cis*-DCE by a laboratory culture of *Dehalococcoides* strain VS (Cupples et al., 2004). The culture was growing at 20 °C with optimal concentrations of molecular hydrogen. The dotted line in Fig. 2 extrapolates the performance of the laboratory culture to cell densities determined in our study. Based on the concentration of *Dehalococcoides* cells in ground water from monitoring wells, the *Dehalococcoides* organisms in the aquifer at the field sites performed as well or better than the culture growing under optimal conditions in the laboratory.

It is possible, but unlikely, that the native *Dehalococcoides* organisms at our survey sites were more efficient than the organisms reported by Lendvay et al. (2003) and Cupples et al. (2004). It is possible, but unlikely, that other organisms were primarily responsible for natural attenuation of chlorinated ethylenes at our study sites. The most likely explanation is that the monitoring wells did not efficiently sample the *Dehalococcoides* organisms in the aquifer, and that the number of *Dehalococcoides* cells recovered in a liter of well water was a small fraction of the number of cells that were exposed to a liter of ground water in the aquifer. Most of the *Dehalococcoides* cells were probably attached to sediment particles.

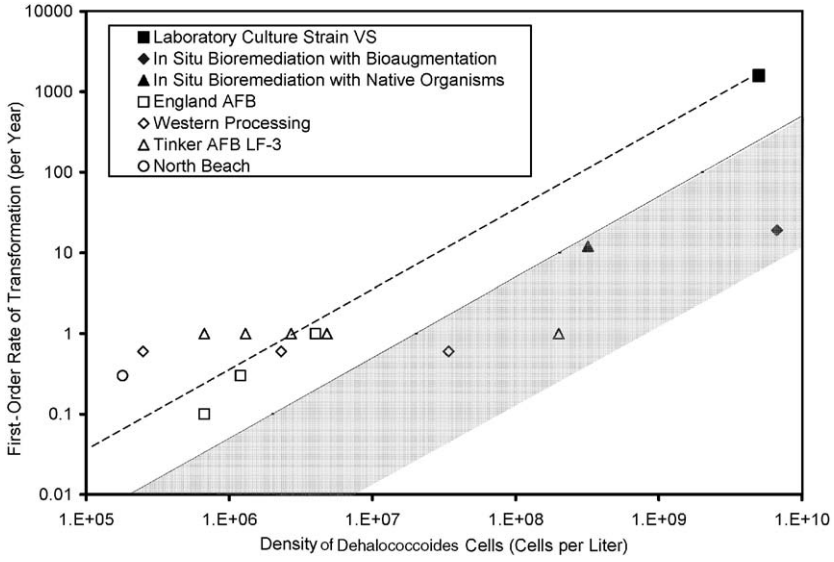

Fig. 2 – Relationship between the density of *Dehalococcoides* cells as determined by quantitative PCR and the first-order rate of attenuation of *cis*-DCE in ground water. The data points with an open symbol are from ground water samples collected at natural attenuation sites as presented in Tables 2 and 3. The data points with a solid diamond symbol or a solid triangle symbol are from sediment samples from a site where biological reductive dechlorination was used to clean up a PCE spill (Lendvay et al., 2003). The data point with a solid square symbol is from a laboratory study of *cis*-DCE transformation by *Dehalococcoides* strain VS growing under optimum conditions (Cupples et al., 2004).

If the relationship between the density of *Dehalococcoides* cells and the rates of *cis*-DCE degradation in the bioremediation study of Lendvay et al. (2003) is extrapolated to a "generally useful" rate of bioremediation for natural attenuation of 0.3 per year, then a density of near or greater than 1×10^7 *Dehalococcoides* cells per liter is necessary to produce a useful rate of natural attenuation. When the density of *Dehalococcoides* cells in wells at our field sites was greater than 1×10^7 cells per liter, the rate at field scale was greater than a "generally useful" rate of 0.3 per year (Fig. 2).

4. Conclusions

Despite the problems that may be associated with the achieved rates of biodegradation at field scale, the PCR assay, and the bacteria sampling bias with water from monitoring wells, there was a consistent relationship between the presence of *Dehalococcoides* DNA in ground water from monitoring wells and "generally useful" rates of dechlorination (greater than or equal to 0.3 per year) of *cis*-DCE and VC along the flow path at sites where the contamination in ground water formed conventional plumes. If *Dehalococcoides* DNA is detected at a density of 1×10^7 *Dehalococcoides* cells per liter of ground water at conventional plumes, we think the association can be used as a screening criterion to identify sites where monitored natural attenuation through natural biological reductive dechlorination may be useful, and further site characterization may be justified.

The relationship between the presence of *Dehalococcoides* DNA and the trend in concentrations of chlorinated ethylenes over time in monitoring wells was not so consistent as that observed for the conventional plumes. If the concentrations of chlorinated ethylenes in the wells were not controlled by the biotransformation of the chlorinated ethylenes dissolved in ground water but by other factors such as the dissolution or desorption of the chlorinated ethylenes from the source material, a PCR assay for *Dehalococcoides* DNA should not be expected to predict the rate of attenuation in concentration over time.

Notice

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Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.watres.2006.05.030.

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